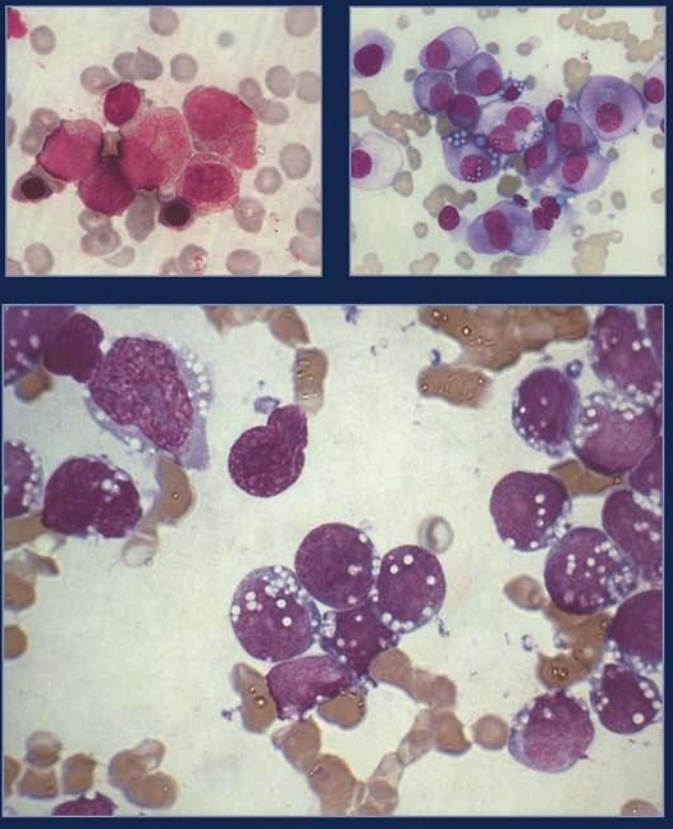
Volume 1

The Clinical Practice of Stem-Cell Transplantation

Edited by

John Barrett and
Jennifer Treleaven



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Foreword

It is an honour and privilege for the President of the European Blood and Marrow Transplantation Group (EBMT) to introduce this book on clinical practice of stem-cell transplantation. It appears at a fascinating and challenging time for this form of treatment. Within a span of 30 years, bone marrow transplantation, once an experimental venture, has become established practice and forms an integral part of the treatment plan for many disease categories. It is estimated that over 30,000 transplants worldwide, at least 14,000 of which were carried out in Europe, took place in 1996 from various donor types and sources. Numbers of transplants are still increasing. Cord blood has recently been introduced as an additional donor source, and new indications such as autoimmune disorders, are on the horizon. The decrease in treatment-related mortality observed over the last few years indicates that there will be an expansion to disease categories not necessarily associated with a lethal outcome.

This period of rapid expansion and change, shown by the shift within a few years from bone marrow to peripheral blood as donor source, produces confrontation between health care and health management. Medical procedure is no longer at the discretion of individual physicians. Budget constraints, cost efficiency considerations and standard operating procedures dictate plans for treatment. It is essential to have tools for coping with this situation. Cooperative national and international groups are one way, a textbook providing a solid background of the latest information another. I hope this book will be useful for all those involved in clinical blood or marrow transplantation for dealing with interactions between basic research, laboratory, nursing care and clinical medicine. The reward will be the well-being of the patients.

Alouis Gratwohl, 1997

Preface

A prerequisite for successful stem-cell transplantation is a coordinated team approach. Procedures are complex, and require haematological, general medical, surgical, psychological, nursing and laboratory input. Management is very interventive, both in terms of diagnosis and treatment, and requires a high degree of understanding between care team and patient.

In recent years, there has been a rapid expansion in the use of stem-cell transplantation worldwide, with new transplant teams coming into being each year. New specialists and new teams need practical information about the application of stem-cell transplantation in clinical practice, and the procedures involved. It is not always easy to obtain such information quickly and concisely. The indications for stem-cell transplantation have expanded considerably, and its place in treatment programmes is now much better defined. As these refinements have occurred and the procedure has become safer, more disease categories have become amenable to stem cell transplantation as a treatment approach, for example, chronic lymphocytic leukaemia, breast cancer, and autoimmune diseases. Also, patients are now better informed about treatment options. Treatment decisions have to be made very rapidly, and there are still many areas of controversy. Protocol books can help, but by their nature they rarely contain reference to the reasons behind the practice laid down. It is also difficult to keep protocol books either up-to-date, complete, or easy to locate on the unit.

With these considerations in mind, we have compiled this book which represents current transplantation practice across the entire discipline. It covers autologous and allogeneic transplantation using marrow, peripheral blood stem cells and cord blood, for the treatment of malignant and non-malignant conditions. There are four main sections: the first covers the place of stem cell transplantation in the treatment of the various malignant and non-malignant conditions. The second deals with the practical aspects of transplantation of relevance prior to the transplant, and the third section deals with practical issues occurring after the transplant. Finally, the fourth section is concerned with more general issues such as out-patient management and setting up transplant units. All of the contributors have had first-hand experience in clinical BMT, in the USA, Europe and Great Britain. We hope that with our broad choice of experts, we have succeeded in avoiding bias relating to a particular unit and that, as far as possible, the views presented reflect those generally accepted.

The aim of this book is to update the reader on advances in these fields and to provide an overview of the relevant literature. It is also to give an easy-to-find and easy-to-comprehend outline of the major problems which may be encountered, and their investigation, diagnosis and treatment, which are largely presented in the form of tables.

The book will be of use to healthcare workers in the field of bone-marrow transplantation, and particularly to those who have hands-on responsibility for patients, including doctors, nurses, pharmacy staff and many others. It is hoped that students of

Medicine will also find it readable, and that it will afford them some insight into the complexities which now face us when dealing with patients undergoing these intensive forms of treatment.

Jennifer Treleaven and John Barrett
May 1998.

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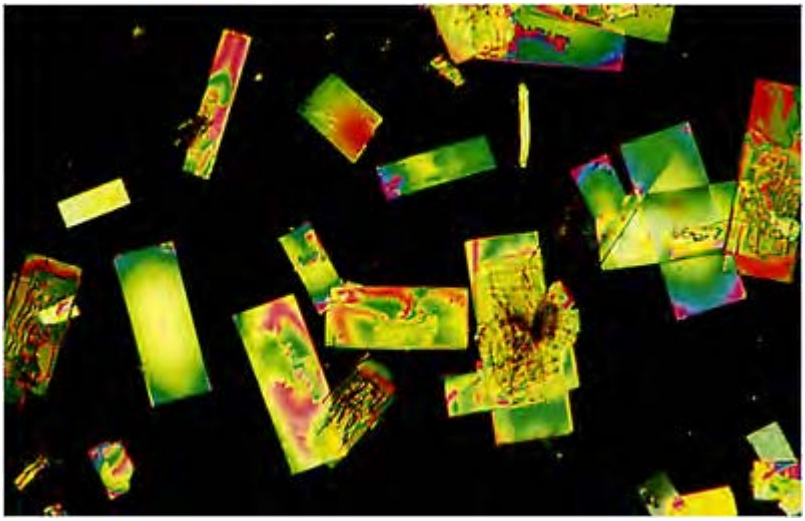
Part 1
The place of stem-cell transplantation in the treatment of malignant non-malingnant disorders



Chapter 1
Introduction

Jennie Treleaven and John Barrett

A brief history of bone-marrow transplantation

Prehistory to 1945

One of the earliest references to the therapeutic properties of bone marrow can be found in the early Irish epic tale *The Tain bo Cuailnge* derived from an ancient pre-Christian oral tradition. The charioteer Cethern, severely wounded and depleted of blood in battle, is treated by the healer Fingin: the treatment involves a three-day bath in bone marrow. According to the legend, this renewed Cethern's strength sufficiently to allow him to resume fighting although its long-term success cannot be evaluated since the hero was overwhelmed in battle shortly afterwards [1]. With the growing perception in the 19th century that the bone marrow was in some way responsible for blood formation came the idea that bone marrow might have healing properties and could be useful in the treatment of anaemia. It was at this time, for example, that the health tonic derived from bone marrow fats 'Virol' became popular (Figure 1.1). In 1896, Quine reported that Brown Sequard and D'Arsenoval administered bone marrow orally in 1891 to treat defective

blood formation [2]. Further attempts using a glycerol extract of animal bone marrow administered orally to treat pernicious anaemia were made [3]. The rationale of treatment with bone marrow was to provide missing nutrients. However, Billings in 1894 and Hamilton in 1895 were sceptical and attributed any positive effects of treatment to the mineral content of the elixir [4,5].

In 1923, Leake and Leake observed some responses to saline extracts of bone marrow in patients with anaemia [6], None of these procedures would have resulted in transfer of living cells and any responses may be attributed to the administration of iron to the anaemic recipient.

The first use of bone marrow administered by a technique likely to result in the transfer of living cells was by Schretzenmayr in 1937. Patients suffering from parasitic infections were treated with intramuscular injections of freshly aspirated autologous marrow, with some benefit [7]. Subsequently in 1944, Bernard injected allogeneic bone marrow into the medullary cavity in patients with bone-marrow deficiency but without success [8].

The devastating and terrifying complications seen in civilian populations following the first use of nuclear weapons in Hiroshima and Nagasaki in 1945 were a strong incentive for radiation biologists to understand and prevent the fatal radiation-induced bone-marrow failure syndrome. Jacobson and Lorenz were among the pioneers in this field. Jacobson showed in 1948 that mice could be protected from bone-marrow failure by shielding a portion of marrow in the hind limbs or the spleen [9]. Later he showed that the syndrome could also be prevented by infusion of spleen cells into an irradiated mouse. A controversy then arose as to the mechanism involved. Jacobson and others believed that the protective effect was due to the transfer of humoral substances that stimulated recovery of the irradiated marrow. Others, including Lorenz, believed that transfer of living cells was responsible for the recovery of the marrow. The year 1954 was a turning point. Lorenz, using a chromosomal marker which identified the transfused cells, demonstrated that the same marker recurred in all the cells derived from the recovering marrow of the lethally irradiated recipient mouse [10]. This proved not only that cellular engraftment had occurred, but also that the transfer of a few cells resulted in complete and stable haematopoietic reconstitution of the recipient. This discovery stimulated further experimental work, defining the biology and mechanisms of marrow transplantation.

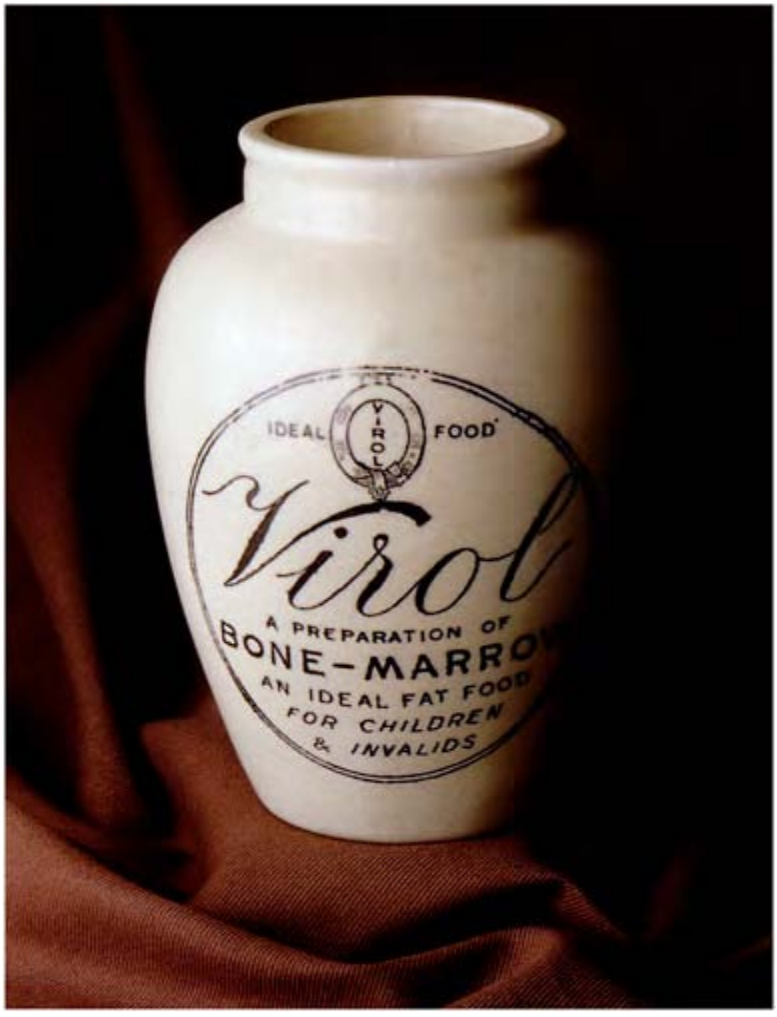


Figure 1.1 Bone marrow has long been considered to have nutritive properties. A replica of the original jars used in the 19th century to contain the nourishing extract made from marrow fats.

Early history 1945–1970: rescue by spleen cell and bone-marrow cell infusion from the 'bone-marrow syndrome' following radiation

In 1961, Till and McCulloch showed that in irradiated mice, marrow re-population originated from multipotential ‘colony-forming units’ which could be detected in the spleen [11]. Dosing experiments demonstrated that such colonies must have arisen from the seeding of a single cell—a stem cell. Single colonies isolated from the spleen could reconstitute haematopoiesis in other irradiated recipients, thus demonstrating that one stem cell could reconstitute the entire myeloid compartment of a recipient mouse. With the ability to save animals from the ‘primary syndrome’ of marrow failure by marrow or spleen cell transplantation came the discovery of the ‘secondary syndrome’, now called graft-versus-host disease (GvHD) [12]. The immunological requirements for GvHD were clearly set out by Billingham in 1966, who defined the central role of alloreacting lymphocytes in the effect [13]. As early as 1956 the idea that allogeneic bone-marrow transplants might exert a therapeutic immunological effect against malignancies was proposed by Barnes and Loutit who observed an antileukaemic effect of transplanted spleen cells in experimental murine models [14].

The first clinical application of bone marrow followed rapidly on the heels of the experimental work. The first attempts to intensify antitumour treatment by myeloablative therapy and autologous marrow transplant rescue were carried out in 1956 by Ferribee in the United States and others in Europe [15]. Treatment success was limited by lack of knowledge of how to administer high-dose therapy and inability to provide adequate supportive care for marrow failure. Early clinical marrow transplant attempts are well reviewed by Pegg [16]. In retrospect, it appears that there may have been at least 6 patients with aplastic anaemia rescued from marrow failure by marrow donations from their identical twin. However, in the absence of a marker of donor engraftment, the role of the donated marrow in the recovery of the patient was treated with scepticism at the time.

Before 1966 there are few reports of an unequivocally successful allogeneic marrow transplant. Out of 417 reported cases reviewed by Pegg, only 3 prolonged survivals following allografts were documented, and it was unclear if any of these patients had directly benefited from the marrow transfusion. Georges Mathé was a pioneer in the early development of clinical bone-marrow transplantation (BMT) (Figure 1.2). He advocated the use of a large radiation dose to eradicate leukaemia and use of substantial amounts of marrow to rescue the recipient [17]. In 1958, 6 physicists were accidentally exposed to large doses of mixed gamma and neutron irradiation at the Vinca nuclear power plant in Yugoslavia. The most severely irradiated died, but of the remaining 5, 4 were judged to have received a radiation dose of between 600 and 1000 rads. Mathé gave them multiple allogeneic bone-marrow infusions from family members. Temporary engraftment was demonstrated using red-cell antigen differences between donor and recipient. The patients survived with autologous recovery, probably protected by a temporary graft from the marrow infusions [18].

In the face of apparently insuperable problems, few investigators had the vision or persistence to make clinical marrow transplantation a primary focus of their research. E.Donnell Thomas working first in Cooperstown, New York and then in Seattle, USA, was the exception (Figure 1.3). In a series of carefully planned experiments in dogs, he defined the radiation doses, marrow-harvesting procedures and methods of support required to carry out marrow transplants in man. He developed a regimen of post-

transplant methotrexate as GvHD prophylaxis and established techniques of transplantation that persist until the present day and which won him the Nobel Prize for Physiology in 1990 [19].

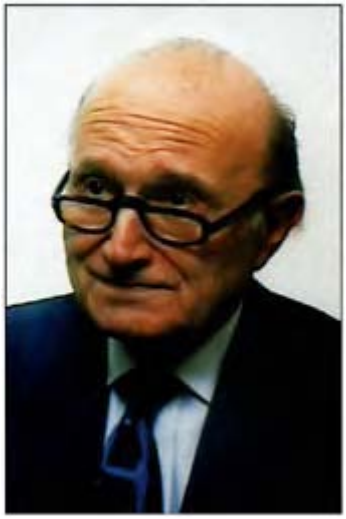


Figure 1.2 Georges Mathé

Figure 1.3 E.Donnell Thomas

However, the breakthrough that changed the whole face of BMT was the elucidation of the human leukocyte antigen (HLA) system, largely attributable to another Nobel

Prizewinner, Jean Dausset [20]. The miniaturization by Terasaki in Los Angeles made HLA typing a practical possibility [21]. With the ability to tissue match donors and recipients, allogeneic transplants between HLA-identical siblings could be carried out—the first was in an infant with severe combined immune deficiency disease in Leiden, The Netherlands [22]. By the end of the 1960s, all the components were in place for a new era of clinical bone-marrow transplantation.

Recent history 1970–1990: BMT as a clinical discipline

The 1970s were a decade of rapid clinical progress with the application of allogeneic BMT to all types of congenital immune deficiency diseases, severe aplastic anaemia, acute leukaemia and chronic myeloid leukaemia (CML). With better remission induction rates, there was renewed interest in autologous BMT for acute leukaemia. By the end of the decade, numerous centres worldwide had active BMT programmes and two influential multicentre bodies had become established: the International Bone Marrow Transplant Registry (IBMTR) (see Chapter 61) and the European Bone Marrow Transplant Group. The potential of BMT as a means of curing patients with otherwise incurable diseases was clear. However, the shortcomings of the procedure—GvHD, graft failure, leukaemic relapse and early toxicity—were now well defined, although even now they still present major problems.

The 1980s were a period of rapid expansion of clinical BMT programmes. Analyses on large patient series were carried out, and as a result it was possible to determine specific patient/donor and transplant-related risk factors for defined diseases. The availability of new drugs, notably cyclosporin for the prevention and treatment of GvHD, the antiviral agents acyclovir and later ganciclovir, and new generations of antibiotics contributed to a gradual reduction in transplant-related mortality [23]. This decade saw the first attempts at transplant engineering—the depletion of T-cells from the marrow inoculum to prevent GvHD. This manoeuvre, while reducing GvHD, was associated with increased problems of graft rejection and leukaemic relapse [24]. With the establishment of large unrelated volunteer donor panels by the Anthony Nolan Research Group in London, Europdonor based in Leiden, The Netherlands and the North American Marrow Donor Pool (NAMDP) in the USA, increasing numbers of transplants were carried out from unrelated donors [25]. In autologous BMT, the demonstration that patients with CML could be reconstituted with autologous peripheral blood cells was the prerequisite information supporting the later widespread use of peripheral blood stem-cell transplants [26]. Autologous transplantation with marrow rescue was used increasingly to treat lymphomas, myeloma and solid tumours. Novel preparative regimens were used to increase the antitumour effect. Techniques to purge the marrow of malignant cells dominated laboratory-based research [27].

Current status in the 1990s: new understanding, new approaches and new refinements

The 1990s have been a period of rapid development in BMT. A major change has been the increasing diversity of stem-cell and donor sources used for transplantation. The use

of peripheral blood stem cells (PBSC) mobilized into the blood by haematopoietic growth factors has overtaken the use of bone marrow for autologous transplants. PBSC transplants have made it possible to perform autologous transplants largely as outpatient procedures because of the rapid haematological recovery with which they are associated [28]. PBSC transplants have reduced the transplant-related mortality of both autografts and allografts. Following the first successful cord blood stem-cell transplant in 1988 [29], increasing numbers of patients (mainly children) have received cord blood transplants. The use of cord blood stem cells as a source of stem cells looks very promising because of the low incidence of acute GvHD from partially HLA-matched donations, and cryopreserved cord blood banks are currently established in several countries. With improved GvHD prophylaxis, stem-cell enriched transplants and better outcomes, transplants from less than fully matched family donors and from unrelated volunteer donors have become commonplace.

New understanding of the pathogenesis of GvHD, and of the basic mechanisms involved in alloreactions, has dramatically changed the way acute GvHD prevention is investigated in experimental studies. Instead of the global immunosuppression conferred by pharmacological agents and non-selective T-cell depletion methods, new approaches focus on the induction of specific tolerance, which prevent the development of acute GvHD during the establishment of donor immunity. It is reasonable to expect that the prevention of GvHD with improved immunological reconstitution post-transplant, and preservation or augmentation of the graft-versus-leukaemia (GvL) effect will result in improved outcomes for allogeneic BMT in the near future. The improved ability to control donor immune reconstitution should make bone-marrow stem-cell transplants safe even in patients lacking a fully matched related donor.

Since it first became accepted in the 1980s that GvL was a clinical as well as an experimental reality, much progress has been made in understanding and characterizing the GvL reaction [30]. While the separation GvL from GvHD is theoretically predicted and has, in limited circumstances, been achieved clinically, our inability to identify antigens driving leukaemia-specific alloresponses, remains the biggest single obstacle to improving the strength and specificity of GvL in clinical practice. However, progress in this field is gaining momentum and we can expect that the next century will see major advances in manipulation of the alloimmune response to the benefit of patients with a variety of malignant disorders.

Lastly, the great strides made in the field of molecular biology have made it possible to transfect genes into human cells. Gene therapy has numerous applications in the field of marrow transplantation—correction of inherited genetic disorders using the patient’s own stem cells or lymphocytes as the vehicle to deliver life-long gene replacement, modification of malignant cells to render them more susceptible to immune or chemotherapeutic control and modification of immune cells to make them more effective [31]. Following the partial success in treatment of the immune deficiency disease, adenosine deaminase deficiency, by infusion of lymphocytes transfected with the adenosine deaminase gene [32], several gene therapy trials have been initiated in other congenital disorders affecting stem cells. However, the problem of achieving adequate and sustained gene expression has yet to be solved.

The future: 2000 and beyond

It will become increasingly necessary to use the term ‘haematopoietic stem-cell transplantation’ rather than ‘bone-marrow transplantation’ which does not adequately describe the diversity of methods now employed to collect stem cells. It can be anticipated that the procedure will continue to become safer, thereby allowing us to safely extend curative treatments to elderly patients, patients only partially matched with the donor and conditions that are not immediately life-threatening. Along these lines a new area of development is the use of transplantation for autoimmune disorders (see Chapter 22).

The techniques that can make these developments possible are already under investigation. Current areas of research include the delivery of measured large doses of $CD34^+$ cells to achieve rapid and stable engraftment even in mismatched donor-recipient combinations. It may eventually become possible to expand stem cells *in vitro*, thus further allowing the opportunity to increase the size of the transplant. With the availability of all the clinically important growth factors required to stimulate both stem-cell proliferation, granulocyte, red cell and megakaryocyte lines (granulocyte-macrophage colony-stimulating factor G-GM CSF; erythropoietin; thrombopoietin; stem-cell factor, kit-ligand), it should be possible not only to significantly reduce the need for transfusion support and eliminate neutropenic episodes, but also to mobilize larger numbers of stem cells for autologous or allogeneic donation. Progress can be expected in the control of T-cell recovery by depletion, and add-back techniques of T-cells selected to avoid GvHD, the use of vaccines or adoptively transferred T-cells to induce antitumour and antiviral responses in the donor or the autograft recipient, and the application of gene therapy to selection or elimination of marked lymphocyte cell populations.

Principles of marrow stem-cell transplantation biology

Autologous and allogeneic transplants

Bone-marrow stem-cell transplants fall into two categories: autologous transplants from the patient’s own cells and allogeneic transplants where the transplanted cells are derived from another individual. Table 1.1 outlines the different limitations and applications of the two approaches. Autologous transplants are mainly used to restore bone-marrow function in patients receiving myeloablative therapy for malignancies. Autografts are being currently explored in the treatment of autoimmune disease where the high-dose therapy is used to provide potent immunosuppression with consequent myelosuppression. In the future, transplantation of gene-modified autologous stem cells and lymphocyte precursors may become more widely used.

Allogeneic transplants offer the opportunity to replace abnormal or malignant haematopoiesis or immune systems with normal haematological and immune cells from a healthy donor. The associated allogeneic immune response between the donor and recipient is responsible for the higher mortality from allogeneic transplants when

compared with autografts, and also for a beneficial antitumour effect and the opportunity to correct congenital or acquired disorders of marrow-derived cells such as haemoglobinopathies, immune deficiencies and aplastic anaemia.

Table 1.1 Comparison of autologous and allogenic stem-cell transplantation

Parameter	Autologous	Allogeneic
Indications	Haematological malignancies and solid tumours. Possible role in autoimmune disorders. Future role in combination with therapy to treat genetic disorders, HIV, etc.	Haematological malignancies, aplastic anaemia, congenital bone-marrow disorders, immune deficiency states, some Inborn errors of metabolism.
Source of stem cells	Autologous marrow or peripheral blood of ells.	Marrow, peripheral blood, cord blood, family donors, unrelated donors, HLA-matched or partially matched.
Preparative regimen	Primarily designed to provide intensive myeloablative treatment to eradicate malignant disease.	Required to provide immunosuppression to allow engraftment. Intensive therapy for malignant disease. Makes 'space' for incoming stem cells.
Post-transplant treatment	Supportive care, transfusions, growth factors, immune manipulation.	Supportive care, transfusions, growth factors, immune manipulation, prophylaxis of GvHD.
Infectious complication risk	Low—mainly in the early post-transplant period.	High—sustained risk of infection for months or years.
Major complications	Preparative regimen toxicity. Disease recurrence/progression. Treatment-related mortality usually <5%.	Preparative regimen toxicity. Disease recurrence/progression. GvHD. Immune deficiency. Treatment-related mortality 5–35% depending on many patient, donor and disease related factors.

Stem cells and growth factors

The basic unit of engraftment is the haematopoietic stem cell neccessary in sufficient numbers to establish complete lymphohaematopoietic recovery in the recipient. The $CD34^+$ surface glycoprotein identifies a primitive cell compartment which includes the pluripotent stem cell capable of sustaining long-term haematopoiesis [33]. However, in practice, a variety of cells are given at transplantation—post-thymic lymphocytes, monocytes, differentiated and undifferentiated progenitor cells. These components of the transplant can affect the outcome as shown in Table 1.2. Stem cells are responsible for the establishment in the recipient of a permanent stem-cell pool, which in turn provides a life-long supply of progenitors of cells in the myeloid and lymphoid series (Figure 1.4). Transplanted stem cells reach the bone marrow through the rich blood supply to the marrow and associate with the marrow stroma in functionally defined

microenvironmental niches necessary for the establishment of long-term haematopoiesis. Haematological recovery following transplantation depends upon a favourable environment, and presence of the haematopoietic growth factors shown in Figure 1.4. It is important to note that in addition to establishing haematopoiesis, the transplanted stem-cell pool is capable of generating new prethymic lymphocyte precursors which undergo maturation and

Table 1.2 Composition of the transplant as it affects the outcome

Component	Outcome
Stem cells	
Early re-populating (more differentiated) cells	Improve early haemopoietic recovery and raduce complications from infection and transfusion
Late re-populating (primitive progenitor) cells	Responsible for long-lasting haematopoietic function
Immune cells	
Natural killer cells	Enhance graft ‘take’ and antitumour effect
T-cells	Enhance graft take and antitumour effect; cause GvHD
B-cells	Responsible for recovery of production
Antigen-presenting cells	
Monocytes and macrophages	May enhance early recovery of immune competence and improve resistance to virus and malignancy
Dendritic cell precursors	
Tumour cells (autografts)	
	Can induce relapse

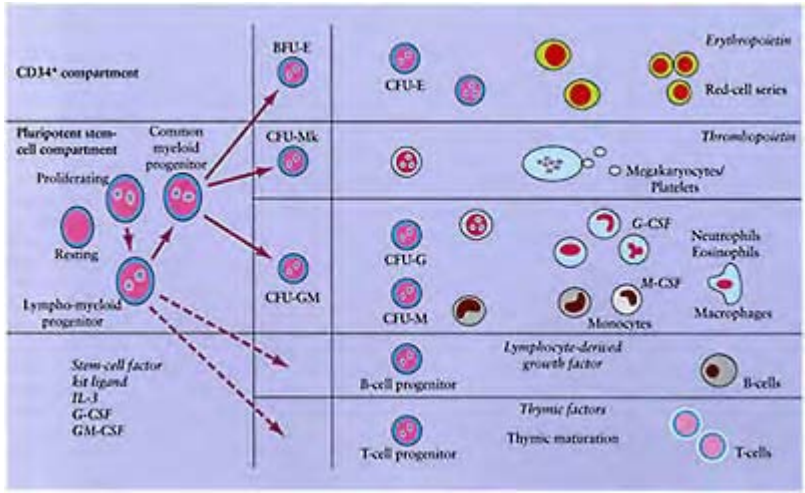


Figure 1.4 The stem cell and its progeny. Haematopoiesis can be subdivided into a pluripotent stem-cell compartment, committed stem-cell compartment, a maturing bone-marrow population and a post-marrow compartment (blood and tissues). Cells expressing the CD34 molecule define an early but broad group of cells which include the most primitive progenitors responsible for sustained haematopoiesis in the recipient. Growth factors and their major sites of action in the developmental process are shown in italics, Although there is some overlap, two functional categories of growth factors can be defined: (1) Early acting factors with activity on the pluripotent stem cells (SCF, kit ligand, interleukin-3 (IL-3), G-CSF, GM-CSF; (2) late acting, more lineage-specific factors including erythropoietin, G-CSF, M-CSF, thrombopoietin. Lymphocyte

development requires the specialized microenvironment of the thymus or B-cell development areas in the gastrointestinal tract, and a constellation of growth factors not illustrated here.

clonal selection in the host and can give rise to new immune cells tolerant to the recipient. There are two roles for the therapeutic use of haematopoietic growth factors in marrow transplantation: (1) to stimulate haematological recovery and reduce the risk of infection and need for transfusions; and (2) to mobilize stem cells into the blood to collect large numbers for subsequent transplantation either in the autologous or allogeneic setting. Currently available growth factors are GM-CSF and G-CSF, and erythropoietin. Stem-cell factor (SCF) kit ligand and thrombopoietin are currently being evaluated in preliminary trials.

Immunological considerations

Transplanted blood and bone-marrow cells contain significant numbers of mature T-lymphocytes. Unless extremely rigorous T-cell depletion has been performed, these cells are always responsible for the initial recovery of lymphocyte-mediated immune function after the transplant. Only after several months do lymphocytes derived from grafted stem cells emerge in the blood. Understanding the interaction between the donor and the patient's immune systems (the alloresponse) requires a basic knowledge of lymphocyte function. Figure 1.5 summarizes the essentials of the process of 'non-self'

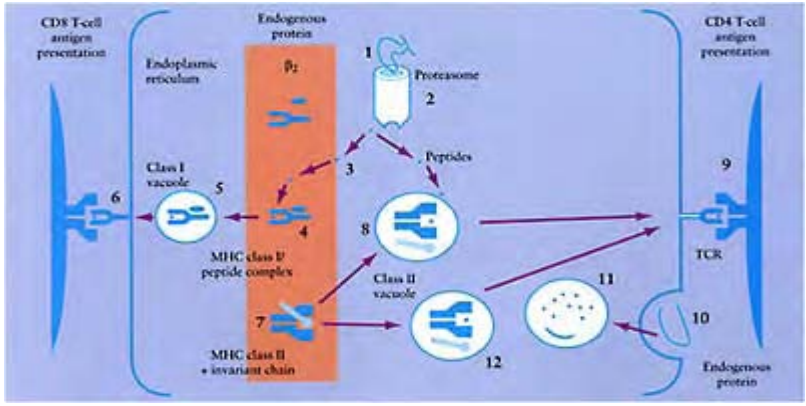


Figure 1.5 Antigen presentation. (1) Antigens derived from the turnover of endogenous proteins derived from all parts of the cell (membranes, nucleus,

cytoplasm) are broken down into peptides by the proteasome. (2) A specialized cytoplasmic organelle of which the proteins are in part coded for by the major histocompatibility complex class I pathway (3–6): Peptides produced by the proteasome enter the endoplasmic reticulum (ER) (3) by an ATP-dependent pump encoded by TAP *genes in the MHC complex. A peptide-MHC class I β_2-microglobulin trimolecular complex is formed (4) which migrates through the Golgi (not shown), generating an MHC class I vacuole (5). The vacuole transports the peptide-loaded MHC molecules to the cell surface, where peptide engaging with the appropriate T-cell receptor initiates T-cell activation (6). The T-cell receptor (TCR) together with CD3 and CD8 (not shown) interact with the entire MHC-β_2-microglobulin-peptide complex in this process.*

MHC class II presentation (7–12) MHC class II molecules originate in the ER bound and stabilized by an invariant chain molecule (7). Some peptides gain access to the post-Golgi class II vacuole displacing the invariant chain to bind to the antigen presenting groove of the MHC molecule. Endogenous antigen presentation to CD4 cells occurs through the interaction of the TCR, CD3 and CD4 with the MHC peptide complex (9). The classical exogenous antigen presentation pathway is shown

in (10–12). Self-proteins or proteins from infectious agents can be phagocytosed by specialized cells such as macrophages, or pinocytosed by other cell types and incorporated into lysosomal vesicles which contact the class II vacuole. Peptides derived from the exogenous protein displace the invariant chain to bind to the MHC class II molecule and are presented at the surface as in (9).

antigen presentation and recognition by an alloreacting T-cell. The central feature is the interaction between the T-cell receptor (TCR) and a peptide antigen presented to the T-cell within the specialized binding groove of a major histocompatibility antigen molecule. These peptide self-antigens are derived from proteins within the cell. Physiologically, the presentation of the wide repertoire of self-antigens during T-cell ontogeny in the thymus is the process whereby self-tolerance is established [34]. In the non-physiological, man-made context of the transplant alloresponse, lymphocytes encountering new antigens on a foreign stimulator cell which do not form part of their own repertoire, perceive the antigen as foreign and initiate an immune response culminating in the elimination of the cell presenting the foreign peptide. In marrow transplantation the alloresponse causes the phenomena of GvHD, graft rejection and the graft-versus-tumour effect (Figure 1.6).

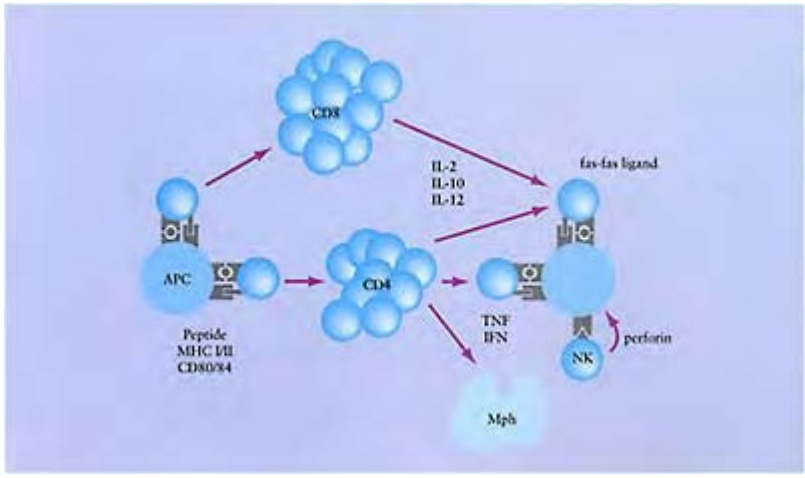


Figure 1.6 The alloresponse. Alloantigens (self-peptides from the recipient) can be presented to the

donor CD4 and CD 8positive T-cells either by host cells with the appropriate MHC molecules, or the intermediary of donor-derived antigenpresenting cells (APC). T-cell activation requires a primary signal from the peptide-MHC-TCR-CD3-CD4/8 molecular complexes, and a secondary signal from the interaction of the B7 molecules on the APC with CD28 on the T-cell. Amplification of the response is achieved by clonal expansion, controlled in particular by IL-2, IL-12, IL-4 and IL-10. The effector function of the T-cell is directed towards a 'helper' pattern by IL-4 and and a 'cytotoxic' pattern by IL-12. Effector activity results in GvHD and a graft-versus-leukemia effect. Tissue damage occurs from direct cytotoxicity through perforin (cell lysis and the fas/fas ligand path (apoptosis). Cytokines released by activated T-cells such as tumour necrosis factor (TNF) and interferons (IFN) cause cell damage and recruit other effector cells such as natural killer (NK) cells and macrophages (Mph), initiating further tissue damage.

Antigens that drive the alloresponses fall into two categories: major histocompatibility complex (MHC) antigens encoded by the MHC gene complex on the long arm of chromosome 6, and minor histocompatibility antigens—the peptides derived from cellular proteins (Table 1.3). While the protein sequences of several hundred MHC molecules is known, the identity of minor antigens is largely unknown [35]. HLA typing is the process of matching donors and recipients at the MHC locus. The purpose of HLA matching is to choose

Table 1.3 Antigens stimulating an alloresponse

Major histocompatibility antigens	HLA-A, -B, -C, DR, DP, DQ
Minor histocompatibility antigens	Known antigens: H-Y, HA-1 to HA-7. Many others
Antigens on malignant cells	Probably exist, but not well described

a donor who does not differ at the MHC loci. Nevertheless, with the exception of genetically identical twins, even perfectly HLA-matched pairs can still exert powerful alloresponses against minor antigens. Because the genes for HLA-A, -B, -C and DR are closely associated, they are inherited in a single haplotype. Donor-recipient sibling pairs identical at all six A, B and DR loci have by chance inherited the same haplotype from the parents (Figure 1.7). Because haplotypes are not perfectly conserved, matched unrelated donor-recipient pairs have more chance of genetic non-identity and consequently more severe alloresponses.

Graft rejection

Graft rejection is the immunological recognition and elimination of the transplanted marrow by the recipient's immune system. Graft rejection manifests either as transient graft take followed by haematopoietic failure, or by a complete absence of marrow recovery (graft failure). Since haematopoietic progenitor cells express HLA class I and II and minor histocompatibility antigens they are susceptible targets for cytotoxic T-cells recognizing major and minor histocompatibility antigen differences. Natural killer (NK) cells can also reject allogeneic marrow.

Graft-versus-host disease

GvHD is the result of a response of donor T-cells to alloantigens of the recipient. The major target tissues are the integument (skin, gastrointestinal tract, the biliary tree and exocrine glands) and the lymphohaematopoietic system. The reaction involves three phases—recognition of the recipient tissues as foreign, amplification of the immune response through clonal expansion of donor-anti-host T-cells, and the effector phase where T-cells damage the target tissue either directly or through recruitment of other effector cells and cytokine production. Acute GvHD occurs early post-transplant and is triggered by cytokines released during the preparative regimen. Chronic GvHD is a complex alloresponse causing abnormalities of immune regulation in addition to tissue damage from infiltrating lymphocytes. The mechanisms of GvHD are reviewed in Chapter 37 and 38.

Factors affecting immune recovery

The most powerful suppressive influence on immune recovery is the presence of clinical GvHD—acute and chronic GvHD are associated with prolonged and incomplete recovery of immune function. The immune defect of GvHD is further exaggerated by immunosuppressive agents used to treat GvHD. The administration of T-cell-depleted bone marrow leads to a lower incidence of GvHD and therefore a higher frequency of ultimately normal immune recovery. However, early immune recovery is compromised.

The state of the donor's immunity has a significant impact on the pattern of recovery of specific responses in the recipient. Transfer of both protective immune function against viruses, and autoimmune responses from the donor to the recipient have been well documented. Specific donor responses from mature B-cells are present early after BMT and persist for many months.

Barriers to marrow engraftment

Marrow may fail to engraft either because the stem cells are defective, or because there is host resistance to stem-cell implantation. Engraftment is determined by competing positive and negative factors associated with the recipient and donor listed in Table 1.4. Immunosuppression is required to overcome the recipient's strong allogeneic response to donor bone-marrow cells and to prevent prompt rejection of the transplant. Large donor stem-cell and lymphocyte doses improve the chances of take. Sensitization of the recipient to the donor, wide disparity of HLA types and inadequate immunosuppression are recipient factors which contribute to graft failure.

The preparative regimen

The preparative regimen given before bone-marrow transplantation has three functions:

1. To immunosuppress the recipient to prevent graft rejection.
2. To provide myeloablation to allow reconstitution of 100% donor haematopoiesis.
3. To confer an antitumour effect.

The choice of preparative regimen depends upon the type of transplant and the nature of the disease treated. For example, transplants for severe aplastic anaemia require only immunosuppression to obtain engraftment, transplants for hereditary disorders with normal marrow cellularity require immunosuppression and myeloablation to permit establishment of 100%

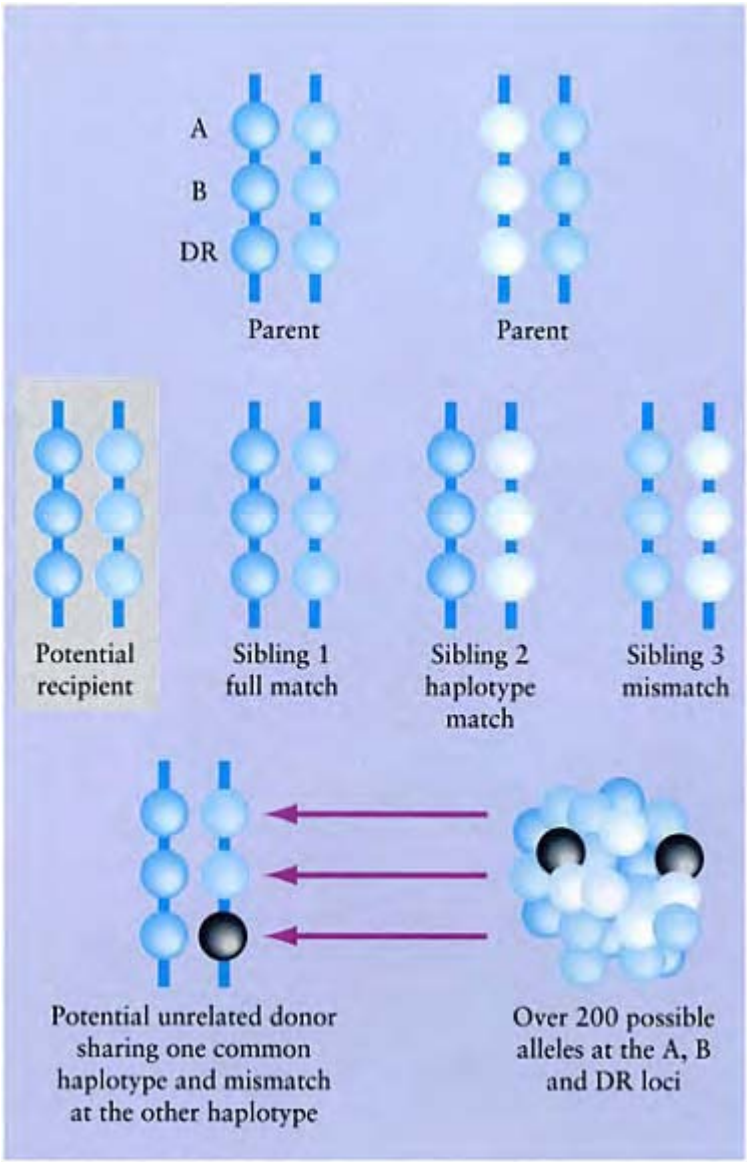


Figure 1.7 The genetic basis of HLA matching. Matching within the family. *Because they are closely linked on the major histocompatibility (MHC) gene complex HLA-A, -B, -C, DR, DP and DQ genes are inherited as a single*

haplotype. Children inherit one haplotype from each parent. Siblings may therefore be fully matched, half matched or fully mismatched with each other. Note that parents can only be half matched with their offspring unless they share a haplotype (e.g. due to intermarriage). Matching from unrelated donors. *A hypothetical haplotype matched unrelated donor, as illustrated. Because they are frequently inherited as a single block, HLA haplotypes are well conserved in the population. It is therefore not uncommon to find shared common haplotypes in the general population. However, genetic recombination results in a wide spectrum of possible HLA haplotypes. This reduces the chances of finding a fully matched unrelated donor because of the very large number of possible alleles for HLA-A, -B and DR (shown lower right). In a Caucasian population, over 1000000 potential donors are needed to identify a fully HLA-matched donor for 60% of patients.*

Table 1.4 Factors affecting marrow engraftment

Haematological
Stem-cell dose
$>10^6$ $CD34^+$ cells/kg associated with better engraftment
Stem-cell quality
Previous chemotherapy decreases take. Cord blood has higher engraftment potential (but low numbers)
Normal recipient environment No fibrosis; no splenomegaly
Available stem-cell niches

Adequate intensity of preparative regimen
Immunological
Donor/recipient compatibility
Incresing incompatibility decreases chance of ‘take’
Recipient sensitization to donor through transfusion strongly increases rejection risk
Low T-cell dose leads to increased risk of rejection
Recipient immunosuppression
Low-intensity preparative regimen increases rejection risk

donor haematopoiesis and transplants for malignant diseases demand an antitumour and an immunosuppressive preparative regimen. The agents used in allograft preparative regimens include irradiation to the lymphoid system (immuno-suppressive) or the entire body (immunosuppressive and myeloablative), alkylating agents such as busulphan and melphalan (mainly myelosuppressive) and cyclophosphamide, antilymphocyte globulin and cytosine arabinoside (mainly immunosuppressive). Preparative regimens for autografts include drugs with specific activity against the malignancy under treatment and typically employ multiple agents with different spectra of non-haematopoietic side-effects so as to allow a large cumulative toxic dose to the malignancy, with dispersed and diminished toxicity to individual target organs.

Clinical considerations

Cure of malignant disease by stem-cell transplantation [36]

The concept behind BMT for malignant diseases is to harvest and store marrow while the malignancy is treated intensively with radiation or chemotherapy which destroys the patient’s marrow. After treatment, the stored marrow is used to reconstitute normal bone-marrow function. The idea is based on two premises: (1) antitumour effect is proportional to the dose intensity; and (2) the limiting toxicity that prevents eradication of the malignancy is bone-marrow failure. The success or failure of autologous transplantation in a particular situation depends partly on the accuracy of these assumptions and partly on the possibility that malignant cells could be reinfused with the transplant. In general, autologous transplants have a low mortality but a high relapse rate. Whether it is useful to purge autologous marrow of malignant cells before reinfusing has not been satisfactorily resolved.

It is clear that allografts, while carrying a higher risk of mortality from the procedure, confer a greater chance of curing the malignancy. This has been called the ‘graft-versus-leukaemia (GvL)’ or ‘graft-versus-tumour (GvT)’ effect—the immune-mediated response which conserves a state of continued remission of a haematological malignancy following allogeneic marrow stem-cell transplants. Because GvHD is intimately

associated with GvL, it can been assumed that similar mechanisms control GvHD and GvL. GvHD requires the recognition by donor T-cells of antigens presented by MHC molecules on the recipient cells initiating clonal expansion of responders and an effector response involving lymphocytes and cytokine. In GvL reactions, the alloresponse suppresses residual leukaemia. The dominant antigens on leukaemia cells driving the GvL response are not known: major or minor histocompatibility antigens co-expressed on GvHD targets (such as normal skin and gut cells) and leukaemic cells could induce a non-specific GvH/GvL alloresponse. The response against either normal or malignant bone-marrow-derived cells may also overlap. Thus, GvL may in part be a graft-versus-marrow effect—involving lymphoid or myeloid lineages or both. Additionally, leukaemia cells may induce a more specific alloresponse if they express antigens either not present or underexpressed on cells of other tissues.

Cure of non-malignant disorders by BMT

Allogeneic bone-marrow stem-cell transplantation can be used to correct non-malignant disorders in two ways: (1) replacement of defective bone-marrow cell lines by normal donor cells; and (2) grafting bone-marrow stem cells capable of generating a permanent population of enzyme-competent cells in the case of hereditary enzyme deficiencies. Autologous BMT is not at present a relevant form of correction for these disorders, but in future gene therapy could allow retransplantation of autologous, genetically corrected, stem cells.

It is easy to understand how BMT can remedy disorders such as aplastic anaemia where bone-marrow failure is corrected by transplanting healthy stem cells, or in thalassaemia major, where the requirement is to ablate defective bone marrow and replace it with that of a normal donor. The mode of correction of metabolic disorders such as lysosomal enzyme defects is more involved. Grafted myeloid and lymphoid cells producing normal quantities of the missing or defective enzyme can correct the metabolic disorder by dissemination throughout the body to sites of accumulated metabolite. Enzyme can be exchanged between donor cells and defective ones and may also be secreted into body fluids [37].

Management

Figure 1.8 shows a flow diagram summarizing the clinical management of the transplant procedure.

Patient selection and transplant planning

The decision to perform a bone-marrow transplant is based upon evaluation of several criteria.

Diagnosis. Whether the treatment decision concerns a malignant or non-malignant disorder, the transplant is only considered an option if less intensive, less risky and cheaper procedures are unlikely to achieve a cure. In malignant disorders, the probability of cure and the success or otherwise of chemotherapy-based procedures must be

evaluated before a transplant is selected as the treatment of choice. In non-malignant disorders such as thalassaemia and sickle-cell disease, where there is no immediate prospect of death from the disorder, the quality of life with or without transplant as well as survival probability must be weighed.

Choice of transplant. Whether to perform an autologous or allogeneic BMT for a malignant disease depends on the chance of cure with either approach and the degree of compatibility between the patient and available donor. If an HLA identical sibling is available outcome following the allogeneic BMT is likely to be optimal. In recent years the opportunities for patients without an HLA identical sibling have widened. The choice rests between an autologous transplant, a marrow transplant from a volunteer donor, a transplant from a mismatched family member, or a partially matched cord blood transplant. Confronted with a high risk of relapse, selection of the allograft option will be favoured over the autograft. If the allograft approach is chosen, immediate availability of a mismatched family donor in a patient with a high risk of disease progression may be a critical feature in favour of selecting a less than perfectly matched transplant over a matched transplant whch is not immediately available. Cord blood transplants are more likely to be used in children.

Rarely, the transplanter is confronted with the dilemma of selecting either an HLA-identical sibling or an identical twin as donor. The low transplant-related mortality from the identical twin BMT has to be balanced against a higher risk of leukaemic relapse.

Risk factors. Sufficient data now exist to predict with some accuracy the probability of the major outcome variables following BMT. These are survival, disease-free survival (DFS), transplant-related mortality (TRM), graft failure, disease relapse, acute and chronic GvHD. Knowledge of these risks helps in planning the type of preparation used for the patient—for example, if there is a high risk of graft rejection, an attempt to enrich for stem cells, avoid T-cell depletion and intensify the immunosuppressive component of the preparative regimen is appropriate. Conversely, if there is a perceived increased risk of GvHD, the marrow sample might be depleted of T-cells and additional GvHD prophylaxis given. Unfortunately, however, these strategies are limited by the interaction of one risk factor with another. Thus, while preventing GvHD, T-cell depletion can increase the risk of relapse and graft failure. The International Bone Marrow Transplant Registry (IBMTR) and the European Bone Marrow Transplant (EBMT) Group have accumulated very large databases on transplanted patients. Both groups have made major contributions to defining the patient, transplant and disease-related risk factors determining transplant outcome. The IBMTR has pioneered comparative database analyses that have helped compare transplant and non-transplant therapies. In addition, there are numerous single and multiple centre studies which have helped to define optimum treatment approaches in specific situations (e.g. allogeneic BMT versus autologous BMT versus chemotherapy for leukaemia).

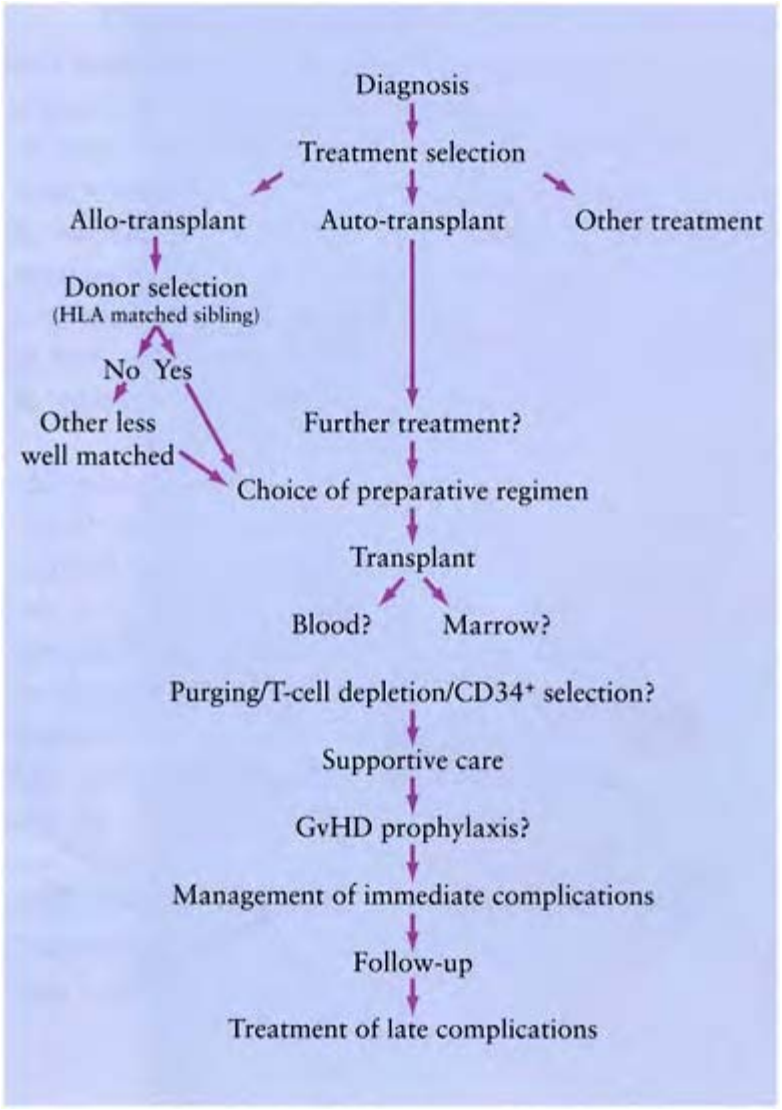


Figure 1.8 Algorithm for the clinical management of bone-marrow transplantation.

Supportive care

The management of patients undergoing transplants is greatly facilitated by the insertion of a semi-permanent intravenous line (see Chapter 28). Good supportive care of the

transplant patient is essential for success of the transplant. It involves the use of prophylactic platelet and red-cell transfusions to prevent thrombocytopenic bleeding and anaemia, antibiotic prophylaxis to prevent infection during the neutropenic period, and prompt treatment of fever with broad-spectrum antibiotics. The preparative regimen-induced nausea, vomiting and mucositis often require a period of intravenous feeding. The boredom and depression induced in the transplant recipient by prolonged hospitalization and the uncertainty of the outcome demand strong support and a unified positive attitude from all members of the transplant team.

Complications

Complications following transplants are frequent and repetitive. They are classified below.

Preparative regimen-related complications. These are some of the first complications to occur after transplant. They vary with the intensity of the preparative regimen and the particular spectrum of toxicities of the agents used. All regimens are associated with some degree of nausea, vomiting, diarrhoea and mucositis. More dangerous immediate complications are veno-occlusive disease, respiratory distress and capillary leak syndromes, renal failure and hepatic failure. Restrictive lung disease and haemorrhagic cystitis occur in the first few months after transplant. The delayed effects of transplant are becoming increasingly well described. They include growth arrest and retardation, gonadal failure, delayed puberty, infertility and an increased risk of second malignancies. Delayed effects of BMT occur from long-term damage to non-haemopoietic tissues by the preparative regimen or from GvHD and immune deficiency after the transplant. Damage to proliferating non-haemopoietic tissues results either in depletion of clonogenic cells, or injury to cellular DNA impairing post-mitotic function and increasing the potential for malignant change.

Transplant-related complications. Bone-marrow failure can occur in autologous transplants because the stem cells transplanted were defective either from previous therapy, or manipulation. After allogeneic transplantation the graft may also fail because it is rejected immunologically. Both types of transplant can result in a period of immune deficiency giving rise to bacterial, fungal or viral infections. Infections are one of the major causes of morbidity and mortality after BMT. Bacterial, fungal, protozoal and viral agents can cause infectious complications from the time of preparation for the transplant which persist for many months afterwards. The pattern of infection is related to its timing in relation to the transplant: infectious complications can be divided into those occurring in the first month after BMT during the phase of neutrophil recovery and repair of mucosal surfaces; those occurring in the first three months often from virus reactivation which can confer a high mortality; and later infections largely associated with persisting cell-mediated immune deficiency from chronic GvHD.

Disease-related complications. Patients transplanted for malignant diseases can relapse at any time following the transplant. Treatment of relapse is challenging. Some patients, however, will respond to immunotherapeutic approaches, some survive second transplants and some may achieve prolonged survival from less-intensive measures.

In the field of non-malignant disorders, recurrence of the original disorder can occur with simultaneous rejection of the transplant. This does not always result in immediate

mortality. For example, in thalassaemia or sickle-cell anaemia, graft rejection is usually accompanied by autologous marrow recovery and survival, albeit with all the complications of the underlying disorder.

Follow-up

Continuing follow-up is critical both for patient safety and to determine true outcome after BMT. The collection and reporting of transplant data to large registries such as the IBMTR is critical to the further development of the procedure. Every transplant is important, contributing to the general pool of knowledge which serves, in turn, to generate the factual basis for improving results in future patients.

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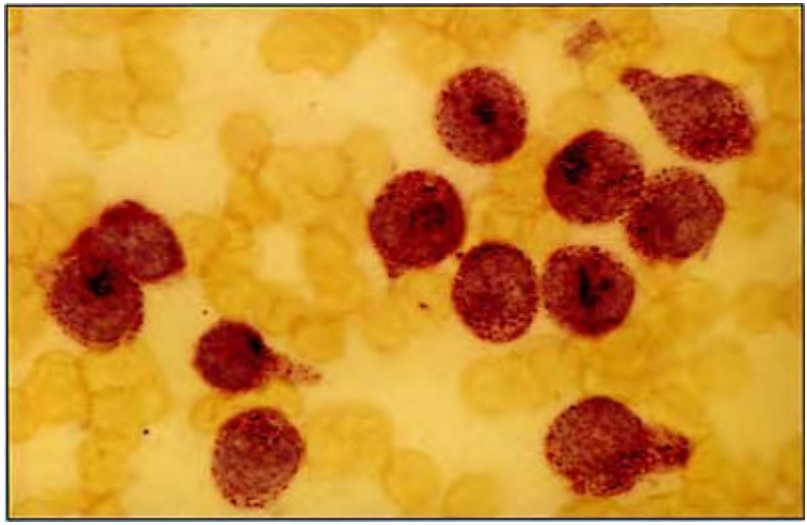

Chapter 2
Theoretical aspects of dose intensity and dose scheduling

Charlotte Rees, Phillip Beale, Ian Judson

Introduction

There is now substantial evidence supporting the role of high-dose chemotherapy prior to peripheral blood stem-cell transplant (PBSCT) or bone-marrow transplant (BMT) in the management of leukaemias [1], lymphomas [2] and some solid tumours, e.g. testicular carcinoma and neuroblastoma [3]. Historically, alkylating agents were the first cytotoxic drugs to be used in high dose as either single agents or in combination with other alkylating agents, because their major dose-limiting toxicity is myelosuppression. Chemotherapeutic drugs now used in high-dose protocols include the alkylating agents cyclophosphamide, ifosfamide, melphalan, carmustine, busulphan, carboplatin, cisplatin and thiotepa. Non-alkylating agents include etoposide, cytosine arabinoside, methotrexate and paclitaxel. The optimal use of cytotoxic drugs has been fundamental to progress in cancer chemotherapy in recent years and this chapter will explain the importance of dose and schedule.

Dose

High-dose treatment is based on the hypothesis that increasing dose or dose rate will increase tumour cell kill, resulting in improved response and survival rates. This assumes that significant increments in dose can be made within an acceptable toxicity range and that such increments in dose are sufficient to eliminate more tumour cells in a higher percentage of patients than would occur at a lower dose level [4]. Dose intensity can be increased both by increasing individual doses and by reducing the dose interval. There is now ample evidence that the dose intensity of combination chemotherapy has an important impact on outcome in adjuvant, curative and palliative settings. The first demonstration of a clear relationship between dose and outcome was made by Kaplan [5] who reported that the higher the dose of radiation applied to a given nodal region in patients with Hodgkin's lymphoma, the lower the recurrence rate. If a total dose of at least 3600 rads was given over a period of five weeks, there was a 90% local cure rate (Figure 2.1). Frei and Cannellos [6] highlighted chemotherapy dose intensity as an important determinant of tumour cell kill with *in vivo* and *in vitro* data (Figure 2.2).

In vitro, alkylating agents exhibited a steep dose-response curve whereas antimetabolites such as

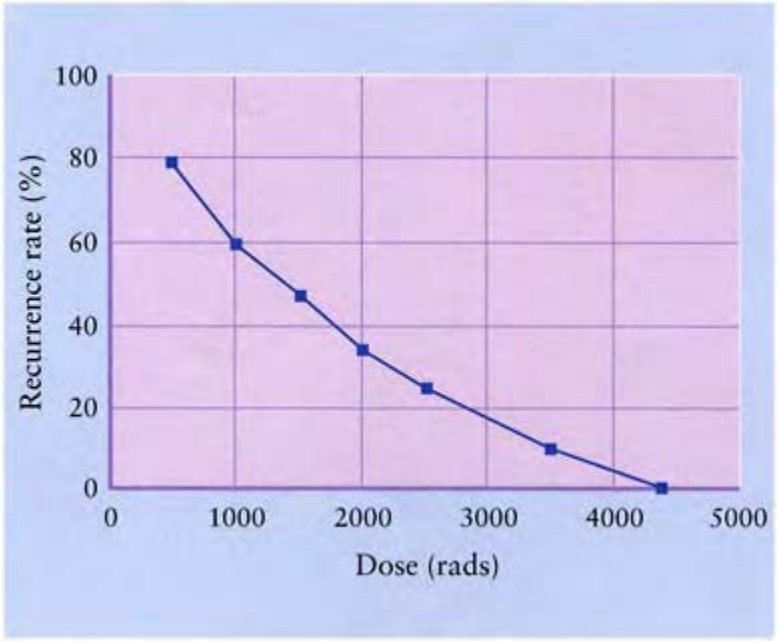

Figure 2.1 Relationship of radiation dose delivered at approximately 1000 rads per week on recurrence rate in Hodgkin's disease. Adapted from Kaplan [5].

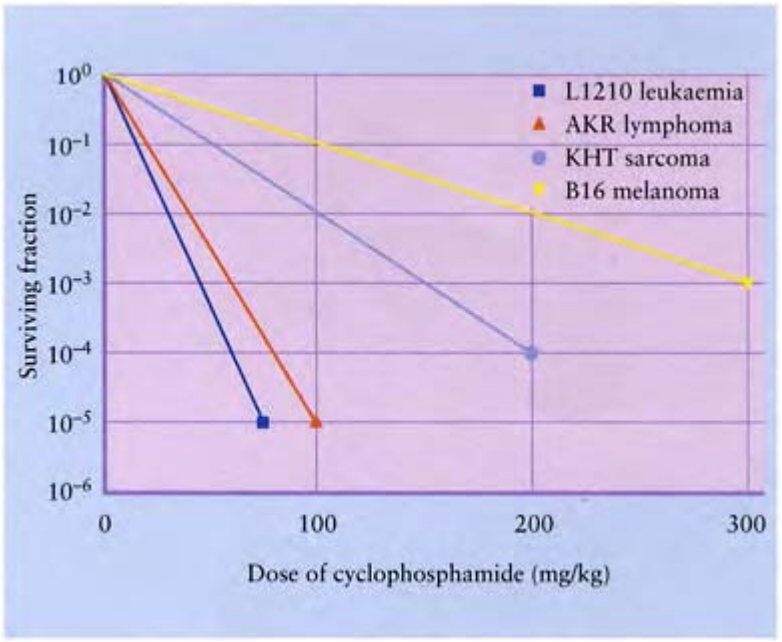

Figure 2.2 Relationship of tumour cell kill to cyclophosphamide dose for various experimental tumour cell lines. Adapted from Frei and Cannellos [6].

methotrexate achieved 1–2 logs of cell kill due to their effect being limited to those cells in S-phase of the cell cycle. Radiation caused a log increase in cell kill with a linear increase in dose, i.e. a straight line. Further studies looking at dose and response utilized *in vivo* excision assays [7]. Mice were injected with tumour cells. Once the tumours were palpable, the mice were treated with different doses of an alkylating agent and then sacrificed 24 hours later. The tumours were excized and prepared as single-cell suspensions and the results were expressed as the surviving fraction compared to untreated controls. The alkylating agents were shown to kill the tumour cells in a log-linear manner with an increasing dose of drug, as demonstrated *in vitro*.

In the clinical setting, the effect of dose is more complex and is in part dependent on the behaviour of the tumour, the patient and the oncologist.

Tumour factors

Tumour factors affecting the response to a given dose are listed in Table 2.1. Tumour populations are heterogeneous, so not all clones of cells may be equally sensitive to a given dose of drug. Sensitive tumours exhibit a linear dose-response curve with alkylating agents, whereas for insensitive tumours, dose may have little effect on

response. It is preferable that the tumour cells are more sensitive to the cytotoxic drug than normal cells including the bone marrow, although myelosuppression is the major dose-limiting toxicity for many cytotoxic drugs. The growth fraction of the tumour, i.e. the fraction of cells which are undergoing cell cycling, also determines response to chemotherapy, particularly for cell-cycle-specific drugs. Solid tumours have a poor vascular supply and a correspondingly low growth fraction which therefore affects their to a given dos

Table 2.1 Factors affecting the response of a tumour to a given dose of chemotherapy

- Tumour heterogeneity
- Sensitivity of tumour cells compared to normal cells
- Tumour burden
- Development of resistance
- Growth fraction
- Hypoxia

responsiveness to high-dose protocols. Drug-resistant tumour populations will also alter the dose-response curve and minimal tumour burden provides the greatest opportunity for cytoeradication as demonstrated by the role of adjuvant chemotherapy for residual micrometastatic disease in breast carcinoma.

Coldman and Goldie [8] showed in a theoretical model that giving dose intensive chemotherapy should reduce the likelihood of resistant cells emerging and subsequently causing treatment failure. They used a somatic mutation computer model for drug resistance and made various assumptions: that drug-resistant cells arise spontaneously and with measurable frequency, i.e. mutation rate; that all cells in the tumour are capable of unlimited proliferation; that stem cells and resistant cells grow with the same kinetics; and that resistant cells are unaffected by the use of the drug. They showed that the probability of achieving a cure varied according to the dose of drugs used and the schedule of administration, dose-intensive regimens being superior and reducing the likelihood of resistant cells appearing.

Drug resistance is considered to be a major cause of chemotherapy failure. In recent years, most interest has focused on mechanisms of cellular resistance. Pglycoprotein (Pgp)-mediated multidrug resistance (MDR) has been extensively studied. Pgp is a membrane protein encoded by the *MDR1* gene which acts as an energy-dependent drug efflux pump, decreasing the intracellular concentration of the cytotoxic drug thereby causing drug resistance [9]. Pgp appears to transport preferentially those agents derived from natural products, e.g. anthracyclines, vinca alkaloids, epipodophyllotoxins and taxanes. Other transport proteins such as multidrug resistanceassociated protein and lung resistance-related protein have somewhat different specificities, although their importance in clinical cancer drug resistance is less clear. The development of inhibitors to combat resistance by this mechanism has shown promise in paediatric and haematological malignancies. Studies by Chan *et al.* [10,11] have yielded convincing

evidence that Pgp predicts outcome of therapy in soft-tissue sarcoma and neuroblastoma. Other mechanisms of resistance include upregulation of target enzymes, increased glutathione, DNA repair and metallothionein, and hypoxia.

Patient factors

Interpatient pharmacokinetic variability affects the response to a given dose (Table 2.2). The bioavailability of an oral drug such as busulphan is determined by nausea and vomiting, gut motility, gut enzymes, prior gastrointestinal surgery, compliance and first-pass metabolism by the liver. Most cytotoxic drugs used in high-dose protocols are administered intravenously. Some cytotoxic drugs such as cyclophosphamide are prodrugs and require conversion to their active metabolites, and the pharmacokinetic parameters of the metabolites are likely to be variable. High-dose chemotherapy may result in saturation of metabolic pathways and also routes of excretion. Renal or hepatic dysfunction will affect drug elimination, and variability in distribution may occur amongst patients, for example, methotrexate accumulation in ascites and pleural effusions. Interpatient variability in protein binding will affect the proportion of free drug which may be important for highly protein-bound drugs.

The marked differences in drug clearance between patients translate into marked variability in drug exposure whether determined by peak levels, steadystate levels or area under the curve (AUC), and the plasma concentration of a drug may not be the best predictor of response or toxicity. There is a significant component of interpatient pharmacokinetic variability that is unexplained as seen from phase I pharmacokinetic studies where the maximally tolerated dose applies to a population of patients and not to an individual [12]. This dilemma is of paramount importance in the high-dose setting where optimal therapy strives to achieve a balance between fatal toxicity and maximal response.

Table 2.2 Factors affecting the pharmacokinetic variability amongst patients

• Bioavailability
• Metabolism
• Excretion
• Protein binding
• Volume of distribution

Treatment factors

There is a sigmoid relationship between dose and response and dose and toxicity (Figure 2.3). Increasing the dose of a drug results in an improved response over a specific dose range. Above this dose range, no further improvement in response is achieved because of the presence of resistant populations of cells. Most antineoplastic drugs have a narrow therapeutic index, and at low dose no therapeutic benefit will be achieved, but at high

dose, toxicity will occur, limiting further dose escalation. The main aim of high-dose chemotherapy is to escalate the dose above the normal range to overcome resistance. BMT and PBSC overcome the major dose-limiting toxicity of myelosuppression.

The oncologist plays a crucial role in determining response to treatment. There is a tendency to reduce dose because of toxicity but not to escalate the dose if patients tolerate treatment without toxicity, and treatment delays and dose reductions may compromise cure rate [13]. Hryniuk and Levine [14] highlighted the importance of dose intensity in the adjuvant treatment of breast carcinoma and Tannock *et al.* [15] showed that in metastatic breast carcinoma, a dose threshold must be exceeded. In the palliative setting, it is important that patients do not experience unacceptable toxicity, but dose intensity remains important [16]. Objective criteria for dose reduction are stated in protocols, but there is considerable subjectivity

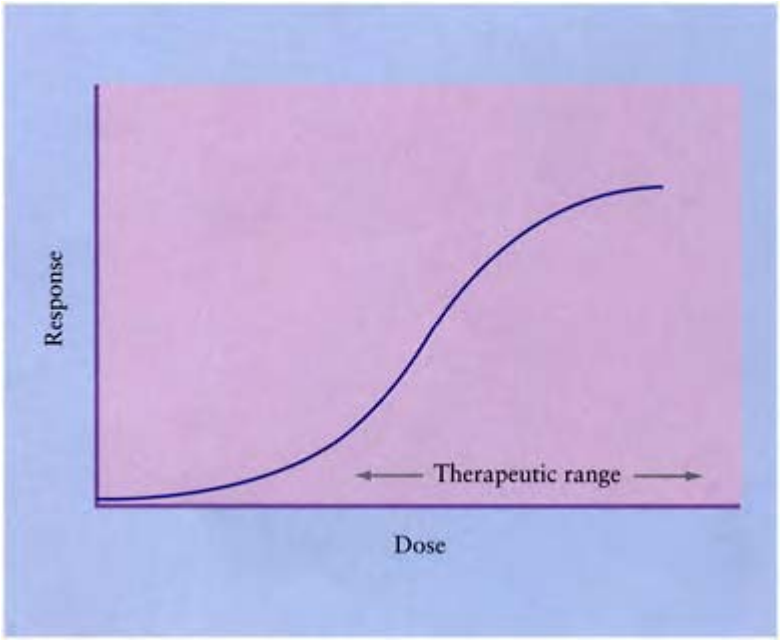

Figure 2.3 Relationship between dose and response.

amongst clinicians in their interpretation [6]. There is also evidence that dose reductions and treatment delays are often not documented in published reports. This may, in part, account for variable responses obtained with identical regimes [17].

Pharmacokinetics and pharmacodynamics

'Pharmacodynamics' refers to the relationship between dose and response, and dose and toxicity. 'Pharmacokinetic' studies are routinely performed as part of phase I trials, but

correlative pharmacodynamic studies have been performed infrequently. Egorin *et al.* [18] showed that with carboplatin, the degree of thrombocytopenia correlated with the area under the curve. In reality, despite greater understanding of anticancer pharmacodynamics [19], individualized dosing of anticancer chemotherapy is still not easy in clinical practice. It has been achieved with the development of pharmacokinetic models and limiting sampling strategies, enabling the pharmacokinetics of a drug to be accurately described from only one or two plasma samples [20]. Where treatment is being given with curative intent, every effort should be made to minimize interpatient variability in drug exposure in order to maximize the benefit while keeping the risk of serious side-effects at an acceptable level. Adaptive control may offer the best way to achieve this goal, but further prospective validation of this approach is required to justify the necessary investment. A scheme for adaptive control of anticancer therapy is represented in Figure 2.4 [12].

It is important to attempt to investigate pharmacokinetic-pharmacodynamic relationships early on in the development of a drug when the drug is being given as a single agent, and to consider the pharmacokinetics and pharmacodynamics of two or more drugs given concurrently. In high dose, cytotoxic drugs cause dose-limiting non-haematological toxicities, for example, veno-occlusive disease (Table 2.3). BMT and PBSCT transplant have overcome the major dose-limiting toxicity of myelosuppression following high-dose chemotherapy protocols but the non-haematological toxicities are more unpredictable [21]. Improved supportive care and the development of haemopoietic growth factors have contributed to the success of transplant programmes and enable multiple cycles of high-dose therapy to be given.

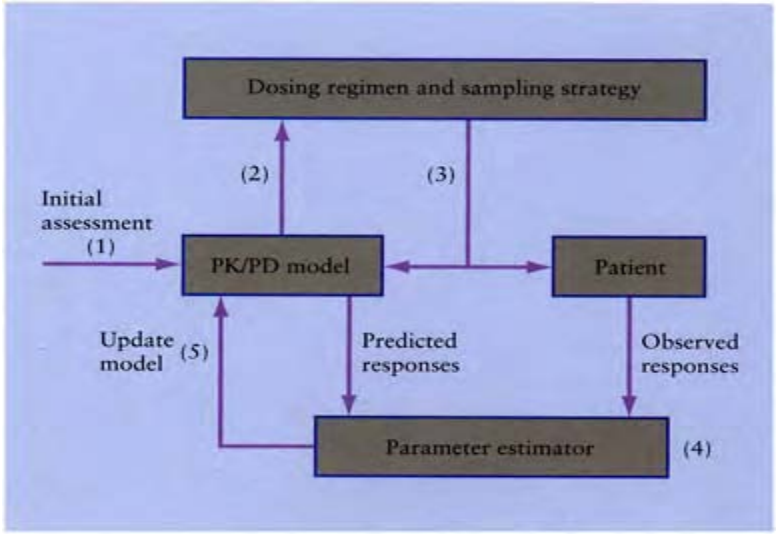

Figure 2.4 A general scheme for adaptive control of anticancer therapy. Adapted from Ratain et al. [12].

Schedules for high-dose chemotherapy

The schedule of administration of high-dose chemotherapy is important for many drugs in determining response, toxicity and emergence of resistance. Most regimens employ multiple agents and the sequence of the drugs may also be important with respect to pharmacokinetic and pharmacodynamic interactions and the subsequent effects upon the host and tumour. When multiple cycles of high-dose therapy are administered, intervals of dosing tend to be individualized due to variation in time to recovery of neutrophil and platelet counts and the optimum schedules are not known. Increasing total dose over many cycles, but reducing dose intensity due to prolonged or severe toxicity may not improve response rates.

Agents that are combined with non-overlapping non-haematological toxicities will hopefully eliminate different subclones within the tumour with different resistance mechanisms, and therefore the effects may be additive. In experimental systems, the isobologram is an *in vitro* approach to the evaluation of two drugs to be used in combination (Figure 2.5). In Figure 2.5, the equitoxic effect doses (ED) of two agents are expressed on the coordinates. A straight line between the extremes represents the total of the ED_{50} of drug 1 and the ED_{50} of drug 2, and in this situation drugs are considered additive. If the sum of the two ED_{50} falls to the right of this line, then the drugs are antagonistic, but if they fall to the left, the drugs are synergistic. However, even if the combination is additive or synergistic, if toxicity is significantly enhanced there will be no benefit *in vivo*.

We shall now consider the drugs which are commonly used in high-dose regimens (Table 2.3), and examine aspects of scheduling in relationship to toxicity, response and resistance mechanisms. We also look at how the pharmacokinetics at high doses may influence the optimum schedule of these drugs. Some drugs are given in ‘high dose’, i.e.

greater than standard dose, but are not used in high-dose regimens followed by haematological rescue.

Cyclophosphamide

In high-dose schedules, cyclophosphamide (CP) 5–8 g/m^2 is usually given as a short infusion over 1–2 hours divided over two to four days. There is no evidence that altering the schedule will alter the response rate. Several studies have shown the induction of metabolism with repeated dosing of cyclophosphamide. Fasola *et al.* [22] demonstrated the area under the curve (AUC) of CP on day 1 to be significantly higher than on day 2. However, Schuler *et al.* [23] found that CP given in divided doses over four days resulted in an increased AUC of the non-protein bound active metabolites by day 4. However, bladder toxicity may be reduced when CP is given as a continuous infusion as opposed to divided bolus administration [24]. Intermittent therapy leads to higher levels of alkylating metabolites in the urine and if the levels of thiols (provided by mesna) are insufficient, then haemorrhagic cystitis may occur.

Table 2.3 Drugs used in high-dose protocols

Drug	Dose	Schedule	Pharmacokinetics/ pharmacodynamics*	Dose-limiting toxicities
Cyclopho sphamide	5–8 g/m^2	Divided dose, given as short infusion	Bladder toxicity may be reduced by giving as a short infusion rather than as divided boluses	Haemorrhagic myocarditis, veno-occlusive disease, pulmonary injury
Ifosfamide	12–18 g/m^2	Continuous infusion	Metabolism not saturable at high doses, renal toxicity less with continuous infusion over 16 days	Haemorrhagic cystitis, neurotoxicity, renal toxicity and mucositis
Busulphan	16 g/m^2	1 mg/kg 4×daily for 4 days	Induces its own metabolism, high AUC predicts for veno-occlusive disease	Veno-occlusive disease
Melphalan	140–180 mg/m^2	Short i.v. infusion	Highly variable AUC. Saturation of drug transport	Gastrointestinal toxicity
Thiotepa	750–900 mg/m^2	Continuous infusion over 4 days or short infusion daily×3	Thiotepa clearance induced with continuous infusion, toxicity more severe with increasing AUC of thiotepa and tepa	Gastrointestinal and CNS toxicity
Carmustine	600 mg/m^2	2-hour infusion	Highly variable pharmacokinetics, high AUC may correlate with pulmonary toxicity	Hepatic, pulmonary, CNS and cardiac toxicity
Mitoxantrone	60–90 mg/m^2	Short i.v. infusion	Metabolism not saturable, neutropenia may relate to plasma residual AUC	Mucosal and gastrointestinal toxicity
Cisplatin	165–250 mg/m^2	Day 1, 8 or continuous infusion over 3 days	Renal toxicity related to C_{max} and AUC, ototoxicity related to peak plasma levels	Peripheral neuropathy, nephrotoxicity, ototoxicity
Carboplatin	AUC 20–30 1600–2000 mg/m^2	1-hour infusion daily×4	AUC correlates with ototoxicity	Veno-occlusive disease, nephrotoxicity
Etoposide	2100 mg/m^2	Continuous infusion or daily×3	Similar AUC, higher C_{max} in daily×3, toxicity may be worse with split schedule	Mucositis
Cytosine arabinoside	1–3 g/m^2× 3–6	12-hourly dosing	Aim to maintain levels within blasts above threshold	Mucosal, gastrointestinal, and CNS toxicity

	days			
Methotrexate	1–33.6 g/m2	4-, 8-, 24-, 36-hour infusion	AUC does not correlate with response, time above threshold predicts for toxicity	Renal toxicity, mucositis
Paclitaxel	250–725 mg/m^2	24-hour continuous infusion	Pharmacokinetics linear over this dose range and schedule, AUC predicts for neuropathy and mucositis	Pulmonary, CNS and renal toxicity
*AUC=Area under the curve				

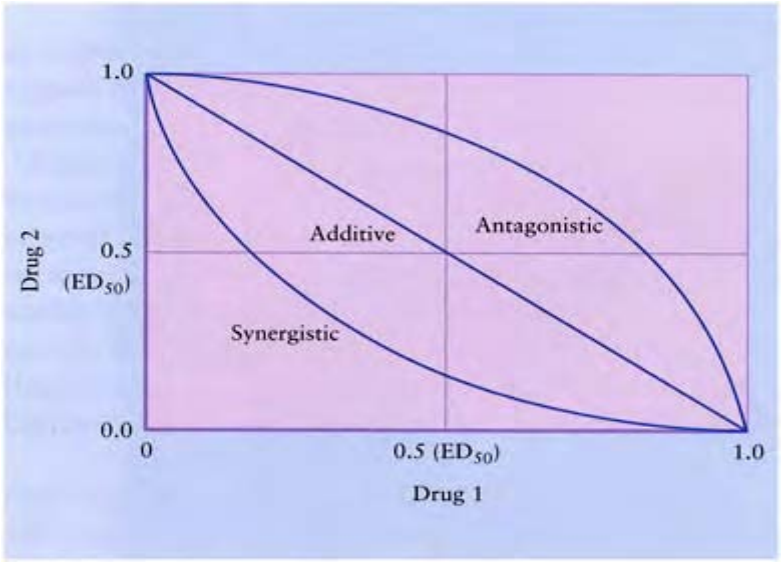

Figure 2.5 ED_{50} isobologram. The equitoxic effect doses (ED) of two agents are expressed on the coordinates. A straight line between the extremes represents the total of the ED_{50} of drug 1 and the ED_{50} of drug 2 and in this situation drugs are considered additive. If the sum of the two ED_{50} falls to the right of this line then the drugs are antagonistic but if they fall to the left, the drugs are synergistic.

Ifosfamide

Ifosfamide is utilized in high-dose protocols with the dose-limiting toxicities of renal damage and central neurotoxicity seen at 16–18 g/m^2. When given as a continuous infusion over 16 days, renal failure is less marked than when given over three to four days [25]. The pharmacokinetics show a significant inter- and intrapatient variability, the mean elimination half-life is 6 hours and ifosfamide induces its own metabolism. There is a linear dose to AUC relationship up to 16 g/m2, and less than 15% of the drug is excreted unchanged in the urine, indicating that metabolism of the prodrug to the active compound is not saturated at these doses [26].

Giving the dose in a fractionated schedule allows the dose intensity to be increased by nearly 50%. This has significant implications, because in clinical phase I–II trials, a number of patients with soft-tissue sarcomas who have progressed after standard dose ifosfamide will respond to high-dose 12 g/m^2 therapy [27].

Busulphan

Busulphan is administered orally at a dose of 1 mg/kg ×4 for four days in high-dose schedules. Children have a more rapid clearance than adults, 35% of adult patients show a decrease in steady-state levels during the standard schedule which may be due to induction of glutathione or glutathione-S-transferase synthesis, or due to induction of its own metabolism [28]. Circadian changes also occur with dosing. Night-time levels are higher in children (1.3–1.5 times more) than day-time levels, but this change is not seen in adults. The AUC after a high dose of busulphan can vary by a factor of seven, and patients with high AUC are more likely to develop veno-occlusive disease (VOD) [29].

Melphalan

High-dose melphalan (HDM) may be administered orally or intravenously. Oral melphalan shows significant inter- and intrapatient variability and after multiple doses there is significant intrapatient variability. Choi *et al.* [30] showed that there were significant differences in pharmacokinetic parameters between the first and third dose of oral HDM when given three days apart. There was a decrease in the peak plasma concentration (2.09 to 1.07 mol/litre), AUC 264.9 to 134 mol/litre min and an increase in oral clearance (25.1 to 53.1 ml/min/kg). The mechanism by which this occurs is not known but it may relate to the effect of prior doses of chemotherapy on the intestinal mucosa or be due to saturable energy-dependent amino acid transport mechanisms. However, when doses are six weeks apart, there is an increase in the peak plasma concentration and AUC.

When given intravenously at high doses (140–180 mg/m^2) over 5–60 minutes there is also significant intrapatient variability, with the AUC ranging from 146 to 1515 mg/ml/min, and plasma clearance from 92 to 961 ml/min [31]. There is no correlation between renal function and pharmacokinetics, and there is no evidence that forced diuresis alters melphalan pharmacokinetics [32]. After successive doses given at six-week intervals, there is an increase in peak plasma levels and AUC, although the effect

appears to plateau after three cycles. This suggests a saturable mechanism, possibly in drug transport.

Due to the highly variable pharmacokinetics, a test dose has been administered prior to HDM and this has led to less than 10% variance in the observed AUC [33]. In addition, at AUC levels greater than 47 mg/litre/min, a correlation between AUC and the relative decrease in the neutrophils was found.

There is no evidence that altering the duration of administration changes the toxicity profile or the efficacy of the drug. There is also no evidence that administration of other chemotherapeutic agents in combination with melphalan alters the pharmacokinetic profile of either drug.

Thiotepa

In high-dose therapy, thiotepa may be administered as either a short infusion over 30 minutes or a longer infusion over 4 hours for three days, or as a continuous infusion over four days, dose range 750–900 mg/m^2. Given as an intermittent bolus, the AUC increases linearly with dose, and 12 hours after dosing there is <0.5% thiotepa in the urine, indicating that there is no evidence of saturability of the metabolic clearance mechanisms [34]. Grade 2–4 toxicity has been shown to correlate with peak plasma concentration and with combined thiotepa and tepa AUC after fractionated thiotepa, given as a 4-hour infusion [35].

However, when given as a continuous infusion with cyclophosphamide (6 g/m^2) over four days, the clearance of thiotepa appeared to be an inducible process. Henner *et al.* [36] showed that during a continuous infusion, 72% of patients had a decrease in the plasma concentrations of thiotepa. However, clearance declined with increasing dose which may be due to the saturation of the metabolic pathway of thiotepa to tepa.

Carmustine

High-dose carmustine forms part of a regimen commonly used in the treatment of metastatic and high risk stage II and III breast carcinoma. This regimen uses cyclophosphamide for three consecutive days (1875 mg/m^2 per day) as a daily 1-hour infusion, cisplatin (55 mg/m^2) as a continuous infusion for 72 hours and carmustine (600mg/m^2) as a 2-hour infusion at the end of the cisplatin infusion. This schedule results in highly variable carmustine pharmacokinetics, with the AUC and clearance varying more than tenfold. Jones *et al.* [37] demonstrated that 60% of patients with an AUC >600 µg/ml.min after a 2-hour infusion developed lung toxicity, versus 11% of patients with an AUC <600 µg/ml.min. When given in this schedule, acute hypotension can occur, associated with intense flushing of the skin of the face and upper chest which is maximal during the infusion and persists for several hours after the end of the infusion [38].

Mitoxantrone

Mitoxantrone is given as a short i.v. bolus (15–60 minutes) in high-dose therapy [maximum tolerated dose (MTD) 60–80 mg/m^2 in combination]. This leads to a high C_{max}

and a long terminal half-life of 50 hours with linear pharmacokinetics up to MTD 80 mg/m^2, indicating that elimination is not saturated at these doses [39]. Due to the long half-life, there must be at least seven days between the infusion of mitoxantrone and the transplantation with bone marrow or peripheral stem cells [40]. Cellular AUC (measured in leukocytes) is much higher than plasma AUC, suggesting that the drug is accumulated in cells and then released slowly or degraded. This behaviour is similiar to other intercalators such as anthracyclines and anthrapyrazoles. Canal *et al.* [41] showed a correlation between both plasma residual AUC and cellular peak concentration of mitoxantrone and the time to recovery of neutrophils to greater than 500 cells/litre.

Cisplatin

Cisplatin has been used in high-dose schedules (165–250 mg/m^2) in the treatment of a number of solid tumours, in particular breast carcinoma [42]. The dose- limiting toxicities are neurotoxicity consisting of peripheral neuropathy, ataxia, visual disturbances, ototoxicity and nephrotoxicity [43]. Nephrotoxicity is related to C_{max} and AUC and can be limited by the use of adequate and prolonged hydration with a urine flow above 200 ml/min. When given over five days in divided doses, peripheral neuropathy is severe and can be disabling, and when the total dose is given as a short infusion over 15 minutes, ototoxicity is limiting due to the high peak plasma levels [44]. However, when administered as a continuous infusion over 24–120 hours, ototoxicity may be less but renal toxicity and peripheral neurotoxicity are significant. Gandara *et al.* [44] used a schedule of 100 mg/m2 on day 1 and 8 as a 3-hour infusion which resulted in significantly less peripheral neurotoxicity (2%). This was confirmed in a randomized trial in patients with melanoma comparing a day 1, 8 schedule with a five-day schedule [45]. More recently, a phase II trial established an MTD of 250 mg/m^2 for cisplatin when given in divided doses on day −12 and −5 in combination with cyclophosphamide and etoposide [46]. This schedule is now employed in a number of high-dose regimens, while continuous infusion over 72 hours is used in others [47, 48].

Carboplatin

Carboplatin in high-dose therapy is usually administered over four days as intermittent intravenous infusions, usually over 1 hour. Pharmacokinetic studies have shown that the AUC does not vary from day 1 to 4 in this schedule (day-to-day variation is 3.3%) and no relationship was found between AUC and toxicities, except that cumulative AUC was predictive of ototoxicity [49]. Hearing loss became significant with an AUC above 30 mg/ml.min and was more pronounced in patients who had been pretreated with cisplatin. In other phase I studies, doses greater than 2000 mg/m^2 per course have been associated with hepatotoxicity and renal toxicity.

Etoposide

The optimal schedule for etoposide is not known although a dose of 100 mg/m^2, given as 2-hour infusions daily for five days, produced a response rate of 89% versus 10% when

500 mg/m2 was given as a 24-hour infusion in previously untreated non-small-cell lung cancer patients [50]. Mross *et al.* compared two schedules of high-dose etoposide with the same total dose, in patients receiving busulphan, etoposide and cyclophosphamide for acute and chronic myeloid leukaemia and myelodysplastic syndrome [51]. Patients received either 30–45 mg/kg on day −4 as a 6-hour infusion, or 10–15 mg/kg on days −5 to −3 as a 1-hour infusion. The only pharmacokinetic parameter to differ between the two groups was the volume at steady state (V_{ss}), which was lower in the single-dose arm and the exposure time with plasma concentrations above 100 ng/ml being higher in the pulsed 1-hour infusion schedule. Haematological and non-haematological toxicities were more severe in the split schedule; maximum bilirubin concentration, time to $>500\times10^9$/litre granulocytes (8.3 versus 14.3 days) and time to $>50\times10^9$/litre platelets (25 versus 35 days) were all worse and one patient developed VOD.

In children, the recommended dose for continuous infusion high-dose etoposide is 2100 mg/m^2 when given over three days [52]. Pharmacokinetic data show a C_{max} of 26–30.1 μg/ml and mean AUC 2061 μg/ml.h, and this compares with intermittent dose pharmacokinetic data (700 mg/m^2 daily×3 as a 30 minute infusion); C_{max}=156 μg/ml and mean AUC= 1740 μg/ml.h. Continuous infusion provides similar exposure but avoids high peak levels. While this suggests that continuous infusion may be a better schedule, no direct comparison has been made in the high-dose setting.

Cytosine arabinoside

Cytosine arabinoside (AraC) has been used in the treatment of leukaemias and lymphomas for the past thirty years. Doses range between 10 mg/m2 given as short infusion every 12 hours for 14 days, to 1–3 g/m^2 once or twice daily for three to six days. High-dose therapy has been studied in newly diagnosed acute myeloid leukaemia (AML) and in relapsed or refractory disease. Ghaddar *et al.* [53] showed that newly diagnosed AML patients given continuous infusion high-dose AraC did not respond more favourably than historical controls treated with low-dose AraC and doxorubicin or amsacrine. Other studies have shown that intermediate-dose AraC produces a similar outcome to high-dose treatment [54].

The cytostatic effects of AraC are dependent upon cellular uptake and intracellular phosphorylation to the nucleotide cytosine arabinoside triphosphate (araCTP). At low doses, AraC cellular uptake is due to facilitated diffusion through membrane receptors, but at high dose, passive diffusion occurs.

Several studies have shown that the retention of araCTP within leukaemic blasts correlates with clinical response to therapy [55]. However, there is marked interpatient variability of AraC concentrations within blasts and this has lead to individualizing the dose and schedule of AraC. This has aimed to achieve a concentration of 100–150 μmol/litre or 185–270 ng/10^7 blasts over a prolonged period of time. Estey *et al.* [56] showed that responding patients with AML treated with high-dose continuous infusion AraC, had levels of 122 μmol/litre against 63 μmol/litre in those who were not responding. In contrast, normal mononuclear cells showed little patient variability with respect to the level of araCTP, and levels fell more rapidly than in blasts after the end of the infusion of high-dose AraC. In leukaemic cells, levels of araCTP increased or remained unchanged after the end of infusion.

Hiddemann *et al.* [57] used a schedule of 45-minute infusions of 0.75 g/m^2 eight times per day with a treatment-free period of 135 minutes between doses, and maintained leukaemic blast levels above 400 ng/10^7 blasts; the AUC for AraCTP was two- to tenfold that achieved by a 3-hour infusion. This schedule utilizes the differences between blasts and normal mononuclear cell pharmacokinetics to reduce toxicity.

The conversion of AraC to araCTP is a saturable process and therefore merely increasing the dose may not lead to increased formation of active metabolites. High-dose AraC is known to improve response rates in relapsed or refractory patients with AML but is associated with higher toxicity.

The optimum dose and schedule of AraC is not known but by utilizing this pharmacokinetic information, a rational approach has been developed to increase the active metabolite levels in leukaemic cells and to decrease toxicity. Trials to confirm the validity of this approach are awaited.

Methotrexate

Methotrexate (MTX) doses of 20–33600 mg/m^2 have been used, but haematological support is not required at high doses because toxicity can be rescued by the use of leucovorin. Schedules employed include a 4-, 8- or 24-hour infusion. Evans *et al.* [58] showed that children with acute lymphoblastic leukaemia (ALL) given 1000 mg/m^2 who achieved a steady state >16 μM were more likely to remain in remission. Similarly, patients treated with 1000 mg/m^2 compared with 30 mg/m^2 were more likely to achieve higher MTX polyglutamate levels within leukaemic blasts, and this was associated with greater antileukaemic activity [59]. In a randomized study comparing two different schedules of MTX in childhood ALL, 12 g/m^2 over 4 hours versus 1 g/m^2 over 36 hours, there was no difference in the antileukaemic activity but there was more toxicity (mucositis) associated with the prolonged infusion [60]. The MTX AUC in these regimens differed dramatically; 6409 μmol/litre.h for the high-dose arm, versus 309 μmol/litre.h for the lower dose. However, the duration of exposure to MTX levels of >1 μmol/litre was 36 hours for 12 g/m^2 versus 45 hours for 1 g/m^2, and this appeared to be a better indicator of therapeutic and toxic effects.

Paclitaxel

Paclitaxel has only recently been used in high-dose protocols. Schedule does appear to be important in determining toxicity when it is used in standard doses. When given over 24 hours, neutropenia was more severe than when the same dose was given over 3 hours [61]. Gianni *et al.* [62] suggested that the degree of neutropenia relates to the time above a threshold concentration (0.05 μmol/litre) and also demonstrated that the pharmacokinetics were not linear up to 250 mg/m^2 which may in part be due to a saturable elimination process. However, these same trials have not shown a relationship between the schedule and response rate. Stemmer *et al.* [63], however, showed in a phase I trial that the AUC was linear from 250 to 725 mg/m^2 when it was administered over 24 hours and also demonstrated that the AUC correlated with the severity of sensory neuropathy and mucositis. Dose-limiting toxicities were pulmonary, renal and CNS. With

increasing doses of paclitaxel, there was no alteration in the AUC of cyclophosphamide or cisplatin that were given as part of the regimen.

Multiple cycles versus single cycles

There are trials underway utilizing two or more cycles of high-dose chemotherapy in an attempt to increase total dose while maintaining dose intensity with manageable toxicities. There are no comparative trials evaluating the effectiveness of this to date.

Sequence dependency

Pharmacokinetic and pharmacodynamic interactions between drugs may be sequence-dependent. This may be due to displacement of drug from plasma proteins, competition for metabolism or changes in renal clearance. There is evidence in preclinical models that drug sequence is important in overcoming resistance, e.g. cisplatin/5-fluorouracil [64]. The sequence cisplatin-paclitaxel at standard doses has been reported to be more toxic than the reverse sequence, whereas carboplatin-paclitaxel is not sequencedependent [65]. Paclitaxel given as a 24-hour infusion with doxorubicin results in greater toxicity than when given as a 3-hour infusion [66].

Conclusion

The use of cytotoxic drugs in high-dose regimens requires an understanding of their mechanism of action, pharmacokinetics and toxicity profile, and this knowledge provides some rationale for the choice of dose and schedule and timing of progenitor stem-cell return. While it is important to exceed and maintain effective inhibitory concentrations in the target tissues, exceeding these may simply result in excessive toxicity, rather than improved activity. Too little is known about sequence and synergy in the high-dose setting to make firm recommendations, and therefore much more research is required. While there is considerable information about the pharmacokinetics of individual drugs, there is little information on the pharmacokinetics and pharmacodynamics of high-dose drug combinations. These data are vital in determining the optimum dose and schedule and it would be appropriate to incorporate these concepts in future high-dose protocols. Improvement in supportive care and the use of growth factors has enabled multiple cycles of high-dose chemotherapy to be administered, although little information is available regarding the optimum schedule or effectiveness of this approach.

Advances in molecular biology have led to greater understanding of drug-resistance mechanisms. High-dose chemotherapy is a strategy to overcome resistance and improve response rates and survival. There is evidence *in vitro* to support this concept, and clinical studies in a number of tumour types have shown that increasing dose improves response rates but not overall survival. However, there are a number of randomized trials in progress with high-dose chemotherapy which will address survival.

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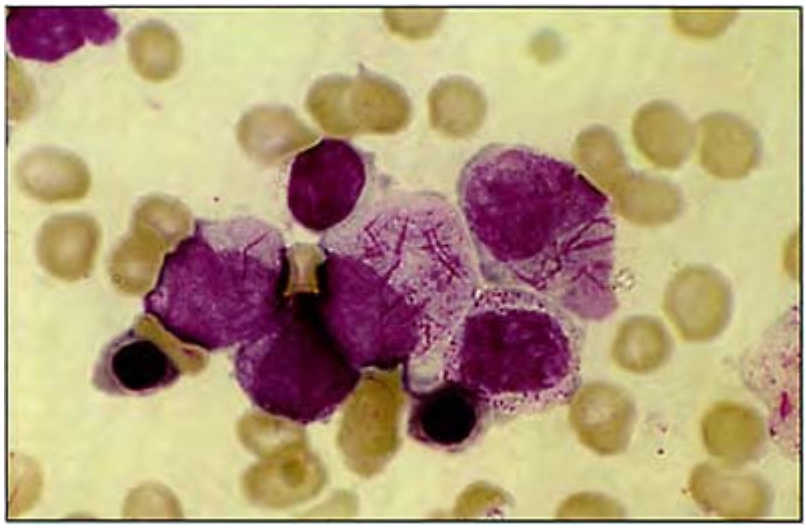

Chapter 3
Acute myeloid luekaemia

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Allogeneic bone marrow

Introduction

Allogeneic bone-marrow transplantation (BMT) has been the standard treatment of patients with acute myeloid leukaemia (AML) for the last 15 years. The procedure was initially used only in patients with advanced or refractory disease because of its toxicity. However, even in these poor-risk cases, long-term survival was shown to be possible in 10–15% [1].

During the 1980s, advances were made in several aspects of transplant care. These included the use of fractionated irradiation [2], use of busulphan as an alternative to total body irradiation (TBI) [3], more effective graft-versus-host disease (GvHD) prophylaxis [4], and improved supportive care, particularly with regard to the infectious complications of transplantation. New conditioning regimes incorporating chemotherapy agents with antileukaemic properties were also shown to be effective [5,6]. As a result,

the outcome of allogeneic BMT has improved with time [7,8], primarily as a result of a reduction in transplant-related mortality.

The role of the graft-versus-leukaemia (GvL) effect following allogeneic BMT in AML

Barnes and Loutit [9] first proposed that bone-marrow grafts exerted an antileukaemic effect in 1956. However, it was only when excessive relapses occurred following T-cell depleted bone-marrow allografts in the 1970s and 1980s [10] that the full significance and therapeutic potential of an immunologically mediated GvL effect was realized. The more recent experience of using donor lymphocyte infusions to re-induce remissions in patients relapsing post allogeneic BMT—while not as effective in AML as chronic myeloid leukaemia (CML)—have further served to consolidate this concept [11,12].

GvL may be considered a separate entity from GvHD but they share many common denominators. It is associated with acute and chronic GvHD but is also seen in the absence of clinical GvHD [13]. Its effect is modulated by GvHD prophylaxis including use of cyclosporin A- and T-cell depletion. Donor lymphocyte infusions can confer a GvL effect without causing acute GvHD [14]. GvL, like GvHD, is related to histocompatibility although it is still conserved in genetically homogeneous populations such as those from Japan [15]. The nature of the GvL effect also seems related to the underlying disease and its stage, being strongest for CML, intermediate for AML and weakest for acute lymphoblastic leukaemia [13].

The pathogenetic mechanisms underlying the GvL process remain unclear, but studies suggest that major histocompatibility complex (MHC)-restricted $CD8^+$ and $CD4^+$ T-cells, natural killer (NK) cells and cytokines may all play an important role. The ability of leukaemic cells to initiate an alloimmune response is of paramount importance and may explain why the GvL effect varies with different haematological malignancies. Both $CD4^+$ and $CD8^+$ T-cells and NK cells recognize peptide antigens on leukaemic cells presented in association with MHC molecules [16]. In the case of CD4, co-stimulation with molecules of the B7 family is required [17] and the presence of these may be critical for production of this effect. Possible leukaemic antigens are numerous and include minor histocompatibility antigens as well as lineage-restricted and leukaemia-specific antigens. Variability in the expression levels of these may also explain interindividual differences in susceptibility to the GvL effect. Some aspects of GvL may also be lymphokine-mediated. Several of these, including tumour necrosis factor (TNF), interleukin-2 (IL-2), interferons (IFN) and myeloid growth factors have antileukaemic activity *in vitro* and *in vivo* (Figure 3.1). This effect may be indirect, by stimulating immunity against leukaemic cells and by increasing sensitivity of such cells to cytotoxic cells, or direct, by cytotoxic action.

How long the GvL effect persists following BMT and the mechanisms by which it reduces leukaemic relapse—either by eradication of the leukaemic clone or by control of growth of residual leukaemic cells—is unknown. Clinical studies suggest that immunemediated antileukaemic effects operate early post-transplant and are not able to control growth of rapidly proliferating leukaemic cells [18].

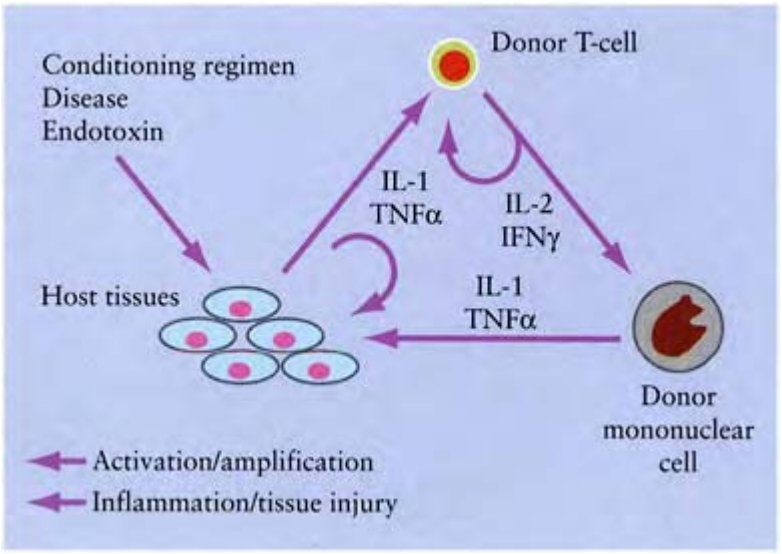

Figure 3.1 The cytokine storm after BMT.

Considerable data now exist to show that transplant recipients developing acute or chronic GvHD have a lower incidence of disease relapse [19,20]. In human leukocyte antigen (HLA)-identical sibling transplants, this antileukaemic effect is maximal when both acute and chronic GvHD occur [21]. An International Bone Marrow Transplant Registry (IBMTR) study on >2000 patients analyzed the roles of GvHD and GvL in preventing relapse and has suggested that several mechanisms are in operation [22]. In AML, patients developing GvHD have a reduced relapse risk which is ameliorated but not lost following T-cell depletion of allogeneic grafts. The risk of relapse is significantly increased following syngeneic transplants even when compared to allogeneic transplants without clinical GvHD (Table 3.1).

Post-transplant immunosuppression with cyclosporin A and methotrexate is the most widely used regimen

Table 3.1 The relative relapse risks following allogenic and syngeneic transplants in patients with AML

Type of transplant	Relative risk	*P* value*
HLA Identical		
No GvHD	1.00	–
Acute GvHD only	0.78	NS
Chronic GvHD only	0.48	NS

Acute and chronic	0.34	0.003
HLA identical,		
T-cell depleted		
No GvHD	1.57	NS
Acute and chronic	0.80	NS
Syngeneic	2.55	0.008
*NS=not significant		

for prevention of GvHD post-allogeneic transplant. Some studies have suggested an increased rate of relapse when these agents are used in combination compared to either alone [23]. However, a study involving >1600 patients treated with this combination has confirmed that there still is an appreciable GvL effect with reduction in relapse and improved leukaemia-free survival (LFS) particularly in patients suffering chronic GvHD but not grades I–II acute GvHD; this effect is most significant for patients transplanted in first complete remission (CR) [24].

The clinical impact of GvL in AML is more difficult to ascertain, although models comparing results following chemotherapy alone, syngeneic transplants and allogeneic transplants aim to do this. Using these types of model, it has been suggested that in adults transplanted in first complete remission, conditioning with high-dose chemotherapy and radiation cures few patients. The effect of GvHD is approximately 15%, and the GvHD independent antileukaemic effect of the transplant approximately 30%, suggesting that immune-mediated antileukaemic effects are as or more important than high-dose chemotherapy and radiation in eradicating disease post-BMT [25].

Attempts to use or augment the GvL effect post-transplant have been used with variable success. This has been done largely by modification of post-transplant immunosuppression or addition of donor leukocytes to the graft. With respect to reduction of relapse, results have been unfavourable and have shown an increase in the incidence of GvHD and early deaths, with no reduction in relapse [26]. Use of lymphokines post-transplant for this purpose has yielded variable results, with IL-2 seemingly offering little or no benefit but IFN reducing relapse risk in one small study [27]. When used to treat established relapse, the experience has been more encouraging with both strategies reported as being successful, although the timing and dose of T-cells required for this purpose is yet to be fully established.

Other methods including *ex vivo* expansion of leukaemia-specific cytotoxic T-cells, and vaccination of donors with leukaemia-specific peptides to induce production of leukaemia-specific T-cells are under development.

There are few data referring to the role of autologous GvL in humans. IL-2 and cyclosporin A have been used with some success but the studies contained insufficient numbers and were inadequately controlled to permit confirmation of this effect [28].

Why GvL is not always successful even in patients who develop GvHD is not known, but there may be several explanations including inhibition of the antileukaemic effect by post-transplant immune suppression, absence of target antigens on leukaemic cells,

resistance of leukaemic cells to lymphokines or extent of the leukaemia at the time of transplant (Table 3.2).

It was clear after the preliminary experience that BMT was likely to be more effective if used at an earlier stage of the disease. It soon became accepted as consolidation treatment for younger patients (<45 years) with AML. Registry data have shown long-term survival rates of approximately 50% in adults following BMT [29,30] compared to 25–40% with intensive chemotherapy [31]. However, the seemingly superior results of BMT must be interpreted with caution. The enhanced survival rates seen with BMT may reflect inadvertent patient selection for HLA-typing or referral to a transplant centre. There is also a time-to-treatment bias, in that the time delay between diagnosis and BMT may favour selection in favour of those already at a lower risk of relapse. It is now well known that there is a major time-censoring effect in AML. Patients who survive four to six months in first complete remission after chemotherapy only have a similar survival at five years as is reported with BMT [32].

Several of the more recent prospective multicentre trials have attempted to overcome selection bias by using donor availability as a biological randomization to either BMT or chemotherapy, and analysing results on a donor versus no donor basis. This may not be a

Table 3.2 Possible reasons why GvL is not always successful even in patients who develop GvHD

- Inhibition of antileukaemic effect by post-transplant immune suppression.
- Absence of target antigens on leukaemic cells.
- Resistance of leukaemic cells to lymphokines.
- Extensive leukaemia at time of transplant.

perfect method, but it is probably superior to anything else, short of a true randomized comparison.

Results of prospective trials

The results of three major multicentre trials appear to indicate a survival advantage for allogeneic BMT over chemotherapy in children with AML, although not all are analyzed according to donor availability.

The Children's Cancer Group (CCG) treated 490 patients aged 0–21 years from 1979 to 1983 and compared allogeneic BMT to two maintenance chemotherapy regimes [33]. Survival estimates at five years for those with a donor versus those without showed a significant difference in favour of BMT (50% vs 36%; $P<0.05$). A later CCG study [34] involving 591 children showed a small survival benefit for BMT but this was not statistically significant when analyzed on a donor versus no donor basis (five-year disease-free survival (DFS) 46% vs 38%; $P=0.06$), although comparisons according to protocol intent or actual treatment did show a survival advantage for BMT.

The AIEOP (Associazone Italiana Ematologia et Oncologia Pediatrica) cooperative study group looked at 161 patients aged 0–15 between 1987 and 1990 [35]. Patients without a donor were randomized to receive either autologous BMT or chemotherapy.

Analysis on an intention-to-treat basis showed allogeneic BMT to be more effective than autologous BMT (ABMT) or chemotherapy in preventing leukaemia relapse and extending disease-free survival (five-year DFS 51% BMT; 21% ABMT; 27% chemotherapy). Bone-marrow relapse was the major cause of treatment failure even in the allogeneic transplant group, in which no toxic deaths occurred.

In adults the benefits of allogeneic transplantation are more difficult to assess as the increased antileukaemic effect is offset by a rise in procedurerelated mortality with age. Furthermore, there is no consensus as to the optimum timing of allogeneic transplantation in AML. The majority of the large trials have focused on the role of transplantation as consolidation therapy in first remission. Of the more recent studies, only two showed a significant survival advantage for allogeneic BMT. In the UK, the Northern Regional Haematology group followed up 149 unselected patients with AML aged 15–55 years [36]. Of those aged 15–40 years who attained remission, 22 out of 28 who had a donor underwent allogeneic BMT, and 24 received chemotherapy. Analysis according to intention to treat showed a four-year event-free survival of 62% in the transplant group compared to 24% in the chemotherapy group.

The European Bone Marrow Transplant (EBMT) leukaemia working party carried out a prospective analysis of transplantation in AML from the time of HLA typing [37]; 168 patients were registered. For patients typed at diagnosis (i.e. unselected patients), the estimated three-year survival figures showed an advantage of allogeneic BMT over ABMT and chemotherapy regardless of whether analysis was performed according to donor versus no donor (44% vs 21%), intention to treat (allo-BMT 48%; ABMT 23%; chemotherapy 0%), or actual treatment (allo-BMT 50%; ABMT 29%; chemotherapy 17%). However, there was no significant difference between the three treatment modalities among the patients typed in first remission, which suggests that at this stage selection in favour of those already cured had occurred.

This lack of a survival advantage for allogeneic BMT in first remission is also apparent in other comparative studies: Archimbaud *et al.* [38] looked at 78 patients aged 15–40 years treated on the same protocol (LYAM-85) at a single institution who were HLA-typed in first remission. Of those who had a donor, 20 out of 27 received a transplant; 31 patients with no donor received cytarabine-based consolidation chemotherapy. There was no significant difference in leukaemia-free survival and overall survival at seven years between the two groups. Although there was an advantage for patients allocated to receive BMT in terms of reduced risk of relapse, this was offset by the higher toxicity of BMT (20–30% toxic deaths compared to 10% in the chemotherapy arm). Other explanations for the lack of a survival difference between those with and without a donor included the fact that one-third of those with a donor failed to undergo BMT in first remission, and the more efficient salvage treatment of those who relapsed after chemotherapy than after BMT (Table 3.3).

The multicentre EORTC/GIMEMA study [39] compared the outcome of allogeneic BMT, autologous BMT and chemotherapy in patients with AML aged 15–40 years in first remission; 168 patients who had an HLA-matched sibling were assigned to allogeneic transplant, while the remaining 254 were randomized between autologous BMT and chemotherapy. Both allogeneic and autologous BMT showed an advantage over chemotherapy in terms of disease-free survival (four-year DFS 55% allo-BMT; 48%

auto-BMT; 30% chemotherapy) but neither improved overall survival because of a lower death rate during remission in the chemotherapy group and successful salvage therapy with autologous BMT in patients relapsing after chemotherapy.

Table 3.3 Allografting in first remmision AML

- reduces relapse risk
- Improves leukaemia-free survival
- may not improve overall survival for all patients due to treatment-related mortality on better salvage rates in chemotherapy arm.

Preliminary results have been reported [40] on the UK MRC AML-10 trial involving a large number of patients (*n*=378) who had a donor available and compared them to patients with siblings who did not have a donor available. All received four courses of intensive chemotherapy, and those with donors were then intended to receive the allogeneic graft. In fact, only 59% actively received the transplant either because of interceding relapse, death in complete remission, other toxicity, or patient refusal. In this study a correction for time delay was made in the analysis. All chemotherapy patients were timecensored to the time when the first transplant was delivered. Although there was a significant reduction in relapse risk, there was no overall survival advantage. Preliminary results from patients treated on the GOELAM 1 protocol [41] also suggest that the three forms of post-remission therapy give comparable results in terms of survival.

Because AML is relatively rare, the number of patients in each treatment arm of any prospective randomized trial is often small which can make it difficult to detect small differences in treatments [41]. Retrospective studies using registry data overcome the problems of patient number but outcome-related variables may differ between treatment groups, and non-uniformity of treatment between centres may exist. There is also the potential for bias in that candidates for BMT who relapse are excluded from retrospective analyses. Comparisons of retrospective data have an inherent unreliability, but with care can be helpful, particularly in circumstances where patient accrual to answer a question prospectively would be almost impossible.

A recent retrospective study by Gale *et al.* [42] compared the results of HLA-identical sibling transplants reported to the International Bone Marrow Transplant Registry (901 patients) with chemotherapy in a comparable group of patients treated by the German AML Cooperative group (196 patients). Although the five-year probability of LFS was higher for transplants than chemotherapy (46% vs 35%; P= 0.01) and the five-year relapse probability was less for transplants than chemotherapy (24% vs 63%), overall survival was similar because of the increased treatment-related mortality in the transplant group (43% vs 7%).

Timing of BMT

Although relapse rates are lowest when allogeneic BMT is done in first remission, this strategy would expose the 20–40% of patients who have already been cured by chemotherapy to the life-threatening toxicity of BMT. Because transplantation also appears to be an effective salvage therapy in patients who relapse, there is a case for

delaying transplantation until relapse occurs. In patients who relapse, transplantation appears to be superior to chemotherapy in terms of overall survival, although no randomized trials have been done. Most studies suggest that these patients cannot be cured by chemotherapy alone, whereas a 20–25% three-year LFS has been reported after allogeneic BMT [43]. In a retrospective comparison conducted by the IBMTR with major trial groups analyzed, the outcome of allogeneic BMT in adults aged under 50 years in second remission was compared with results after chemotherapy [44,45]. The transplant cohort consisted of 257 patients reported to the IBMTR, and the chemotherapy cohort included patients treated on the MRC, ECOG and MD Anderson protocols. While the study was not designed to determine the best treatment strategy in patients with AML who relapse, analysis showed a significantly higher three-year probability of LFS with transplantation in patients aged under 30 years with a first remission duration of longer than one year (41% vs 17%) and those aged over 30 years with a first remission lasting less than one year (18% vs 7%) (Table 3.4).

For patients not transplanted in first remission who relapse, there is debate about whether transplantation is best performed in untreated first relapse or second remission. Transplantation in untreated first relapse has been shown to be feasible. The report by Clift *et al.* [46] summarizing the Seattle experience in transplanting patients with AML during untreated first relapse looked at 126 patients and quotes a five-year probability of relapse-free survival of 23%. Earlier reports from Seattle [47] showed that the results of transplantation in first relapse were not demonstrably inferior to those obtained in second remission, and since less than 50% of patients achieve a second remission there is little to be gained by delaying transplantation until after re-induction. However, this approach requires prompt access to a transplant centre and other complications at the time of relapse such as infection may increase the risks of the procedure.

Although concerns over toxicity may favour delaying BMT until relapse in order to avoid unnecessary risk to those patients already cured, some would argue that allogeneic BMT is most effective as consolidation therapy when performed soon after induction. If BMT is performed early, patients will have had less exposure to organ-damaging chemotherapy and a shorter duration of neutropenia, so are less likely to have acquired harmful fungal infections. Early allogeneic transplantation has been shown to be an efficient consolidation therapy for adults. Jourdan *et al.* [48] studied a cohort of patients reported to the SFGM registry with a mean age of 31

Table 3.4 IBMTR analysis of 257 adults in second remission of AML

Significantly higher three-year probability of LFS with transplantation in:
• Patients aged under 30 with first remission duration longer than 1 year (41% vs 17%).
• Those aged over 30 with first remission lasting less than 1 year (18% vs 7%).

years and who had undergone transplantation in first remission within 100 days from diagnosis. The five-year probability of overall survival was 71%, and transplant-related mortality was low at 14%. Despite concerns that this strategy may have recruited higher risk patients who might have relapsed prior to a delayed transplant, or that patients may not have received sufficient chemotherapy, the five-year probability of relapse was only

17%. However, although transplant-related mortality may be lower with early transplants it is unpredictable, and therefore there is still a case for reserving allogeneic BMT as salvage therapy in those who relapse.

Risk stratification

The fact that intensive chemotherapy, especially when this involves high-dose cytarabine, has been shown to give comparable overall survival rates to those of allogeneic BMT in first remission [49] raises the question of whether any patient with a donor in first remission should be exposed to the risks of a transplant.

There is a need to identify those patients who are likely to do well with intensive chemotherapy alone in order to avoid unnecessary exposure to the toxicity of BMT, as well as those likely to fail on standard treatment who may benefit from early transplantation. While none of the previous studies stratified patients according to risk, two recent studies have looked at the impact of cytogenetic abnormalities at diagnosis on the outcome of BMT performed in first remission. Karyotype at diagnosis has been shown to be an important prognostic factor. A retrospective study from the IBMTR [50] examined the impact of cytogenetic abnormalities on the outcome of 1516 HLA-identical sibling transplants concluded that relapse rates were highest and LFS lowest in those with poor risk cytogenetics, compared to those with no abnormality, or with good/intermediate risk karyotypes. A prospective study by Ferrant *et al.* [51] showed similar results. While a favourable karyotype is associated with a good outcome, it is difficult to be certain that this is due solely to the transplant as patients with these karyotypes also show improved survival when treated with chemotherapy alone.

This issue has been part of the analysis of the UK MRC AML-10 Trial analysis [40]. Based on a ‘donor versus no-donor available’ analysis, although there was a reduced risk of relapse in good risk patients (defined only as favourable karyotypes t8:21 (Figures 3.2 and 3.3), t15:17 and inv 16, with or without additional abnormalities, this was more than counterbalanced by an increased procedure-related mortality. Overall survival was thus significantly inferior in the ‘donor available’ arm. Similarly, poor risk patients (complex karyotype, abnormalities of chromosome 5 or 7, or patients with >20% blasts in the marrow after course 1 of chemotherapy) did not benefit because the relapse risk was high (Table 3.5). There was a small but significant advantage in standard risk (all other groups) patients.

Current place of allogeneic BMT in AML

Allogeneic BMT remains the most effective antileukaemic treatment for AML. It is now clearer, that for those patients with the highest risk of relapse, it is much less efficient at curing the disease. However, new approaches either to myeloablation or augmenting the GvL effect may improve results. Alternative treatments without transplantation do not offer much hope for this difficult group of patients.

It now seems reasonable to delay transplant in good risk patients until they fail treatment. These patients tend to be young, and late effects are a serious consideration. In

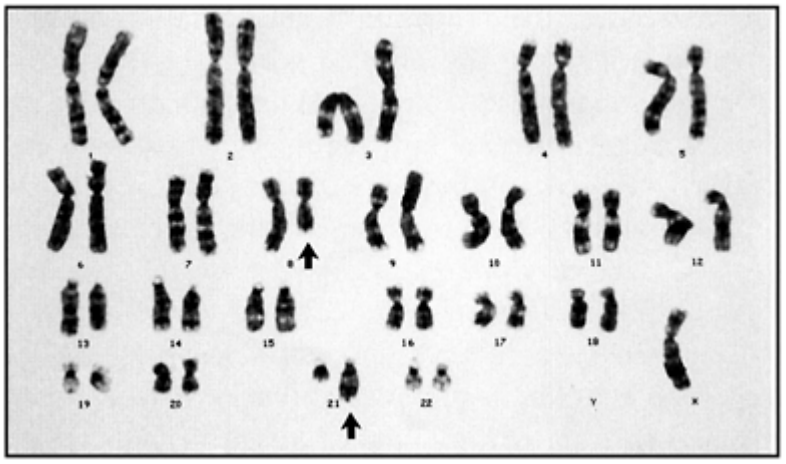

Figure 3.2 G-banded karyotype showing the t8;21 (q22; q22) chromosome translocation associated with acute myeloid luekaemia.

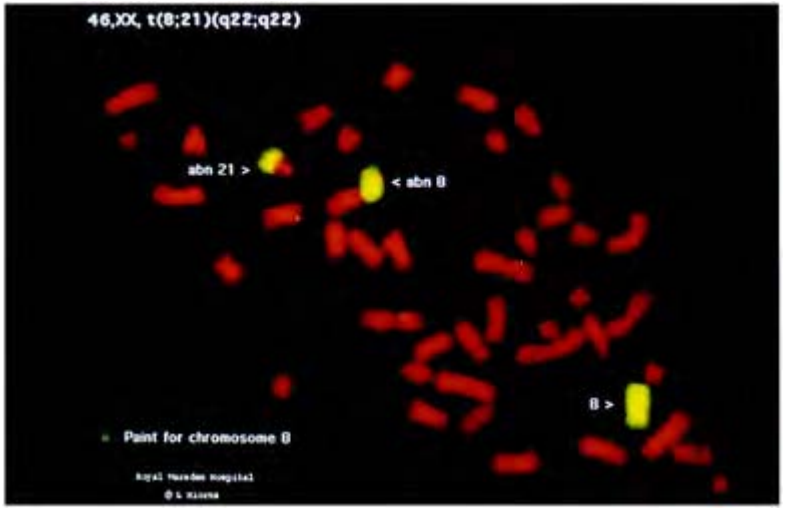

Figure 3.3 Fluorescence in-situ hybridization showing the t8;21 (q22; q22) chromosome translocation associated with acute myeloid leukaemia.

Table 3.5 Prognostic features in AML

Good risk
• Chromosome t8:21, t15:17 and inv 16, with or without additional abnormalities.
Poor risk
• Complex chromosome karyotype, or abnormalities of 5 or 7
• >20% in the marrow after course 1 of chemothrapy

the MRC trial, these patients had a one-year survival from relapse of 90%, so this appears to be a reasonable, secure strategy.

Standard risk patients should be encouraged to receive an allograft as part of first remission treatment. The salvage rate of these patients is less predictable, and thus reaching a transplant in CR2 is less certain. It could be argued with some justification that, even in these patients, outcome is unclear; results after chemotherapy may continue to improve, and transplants, where possible, should continue to be performed within a clinical trial context. It should also not be forgotten that the relapse risk in good risk disease has reduced, indicating that further chemotherapy—with minimum toxicity—may still be indicated for these patients.

Autologous transplantation

For patients who lack a suitably matched donor, autologous transplantation of haemopoietic stem cells is one of the alternative treatment strategies. The vast majority of evaluable data have been with the use of bone-marrow-derived cells, but the potential merits of peripheral blood-derived cells will be discussed later. The international experience of autografting in AML is now considerable, but unfortunately the majority of this effort has been outside the context of randomized trials which are the only way to establish therapeutic value.

Background rationale

In the late 1970s, it was not known whether bone marrow collected after substantial prior chemotherapy would establish durable engraftment. At that stage, very little experience was available of using cryopreserved stem cells in the face of myeloablative (TBI-based) cytoreduction. There was also considerable cynicism about the use of potentially diseased marrow with no technology available to eliminate contaminating leukaemia cells. The first two issues were tackled in the seminal study of Dicke and colleagues in Houston, who used autologous marrow to support TBI-based myeloablation for patients who had already failed conventional treatment for relapsed disease [52]. Although complete remissions were obtained, these were short-lived and it was clear that if this approach to treatment was to be useful it would not be as a treatment of chemoresistant disease. This was subsequently confirmed in the early data collected by EBMT and such an approach is now regarded as pointless. This was about the time that allogeneic BMT for advanced disease had reached the same conclusion and the superior results of transplantation in

remission were emerging. This prompted the early enthusiasts to conclude that autografting should move into the setting of remission. The first single-centre nonrandomized studies started to appear in the early 1980s. The rationale of the approach on the positive side was that myeloablation itself (including TBI) could safely be applied in patients up to 55–60 years of age as evidenced by syngeneic transplant experience. Because of the absence of GvHD and other sequelae of the allograft approach, the morbidity would be less. This would mean that a substantial proportion of cases with AML who entered remission could be offered this approach. On the negative side, there was concern that the harvested autograft would inevitably be contaminated with occult disease. Mathematical arguments at the time suggested that while there may be occult disease present which would be re-infused, this would be so small as to possibly be of no clinical relevance. Many investigators felt—and to this day feel—that a process of purging the marrow *ex vivo* is a prerequisite for a successful and ethical autograft.

Non-randomized studies in first remission

Several individual groups reported series of patients in whom autologous BMT was used as consolidation treatment in first remission [53–59]. These patients had received widely differing types and amounts of conventional chemotherapy prior to the autograft and a variety of myeloablative preparative regimens for the autograft. The overall outcomes were surprisingly similar, being a durable survival of 45–55%. Some of the early chemotherapy combinations, such as TACC, high-dose melphalan and single course BACT have been less successful than the more widely adopted cyclophosphamide/TBI, or busulphan/cyclophosphamide schedules. Given the substantial interpatient variability, it is not possible to identify a ‘best choice’ myeloablative protocol.

The procedure has turned out to have a low immediate procedural mortality of 6–8%. Pneumonitis is an unusual complication. While cytomegalovirus (CMV) reactivation was common, CMV disease was not. A consistent feature was that platelet regeneration was found to be very slow, and while this undoubtedly contributed to treatment-related mortality, it was not found to be an indication of impending relapse. It seems to be a phenomenon associated particularly with AML, since heavily pretreated patients with other diseases do not have such a slow recovery. Various correlations with cell dose, colony-forming cells of the granulocyte-macrophage (CFU-GM) or ethyroid lineage, (BFU-E) and other parameters have not shown a consistent correlation. Platelet count at harvest emerged as the only patient variable in one study [60] (Table 3.6).

Most of the patients who relapse after autograft do so in the first 18 months. In this respect, the pattern of relapse risk bears a closer relationship to that seen after allogeneic BMT than after chemotherapy. The time spent in remission before autograft was shown, from registry data, to correlate with outcome of the autograft: the shorter the time period, the higher the risk of relapse. Two important conclusions could be drawn from this. First, it is well known that in the early 1980s the pattern of relapse with chemotherapy indicated that the highest risk period was in the months immediately after achieving remission. Thus, patients autografted later in complete remission are inherently at lower risk of relapse. This ‘time-censoring’ effect, which has already been raised in relation to allogeneic BMT, will be mentioned later. An alternative conclusion could be that autograft outcome could be optimized if preceded by consolidation therapy, in order to

achieve maximum cytoreduction *in vivo* (Table 3.7). Both of these propositions are likely to be correct

Table 3.6 Aspects of autografting in AML

• Low procedure-related mortality of 6–8%.
• Pneumonitis unusual.
• CMV reactivation common. CMV disease not.
• Platelet regeneration very slow, but does not indicate impending relapse. Platelet count at harvest may be relevant.
• Most patients who relapse after autograft do so in the first 18 months.

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Studies in second remission

Several series and registry data suggest that 30% of patients who receive an autograft in second remission will become long-term survivors [61–63]. In contrast with the first remission experience, many of these patients received busulphan-cyclophosphamide together with purged marrow. It was in this context that cyclophosphamide derivative purging was pioneered. This will be discussed later, but it should be

Table 3.7 Outcome after autografting in AML

• The shorter the time spent in remission before autografting, the higher the risk of relapse. Thus, patients autografted later in complete remission are inherently at lower risk of relapse.
• Autograft outcome can thus be optimized if preceded by consolidation therapy.

Table 3.8 Major determinants of duration of second remission in AML

• Age of the patient
• Duration of first remission
• Other risk features (e.g. karyotype)

noted that in a multicentre UK experience using busulphan-cyclophosphamide with unpurged marrow, the same results were achieved [64].

Although there is consistency between different series in second complete remission, there are very substantial selection factors in operation making it almost impossible to conclude what contribution autografting is making compared with alternative approaches to treatment. The major determinants of duration of a second remission are patient age and the duration of first remission. More recently it has been recognized that other risk features (e.g. karyotype) may be influential in this setting (Table 3.8).

Autograft as primary treatment of relapse

Early experience clearly indicated that using autografting as the last ditch treatment of patients who had relapsed and proven refractory to further treatment was pointless. There is a dilemma about what to do for a patient who relapses in whom marrow has already been stored. Overall, 50% of cases will not achieve a second remission, and even in those who do, a proportion will become medically unfit or relapse shortly thereafter. In other words the chances of reaching an autograft in second complete remission are by no means certain. If patients relapse late in first complete remission with favourable disease (i.e. karyotype) and are young, they will have an excellent chance of achieving second complete remission. On the other hand, if the relapse occurs early—as the majority now do—there is a much poorer chance of achieving second complete remission.

This raises the issue of using the autograft as the primary treatment of relapse. The logistic barriers to this approach are considerable. There are likely to be very few centres who can immediately provide a transplant bed. The experience of such an approach is limited to a prospective study based in Seattle [65], the overall result of which suggested that survival from an immediate autograft was about 20%. This probably compares favourably with a strategy of inducing remission first, where, assuming a 50% second complete remission rate, no other patient loss, and a 30% survival, 15% of relapsed patients will be salvaged. It should, however, be emphasized that concerns have been expressed about whether the patients in this study were typical, or biased by patients who relapsed ‘slowly’.

Is purging necessary?

There has been enjoyable debate about whether various methods of purging the graft *ex vivo* are possible, measurable or clinically necessary. For the advocates it is quite illogical to re-infuse the same tissue—even in small amounts—from which the anticipated relapse will occur. More recently, gene marking studies have elegantly shown that it is at least possible for contamination of the graft to contribute to subsequent clinical relapse [66]. On reflection, this is a remarkable finding in that it was demonstrated in a patient with a t8:21 translocation in which the harvested marrow was polymerase chain reaction (PCR)-negative, yet it still proved possible to effectively genetically mark the malignant cells in this PCR-negative collection.

There is no conventional way to purge in AML. Although immunological methods have advocates, usually based on an anti-CD33 linkage [67], it is accepted that the phenotypic heterogeneity of AML and the immunological differences between the blast and clonogenic populations, restrict the potential of this approach. There was considerable excitement when it was shown to be possible to incubate the autograft in a 10-day long-term culture system, without prejudicing eventual engraftment [68]. The clinical results were better than the average results in recipients of unpurged marrow, but it is not clear what degree of patient selection might have been operating. This point is given some relevance by the demonstration that good growth in a conventional long-term culture system predicted a low relapse risk in patients who subsequently received an autograft, whereas poor growth was associated with a high risk of relapse [69].

The main impetus to purging in AML came from the demonstration that treatment with the cyclophosphamide derivative 4-hydroperoxycyclo-phosphamide (4HC) of syngeneic grafts on Brown Norway rats contaminated with leukaemic cells improved survival after infusion [70]. The effect was dependent upon the dose of 4HC used. 4HC is an active metabolite of cyclophosphamide to which Brown Norway leukaemia cells are particularly sensitive. Important dose-finding studies were conducted in Baltimore which concluded that a standard dose of 100 μg was compatible with haemopoietic regeneration [71]. A feature of most studies of pharmacological purging is some delay in haemopoietic recovery.

An alternative approach, principally advocated by Gorin and colleagues [72] is that the dose should be individualized based on pre-testing with a CFU-GM dose-response. Under these circumstances the individual dose is the one which eradicates 95% of CFU-GM. Clearly, the robustness of residual haemopoiesis is an influential factor, and one could speculate that there is greater marrow reserve in patients who qualify for the higher dose, and this in itself may be a favourable characteristic.

No prospective randomized trials have been undertaken to specifically evaluate purging. There are many practical difficulties in this. The pioneering work in Baltimore was in the setting of second remission. As mentioned before, the 30% or so survival achieved is difficult to evaluate, since similar results have been obtained without purging. Several of these patients, however, have had longer second than first remissions. This so-called ‘inversion’ strongly suggests that there has been some modification of the disease process, but it does not mean that the purging component was responsible.

Enthusiastic analysis of the EBMT registry has shown a reduced relapse risk in patients conditioned with total body irradiation, who received purged versus non-purged marrow [73]. Because of delayed haemopoietic recovery, there is some extra mortality, so the overall survival benefit is not significantly greater. In the subset who received adjusted dose purging, the advantage was greater. It should be restated, however, that the selection biases in these patients could be considerable.

Subgroup analysis of this data set has suggested two subsets of patients in whom purging appeared most beneficial. These were patients who were originally slow to enter complete remission (>40 days) and those in whom autografting was performed early (<6 months). It is not clear whether there are patients who are in both subsets. The advantages are substantial (37% vs 58% and 35% vs 54%, respectively, for relapse risk). This sort of analysis only has value in suggesting in which setting a prospective trial might be viable. With modern induction chemotherapy in patients under 60 years of age, only about 20% of patients presenting will fail to enter complete remission after one course of treatment, and thereby be candidates for such a protocol.

These data raise an interesting issue. These early patients who had purged marrow returned have a reduction in relapse risk from 55% to 37% simply by the purging. However, data on twin transplants—who provide a perfectly clean graft, without any GvL effect—have a 50–60% relapse risk. This discrepancy cannot be explained by a mechanism of *in vitro* cell kill alone. There might be an additional immunologically mediated mechanism. Preliminary evidence has suggested that natural killer (NK) cell activity is enhanced in the post-transplant period in recipients of purged marrow [74]. To date there are no data available to say whether there is an even more marked effect with adjusted dose purging.

Timing of autologous bone-marrow transplant

From what has already been discussed, it can be seen that there are a number of different time points when autografting can be used in the natural history of the disease. If it is to be undertaken in first complete remission, the choice is between early in consolidation, where the chances of there being residual disease are greater, but the chance of patient loss to protocol are least, or following some consolidation which maximally cytoreduces the patient before autograft and will perhaps act as *in vivo* purging of the graft. However, this poses a greater risk of patient loss to toxicity. The autograft could be reserved only for patients who fail first-line treatment, in which case stem cells should be stored in first complete remission and the choice is to use them to support high-dose treatment as primary treatment of relapse, or to consolidate those who achieve a second complete remission. Based on available evidence, one could calculate that any of these strategies would produce the same number of survivors overall, but such strategies require prospective evaluation because there is no information about the number of patients who might not comply with these options for various reasons unrelated to relapse of disease. The denominator group of patients from which all of these single-centre studies is derived is unknown.

Selection biases

The experience of the early and mid-1980s strongly suggested that autografting conferred a useful antileukaemic effect in spite of the possibility that reinfusion of occult leukaemia could be taking place and there was a loss of any GvL effect. It remains unclear whether the effect is merely one of effective cytoablation or whether there may, in fact, be an immunological component. The latter possibility is supported by the observation that there is an increase in NK cells in the post-autograft period which are capable of lysing autologous blasts *in vitro* [75]. This phenomenon is seen after allogeneic and autologous transplantation but not chemotherapy alone.

It is clear that the reservation expressed about patient selection makes it extremely difficult to conclude what the benefit, if any, of autografting may be over alternative therapies. Time-censoring was initially thought to be the largest bias. If patients are not receiving the autograft till four to six months into remission, they already have a 35–40% prospect of surviving five years. In addition conventional chemotherapy has improved due to a trend to apply it more intensively. By the late 1980s, it was clear that prospective trials were needed.

Prospective trials of autologous BMT in AML

A number of large multicentre trials have been completed or are coming to a conclusion. All are addressing the role of the autograft in first complete remission, with some variations [76–79]. The designs are usually to randomize patients after one or two consolidation courses to autograft versus further chemotherapy. In most, bone marrow is cryopreserved unpurged. In the studies conducted by the MRC and HOVON, the design is to randomize the addition of autograft or not to either three or four courses of chemotherapy. The MRC design only randomizes patients in whom marrow has been

harvested, thereby leaving the option to salvage patients in the chemotherapy only arm with an autograft in second complete remission.

In the nine trials so far reported, in published or abstract form, a substantial number are children (Table 3.9). This large trial experience has produced some common themes. First, the protocols and randomizations have been difficult to complete, with usually only 30–40% of available patients being randomized. In addition there is further default in the delivery of the allocated treatment. The reasons for this are not usually relapse, but haematological or other toxicities. It is noteworthy that in the MRC Trial assessing allogeneic BMT when a donor was available it was more likely that the allograft would be carried out (60%), perhaps revealing a lack of conviction of the benefits of randomization to the autograft option.

This defect has in practice changed the nature of the question being asked. Information can only be obtained in patients willing, and considered fit, to undergo the process. This may not represent the other patients, and should be extrapolated with caution.

It is fairly clear, on the intention-to-treat basis, that an autograft can reduce the relapse risk. Since not all patients received the autograft, the antileukaemic effect is certainly underestimated. While this may convert into a superior event-free survival, there are two factors operating which prevent the powerful antileukaemic effect from being—as yet—converted to an overall survival advantage. In some of the trials—most obviously POG and MRC in adults—there was an excess risk of dying in the autograft arm, ranging from 12–15%. The causes were numerous, but were regularly associated with poor engraftment. Given that not all patients received the procedure, this is likely to be an underestimate of the problem. The second issue is that, at least for some patients, relapse from the chemotherapy arm could be salvaged into a durable second remission. This was true for the EORTC trial where the chemotherapy arm was perhaps too weak, and for children in the MRC Trial. Whether these salvages will be durable is unclear, but they will certainly require long-term follow-up.

It can probably be concluded, at the moment, that in children intensive chemotherapy alone should be used as first line and that the potential for later toxicity does not justify deploying the autograft in first remission. The MRC experience was that children have a realistic chance of salvage after relapse. The total experience indicates in all subgroups of patients

Table 3.9 Rondomized studies of autografting in AML

Study group	Non-randomized (n)	Purged marrow	Autografting versus chemotherapy	Autografting versus no further treatment	Adults or children
BGMT 84 [76]	3577	No	Yes		Adults
BGMT 87 [77]	77	No	Yes		Adults
EORTC/GIMEMA [39]	254	No	Yes		Adults

GOELAM [41]	151	No	Yes		Adults
AIEOP [35]	72	No	Yes		Children
POG [78]	232	Yes	Yes		Children
CCG [79]	412		Yes		Children
MRC-10 [40]	381	No		Yes	100 Children
HOVON [4]	124	No		Yes	Adults

that more treatment can further reduce the relapse risk. The key is to achieve this without mortality and with minimum morbidity. Peripheral blood stem cells require careful evaluation as to whether that approach can reduce the prolonged cytopenia and thereby reduce mortality without increasing the relapse risk.

General conclusions

Stem-cell (bone-marrow) transplantation has been a crucial development in the treatment of AML in the younger patient, not least because of the lessons which have been learned about supportive care. The outcomes for patients who have received an allograft or an autograft have been remarkably consistent when they have been evaluated in prospective multicentre trials. However, there can be no doubt that transplant recipients are a selected patient population with an already reduced risk of relapse. Over the last decade, conventional chemotherapy has increased in intensity with improved results such that it is difficult to be sure how much more advantageous transplants might be. Against this background there has been increasing recognition that prognostic factors (e.g. karyotype) have a substantial influence on relapse risk and are arguably more important than the treatment used.

When the appropriate analyses are done, for example, analyzing the allograft contribution on a donor versus no donor available basis—which is a surrogate for intention-to-treat analysis—and bearing in mind risk-group subdivisions, transplantation may have a limited role in good risk and bad risk disease but should still be offered to patients with standard risk disease. Even in that context there is still some uncertainty of benefit, so it would still be beneficial to conduct the procedure within the context of a prospective trial.

The investment by several trial groups in prospective trials of autologous BMT has been considerable. In most but not all, the antileukaemic effect is substantial. A large component of this experience has been in children where, although the relapse risk can be reduced, without necessarily conferring a significant risk (the MRC experience, for example), there is a reasonable prospect of salvaging such patients if they relapse. It is quite possible that for other patients the reduction in relapse may, on longer follow-up, convert into a survival advantage. In spite of all this effort many questions remain unanswered.

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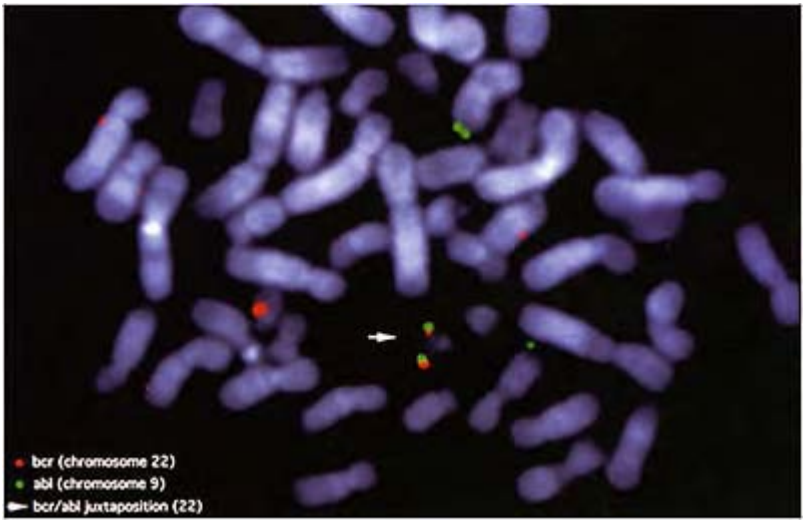

Chapter 4
Chronic myeloid luekaemia

John Goldman

Introduction

The term ‘chronic myeloid leukaemia’ (CML) describes a specific form of leukaemia characterized by progressive splenomegaly, leukocytosis, anaemia, marrow hypercellularity and the finding in most cases of the Philadelphia (Ph) chromosome (Figure 4.1) in all dividing cells of the myeloid series and in some B-lymphocytes. Between 5 and 10% of patients with CML have variant features, such as typical CML without the Ph chromosome, haematologically atypical CML (also Ph-negative), juvenile chronic myeloid leukaemia and various forms of the myelodysplastic syndrome. It is likely that Ph-positive CML together with the rare Ph-negative *BCR/ABL*-positive CML patients are a relatively homogeneous group, and indications for and results of bone-marrow transplant (BMT) in this group cannot necessarily be extrapolated to other patients.

Chromosomal and molecular features of Ph-positive CML

The observation that the Ph chromosome is present in nearly all marrow metaphases in the majority of patients with newly diagnosed CML, and the fact that Ph-positivity usually persists despite conventional treatment, are consistent with the notion that the Ph chromosome is a reliable marker for leukaemic cells in an individual patient. Although there are well-documented cases of Ph-negative leukaemia preceding recognition of the Ph chromosome and some suggestion that Ph-positive haemopoiesis may on occasion be preceded by clonal Ph-negative

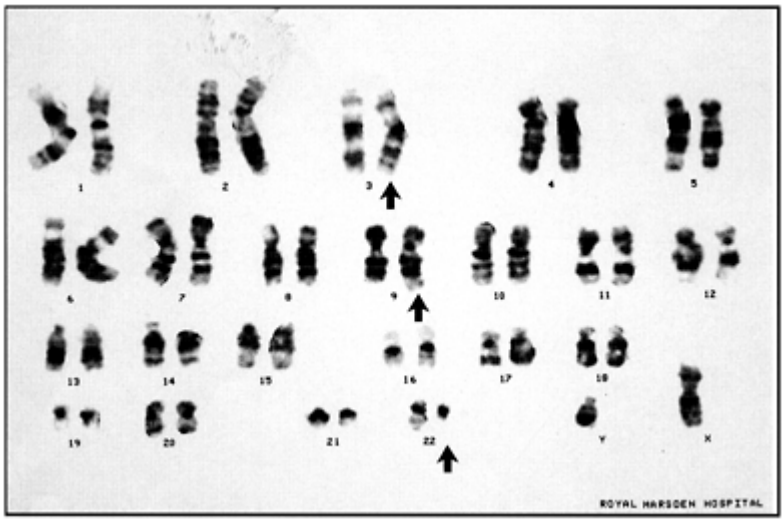

Figure 4.1 The Philadelphia translocation.

haemopoiesis in individual patients (reviewed in [1]), one may, for practical purposes, assume that a Ph-positive cell is part of the leukaemic population and that a Ph-negative cell is not. Ph-positive cells can be recognized by standard cytogenetic studies of metaphase preparations or by 'fluorescence *in situ* hybridization' of interphase cells using *BCR* and *ABL* probes [2],

There have been major advances in the last few years in our understanding of the molecular changes underlying CML. In 1973, Rowley showed that the Ph chromosome was due to a reciprocal translocation of genetic material involving chromosomes 9 and 22 [3] (Figure 4.1), and the precise position of the breakpoints was later shown to be consistent in different patients [t(9;22)(q34;q11)]. The generation of the Ph chromosome is associated with a break in the *BCR* gene on chromosome 22 and the translocation brings the *ABL* proto-oncogene, normally located on the long arm of chromosome 9, into juxtaposition with the 5′ portion of the *BCR* gene that remains in its normal location [4]. The result is a chimaeric gene, termed *BCR/ABL*, formed partly of *BCR* and partly of

ABL sequences (Figure 4.2). This gene is expressed in leukaemic cells as an 8.5-kb mRNA and a 210-kDa protein (p210BCR/ABL) with tyrosine kinase activity.

Because the breakpoint in the *BCR* gene occurs within a relatively small region in different patients with CML, rearrangement of the *BCR* gene (and by implication formation of a chimaeric *BCR/ABL* gene) can be detected by Southern blotting with a BCR probe. In practice, use of the polymerase chain reaction

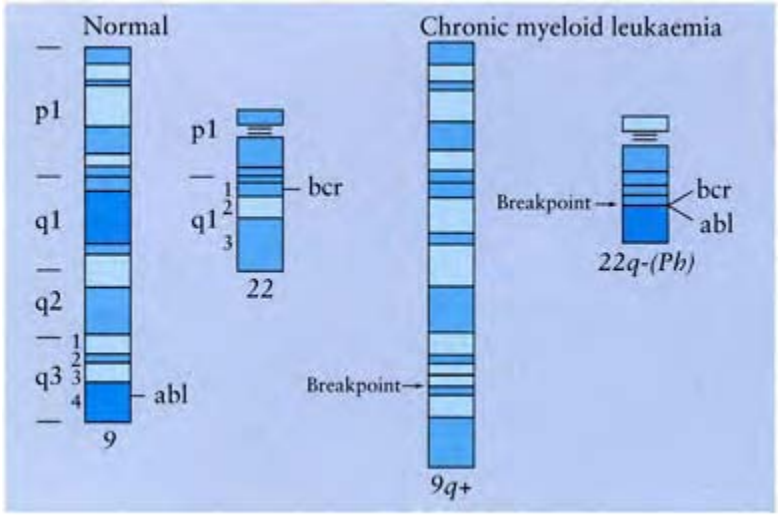

Figure 4.2 The BCR/ABL *gene*.

(PCR) is the best way of detecting cells with a *BCR/ABL* gene [5]. Ninety-eight percent of CML patients have one or other of two precisely defined mRNAs which can readily be reverse transcribed to cDNA and amplified by PCR [6]. Thus, reverse transcription PCR (RT-PCR) technology lends itself well to confirming the presence of the *BCR/ABL* gene in CML and to detecting minute quantities of leukaemia-specific transcript that may persist after treatment with interferon-α (IFN-α) or BMT. It may not readily reveal the lineage of origin of such 'minimal residual disease' and it could yield 'false-negative' results if leukaemic cells survive that did not express mRNA.

Atypical forms of CML

About 8% of patients with haematologically acceptable CML have leukaemia cells that are cytogenetically normal or show chromosomal changes other than a Ph chromosome [6,7]. About half these patients prove to have a *BCR/ABL* chimaeric gene with corresponding mRNA and p210BCR/ABL indistinguishable from Ph-positive CML. They can thus be regarded as examples of 'cytogenetically occult' but otherwise classical CML. A few such patients have been treated by allogeneic BMT and clinical results do not differ from results of transplanting Ph-positive CML. The remaining Ph-negative patients, who lack evidence of BCR gene involvement, probably have a prognosis poorer

than that of 'BCR/ABL-positive' CML and should therefore be considered for treatment by allografting if possible at the earliest opportunity.

Most patients with the rare chronic neutrophilic leukaemia (CNL) have cytogenetically normal cells; very rare patients with CNL have a Ph chromosome and a chimaeric *BCR/ABL* gene in their leukaemia cells; the breakpoint in the *BCR* gene is downstream of the breakpoint usually found in CML [8]. In these cases the *BCR/ABL* gene encodes a protein larger than that found in CML, namely p230BCR/ABL.

Juvenile chronic myeloid leukaemia (JCML) is a rare myeloproliferative disorder of children characterized by hepatosplenomegaly, polymorphic rashes and leukocytosis with increased levels of foetal haemoglobin [9]. The bone marrow is hypercellular but the basophilia, eosinophilia and Ph chromosome found in adult-type CML are all absent in jCML. A small number of patients with JCML have been treated by allogeneic BMT and this is the treatment of choice for this otherwise rapidly fatal disease [10].

About 20% of adults and 2–3% of children with acute lymphoblastic leukaemia (ALL) have a Ph chromosome, which is associated with a notoriously poor prognosis. In adults with Ph-positive ALL, the *BCR/ABL* chimaeric gene is usually identical to that found in CML but more than half the children with Ph-positive ALL have a smaller *BCR/ABL* gene; this is formed by juxtaposition of the first exon of the *BCR* gene with the bulk of the *ABL* gene [4]. The result is a smaller chimaeric gene that is expressed as a protein of 190-kDa (in contrast to the p210 of CML). The majority of patients do achieve complete remission with standard combinations of cytotoxic drugs, children more commonly than adults, but relapse occurs early and very few such patients become longterm disease-free survivors. Thus, patients with Ph-positive ALL should be considered for allogeneic BMT in first remission. Results with small numbers of patients suggest that the probability of cure is considerably higher than after treatment with chemotherapy alone [11,12].

Biological aspects of Ph-positive CML

Normal haemopoiesis in man is believed to depend on the presence in the marrow of a population of haemopoietic stem cells which are termed 'pluripotential' because they can apparently differentiate along either myeloid or lymphoid lineages. Several lines of evidence suggest that CML is the disease *par excellence* of the pluripotential stem cell. For example, the Ph chromosome is found mainly in the progeny of the myeloid progenitor cells in the marrow but also in cell of B- and occasionally of T-lineage. The finding that CML in transformation can manifest predominantly myeloid or lymphoid features, and the observation that the lymphoid features, although usually showing a pre-B phenotype, may on occasion be characteristic of the T-lineage, are consistent with the notion that the target cell for transformation is pluripotential (or is descended from a leukaemic cell with such potential) [13]. Moreover, study of leukaemic cells in females with CML who are heterozygous for glucose-6-phosphate dehydrogenase isoenzymes, as well as study of the distribution of apparently clonal chromosomal abnormalities in patients who are somatic mosaics provide further, albeit circumstantial, evidence that the disease was of clonal origin (at least in the cases studied) and therefore was presumably derived from a single pluripotential stem cell (reviewed in [1]).

The further evolution of CML that occurs after diagnosis presumably involves additional acquired molecular changes that occur in the 'chronic phase' stem cells.

However, the additional chromosomal changes that occur during chronic phase or as a prelude to disease transformation do not provide any useful clues as to the nature of the new molecular events. Transformation probably involves activation of one or more proto-oncogenes or inactivation of one or more tumour suppressor genes. To date, only *p53, p16, EVI-1* and *RB* have been implicated in disease progression [14,15,16] and none of these in a consistent manner. It is likely that the range of genes that may contribute to blastic transformation is relatively large. At the clinical level there is typically reduced responsiveness of the leukaemic clone to conventional treatment in association with increasing numbers of blast cells that must be genetically different from the blast cells present in uncomplicated chronic-phase disease.

This molecular and kinetic concept of the pathogenesis of CML has some relevance in the design of regimens for cytoreductive therapy before allogeneic BMT. It also provides a framework in which to consider the possibility that the duration of disease before allografting might influence the probability of survival after transplant (see below). Most importantly, it provides a partial explanation for the observation that although eradication of CML is much more difficult if the transplant is performed in accelerated or blastic phases, occasional patients transplanted in chronic phase relapse directly to blastic transformation without a preceding period of chronic-phase relapse [16].

Conventional treatment for Ph-positive CML

Radiotherapy was introduced as standard palliative therapy for CML in the first decade of this century. Busulphan was introduced in the 1950s and began to replace radiotherapy in the 1960s. In the last decade, many clinicians have recommended use of hydroxyurea rather than busulphan as primary treatment of CML, because hydroxyurea is easier to administer and lacks some of the serious and occasionally fatal side-effects of busulphan. Patients treated with hydroxyurea may survive somewhat longer than those treated with busulphan. However, IFN-α should now be the first choice for management of a newly diagnosed patient with CML.

IFN-α is a glycoprotein of biological origin with antiviral and antiproliferative actions. It was first used as treatment for CML in the early 1980s. It was shown to reduce the leukocyte count and induce haematological remission in the majority, but not in all, patients; of considerable interest was the observation that it could induce Ph-negative haemopoiesis in about 10% of patients. Subsequently, its efficacy was compared with cytotoxic drugs in a number of controlled studies [17,18]. For the present, it can be concluded that compared with hydroxyurea IFN-α prolongs survival by one to three years. Patients who achieve complete cytogenetic remission have the longest survival [19]. This is probably due to two distinct factors: first the fact that cytogenetic response to IFN-α may identify a subpopulation of CML patients who have a relatively non-aggressive disease and thus would survive longer than average with any treatment, and second the probability that IFN-α reduces the leukaemia stem-cell mass and thereby the chance of disease-transformation. It is likely that patients who do achieve haematological but not cytogenetic responses may also have their survival prolonged. Haematological non-responders may not derive appreciable benefit from IFN-α treatment (Table 4.1).

Table 4.1 Imterferon-α for chronic myeloid luekaemia

- Prolongs survival by one to three years. Those in complete cytogenetic remission have the longest survival.
- Cytogenetic response may identify patients with relatively non-aggressive disease who would survive longer than average with any treatment.
- Reduces leukaemia stem-cell mass and thereby chance of disease transformation.
- Patients who achieve haematological but not cytogenetic responses may also have prolonged survival.
- Haematological non-responders may not benefit from IFN-α treatment.

Allogeneic-bone marrow transplantation

Although the hazards of allogeneic BMT are still considerable, it is generally agreed that a youngish patient with CML who has an human leukocyte antigen (HLA)-identical sibling should be offered treatment by transplantation. The outstanding questions are: when to do the transplant, how to induce cytoreduction, how to prevent graft-versus-host disease (GvHD) and what to do for the patient who relapses after BMT? There are other questions: what should be the upper age for BMT for CML and how should the patient lacking an HLA-identical sibling be handled?

When to perform the allograft

The results of allogeneic BMT performed in transformation are generally poor [20]. This led to the recommendation that patients with HLA-identical sibling donors should be offered the option of treatment by BMT as soon as convenient after diagnosis (Figure 4.3). An analysis performed by the International Bone Marrow Transplant Registry (IBMTR) [21] showed that the probabilities of survival at four years for patients allografted in chronic phase, accelerated phase and blastic transformation were respectively 49%, 32% and 12%. The corresponding figures for leukaemia-free survival were 45%, 28% and 12%. In 1986, the Seattle transplant group reported that survival was significantly better for chronic-phase patients transplanted within one year of diagnosis than for patients still in chronic phase transplanted later [22] and the IBMTR subsequently showed a similar inverse relationship between duration of disease before BMT and probability of survival [23]. The difference is attributable more to differences in transplant-related mortality than to differences in relapse. Moreover, these statistics take no account of the survival of those patients in whom transformation supervened more than one year from diagnosis before transplant was undertaken, a population that might have biased the analysis in either direction. Thus, for the present, it seems reasonable to recommend to a newly diagnosed patient who has an HLA-identical

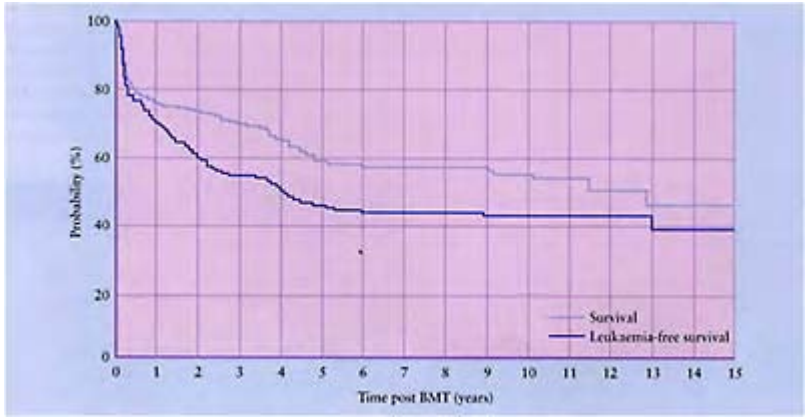

Figure 4.3 Survival and leukaemia-free survival after allogeneic BMT using matched sibling donors.

sibling that BMT should ideally be carried out within one year of diagnosis.

Cytoreductive regimens

It was believed for many years that the objectives of conditioning regimens preceding BMT were to induce immunosuppression and to eradicate every last remaining leukaemic stem cell. The recognition of the clinical relevance of a graft-versus-leukaemia (GvL) effect (see below) invalidates this belief. This said, most conditioning regimens continue to rely on a combination of high-dose chemotherapy and radiotherapy. The initial attempts to transplant CML were directed mainly at patients with advanced disease. The standard conditioning involved cyclophosphamide (usually 60 mg/kg daily×2) followed by total body irradiation (TBI), usually 10 Gy [20]. When attention was turned to allografting patients in chronic phase, the same protocols were adopted [22–25]. Thus, for example, the Seattle group has in general made use of cyclophosphamide and TBI, either single dose or fractionated with six daily 200 cGy fractions or seven daily 225 cGy fractions [22,26]. It is interesting to note that the low-dose TBI was associated with a 36% actuarial relapse rate at four years; the high-dose TBI was associated with no risk of relapse [26]. At the Hammersmith Hospital, 200 cGy fractions given twice daily for five or six doses have been used [25]. At the Sloan Kettering Cancer Center in New York, the standard protocol comprises cyclophosphamide with ‘hyperfractionated’ TBI—the radiotherapy is given as 120 cGy fractions three times daily to a total dose of 1320 or 1440 cGy [27]. It is worth noting that the major objection to use of shielding to protect the lungs above a given cumulative dose of radiotherapy, namely the risk that individual leukaemia cells in the ribs might thereby escape destruction, loses much of its force once one accepts the concept that GvL effects play an important role in leukaemia eradication. In general, a wide variety of different radiotherapy schedules is in use.

Radiotherapy is not an essential component of conditioning for CML patients. The Baltimore group accumulated extensive experience with the use of busulphan (usually 4 mg/kg daily×4 days) followed by cyclophosphamide (usually 60 mg/kg daily×4 days) (BU/CY) [28,29]. The Fred Hutchinson Research Center compared BU/CY with cyclo/TBI in a randomized prospective study in patients undergoing BMT for CML. There was no significant difference in leukaemia-free survival in the two arms [30]. Although BU/CY may be unduly toxic for patients who have already received substantial amounts of chemotherapy before BMT, it is certainly an effective schedule and deserves further study. Table 4.2 summarizes some points regarding conditioning therapy.

Graft-versus-leukaemia effect

There is experimental evidence from animal model systems that GvHD may exert an antitumour or antileukaemia effect [31], a so-called 'GvL effect'. In the clinical setting, patients allografted for acute leukaemia who sustain GvHD may have a lower probability of relapse than those without GvHD [32,33]. It is assumed that T-lymphocytes in the donor marrow are capable of mediating GvHD on the one hand and GvL on the other [34,35]. Whether the same sub-populations of T lymphocytes mediate the two diverse effects is unknown. A similar trend, a lower incidence of relapse in those with GvHD, is seen in patients allografted for CML in chronic phase with marrow from HLA-identical sibling donors but the overall relapse rate is low and the trend is not significant [36].

The concept that a GvL effect plays an important role in the cure of leukaemia after allografting for CML in chronic phase gains further support from the results of T-cell-depleted transplants. There are now a number of different studies in which patients in chronic phase were transplanted with donor marrow cells that had been depleted *in vitro* of T-lymphocytes by different methods. In every study, the probability of relapse in patients

Table 4.2 Conditioning for BMT in CML

- Radiotherapy not essential.
- Busulphan (4 mg/kg daily×4 days) followed by cyclophosphamide (60 mg/kg daily×4 days) (BU/CY) used by Baltimore group.
- The EBMT Group compared BU/CY with cyclo/TBI in a randomized study and found no significant difference in leukaemia-free survival in the two arms.

receiving T-depleted marrow cells was higher, usually very significantly so, than in comparable patients who received unmanipulated marrow cells [37–40].

Using published clinical data, it is possible to some extent to identify the antileukaemic effects of GvL operating independently of the antileukaemic effects of GvHD. Thus, for example, if one estimates that the actuarial probability of relapse for patients in chronic phase allografted with unmanipulated marrow is 10%, for patients allografted with syngeneic marrow 30% and for patients allografted with T-depleted marrow 50%, the contribution towards cure of the T-cell component must be 40%, of which the non-syngeneic component is 15%. This can be broken down further if one accepts that there is a difference in probability of relapse in recipients of unmanipulated

marrow cells according to whether the patient does or does not sustain GvHD, e.g. 5 versus 15%. In this example, the difference between the relapse rates in these two patient categories (10%) must be computed as the antileukaemic contribution of GvHD and the difference between the relapse rates for syngeneic transplants and that for non-GvHD allogeneic transplants (25−15=10%) may be computed as the antileukaemic contribution of the GvL effect.

The mechanisms by which T-lymphocytes exert a GvL effect remain highly speculative [35,41]. It is possible that they release cytokines, such as interleukin-2, interferon-α or transforming growth factor-β, that selectively suppress the proliferation of Ph-positive cells. It is possible that T-cell or natural killer cells act directly against leukaemia cells. A third possibility is that CML cells express leukaemia-specific antigens, possibly coded by the *BCR/ABL* chimaeric gene, that provoke a true leukaemia-specific T-cell response.

Are some patients really cured?

The rationale for advising patients with CML to undergo allogeneic bone-marrow transplantation is based on the belief that the probability of cure is high if they survive the transplant procedure. This seems in practice to be true, but longer follow-up of treated patients is still required before one can be certain. The evidence in favour of cure derives from three interrelated sources.

Evidence from survival curves

Transplant centres worldwide began routinely to perform allogeneic BMT for patients with CML in chronic phase in the early 1980s. This means that an appreciable proportion of survivors have now been at risk of relapse for ten to fifteen years. It appears that patients destined to relapse usually do so within three years of diagnosis and relapse later is very rare. This means that a Kaplan-Meier plot of survival or disease-free survival tends to flatten out by three years [22,25]—an observation that supports, but by no means proves, the reality of cure.

Transient cytogenetic relapses

A small number of patients has now been reported in whom Ph-positive metaphases were identified in the bone marrow from 6 to 24 months after allografting, but in whom subsequent cytogenetic studies were entirely normal [41–44]. Some authors have termed this sequence of events ‘transient cytogenetic relapse’. The mechanism underlying this sequence is obscure. It is, however, consistent with the concept of an ongoing GvL effect, which presumably continues to operate for months or years after the transplant. It may be speculated that this GvL effect could remain operative for the life of the patient and that cure is, in effect, no more than permanent suppression (but not eradication) of leukaemic stem cells.

Evidence from RT-PCR studies

Perhaps the best evidence favouring leukaemia eradication as evidence for cure derives from studies using the RT-PCR (see above). The majority of patients in complete cytogenetic and haematological remission after BMT with marrow depleted of T-cells remain positive when studied by RT-PCR for months or years after transplant [45]. This correlates with their known increased risk of relapse. In contrast, CML patients allografted in chronic phase with unmanipulated marrow cells may be positive when studied by PCR for the first six or nine months post-BMT but are usually negative thereafter [46,47]. These observations can be interpreted as reflecting residual leukaemia cells that diminish in number during the months post-transplant.

Relapse after allogeneic BMT

The actuarial probability of relapse after allogeneic BMT performed in advanced phases of CML is 30–60%. The corresponding figure for patients allografted in chronic phase with unmanipulated allogeneic marrow is 10–20% (Table 4.3). It is likely

Table 4.3 Relapse after allogeneic BMT

• The actuarial probability of relapse after allogeneic BMT performed in advanced phases of CML is 30 to 60%.
• The corresponding figure for patients allografted in chronic phase with unmanipulated allogeneic marrow is 10–20%.

that the details of the conditioning regimen influence the probability of relapse, but no factors intrinsic to the patient or the disease appear to be significant. Relapse, when it occurs, seems to proceed in an orderly manner. It may be detected first by the use of quantitative RT-PCR which measures approximately the numbers of *BCR/ABL* transcripts in the blood or marrow. Thus, a patient whose RT-PCR studies were previously negative may on serial monitoring show steadily rising *BCR-ABL* transcript numbers [48]. Thereafter, Ph-positive metaphases may be found in the marrow. The marrow at this stage may be unduly cellular in comparison with other transplanted patients. Only when the marrow has become 100% Ph-positive will the leukocyte count begin to rise. It is of interest that the relapse is usually restricted to cells of the myeloid series; the lymphoid system at relapse is still predominantly or totally of donor origin [49]. The use of T-cell depletion greatly increases the risk of relapse, but to different degrees according to the method of T-cell depletion. Thus, counterflow elutriation, which removes lymphoid cells from donor marrow by physical means, is associated with a smaller increased risk of relapse than is T-cell depletion with the pan-lymphoid monoclonal antibody Campath-1M [50] Table 4.4 summarizes approaches which have been employed for T-cell depletion.

Occasional patients have been reported who were transplanted in chronic phase and who subsequently relapsed in blastic transformation [16]. This observation is not easily explained. It is possible that such patients relapsed first into a chronic phase that escaped detection and transformation then supervened. Alternatively, the transformed leukaemic

stem cell may have been present in the patient's marrow at the time of transplant and remained latent for some months or

Table 4.4 Techniques for T-cell depletion

Incubation with monoclonal antibodies
Anti-lymphoid Campath series antibodies: anti-T-cell, CD3, CD8, T10/B9
Soy bean lectin agglutination and E-rosette formation
Counterflow elutriation

years before manifesting its aggressive proliferative potential. The most intriguing and, in some ways, worrying interpretation is the hypothesis that leukaemic stem cells survive the allograft procedure in the majority of (or all) patients, and that such stem cells remain susceptible to transformation for the life of the patient. The relative paucity of relapses more than four years from allografting [51], and the PCR findings cited above to some extent militate against this last interpretation.

Relapse in cells of donor origin

Two patients have been described in some detail in whom relapse appears to have occurred in cells of donor origin [52,53]. In both cases, the donor was of the opposite sex from the patient and cells at relapse showed both the Ph-chromosome and the sex complement of the donor. It may be relevant to note that both patients had been in blast-cell transformation before transplantation and both relapsed to transformation. These cases could thus be examples of '*in vivo* transfection' of donor stem cells with DNA sequences causing transformed leukaemia [54]. It is, however, difficult to envisage such a complicated mechanism, since most workers currently believe that the oncogene changes associated with the Philadelphia chromosome are distinct from those that underlie progression to transformation (see above).

Treatment of relapse after BMT

The management of patients who relapse is not straightforward. If they relapse in myeloid transformation, no special measures are likely to be effective. Relapse to lymphoid transformation may be treated as conventional lymphoid transformation, and the patient may be expected to be restored to complete remission in some cases. Various approaches can be considered for patients who relapse in chronic phase. Some patients have been subjected to second transplant procedures with variable results [55]. Occasional patients have been treated successfully with IFN-α [56], but this agent does not usually restore Phnegativity. Symptoms can be prevented by standard chemotherapy with hydroxyurea, but this drug probably does not delay progression of the disease and patients must be at risk of transformation.

The best method of treating patients who relapse to chronic-phase disease is probably transfusion of T-lymphocytes collected from the original donor by leukapheresis [57–59]. About 70% of patients so treated achieve complete haematological remission which can

usually be confirmed at the molecular level [60]. In almost all cases these remissions have proved durable. The technique however carries the risk of two complications: marrow aplasia and GvHD. A minority of patients who receive donor lymphocyte transfusions develop pancytopenia and marrow hypoplasia which may resolve spontaneously but can if inadequately treated prove fatal (Table 4.5). The risk seems to be smaller in patients treated in molecular or cytogenetic relapse before the onset of haematological relapse. This complication can be treated by transfusion of additional marrow- or blood-derived donor stem cells. Some patients develop GvHD after DLT, which may be severe. The risk of this complication may be minimized by repeated transfusions of low numbers of donor cells at intervals of weeks or months on an escalating schedule [61].

Use of donors other than HLA-identical siblings

Because CML can be cured only by BMT, and because the transplant can, if necessary, be delayed for weeks or months after diagnosis, CML is the ideal disease in which to consider a transplant using a closely HLA-matched relative of the donor or a matched unrelated volunteer donor, since donors in both categories may take some time to identify. The Seattle group used five-antigen-matched related donors for transplanting patients with acute leukaemia in first remission. They reported survival comparable to use of genetically HLA-identical siblings [62], but the intensity and severity of GvHD are undoubtedly greater in the former case. Nonetheless, BMT using such a donor can

Table 4.5 Relapse after BMT

• Transfusion of T-lymphocytes collected from the original donor by leukapheresis effects complete haematological remission in about 70% of patients who relapse into chronic phase.
• Problems may include severe GvHD and marrow hypoplasia or aplasia.

be considered for younger patients with CML in chronic phase.

In recent years a relatively large number of patients with CML in chronic phase have been treated by allogeneic BMT using ‘matched’ unrelated or volunteer donors to transplant patients with CML [63–66]. The general approach has been to perform class I typing and if possible also class II typing on a relatively large number of volunteers, and to enter the results on a computer database. When a candidate patient is identified, the database is searched for possible matches and the individuals are then summoned for confirmation of HLA typing by molecular methods. The procedure is laborious and time consuming and it would undoubtedly be more efficient if donor panels comprised appropriate numbers of volunteers fully typed by molecular methods.

Because increasing sophistication of tissue typing may be useful in reducing the risk of graft failure or GvHD but has the obvious disadvantage of reducing the number of ‘perfect’ matches, attention has focused recently on the development of *in vitro* tests that may predict GvHD. The mixed lymphocyte reaction has little value in this regard. In contrast, assay of the frequency of alloreactive cytotoxic T-lymphocyte precursors in the peripheral blood of the donor does correlate with the severity of GvHD and may be used as a basis for selecting donors [67] (Table 4.6). It seems possible that further exploitation

of this technology could help to identify suitable donors who are to some degree histoincompatible by standard tests.

The clinical results of BMT using 'matched' unrelated donors are still inferior to results of sibling donor transplants (Figure 4.4). Thus, in general, at two years the actuarial survival is about 50%, leukaemia-free survival about 40% and relapse between 5 and

Table 4.6 Tests that may predict GvHD when using an unrelated donor

• The mixed lymphocyte reaction has little value.
• Assay of the frequency of alloreactive cytotoxic T-lymphocyte precursors in the peripheral blood of the donor correlates with the severity of GvHD and may be used as a basis for selecting donors.

20% [68]. The variable relapse rate reflects the different techniques employed for the prevention of GvHD. Thus, if only cyclosporin A and methotrexate are used post-transplant, the relapse rate is less than 5% but the incidence of acute and chronic GvHD relatively high; conversely in centres where *in vivo* or *ex vivo* T-cell depletion of donor marrow is employed, the incidence and severity of acute and chronic GvHD are reduced but the relapse rate at two years may approach or even exceed 20%. This incidence of relapse may be acceptable if the patients can be monitored closely post-BMT by cytogenetic and molecular methods and early relapse is treated successfully with DLT.

The probability of success following BMT using an unrelated donor seems to depend on a number of interrelated factors, as listed below.

- Phase of disease. As with transplants using matched sibling donors, the probability of survival is higher and the probability of relapse is lower if the transplant is performed in chronic phase than in advanced phases of CML.
- Duration of chronic-phase disease. The probability of survival is greater if the transplant is performed within one year of diagnosis than if it is performed later.
- Degree of histocompatibility between donor and recipient. Based on results of serological matching for HLA A, B and DR, the results of transplant using unrelated donors are better if the donor and recipient are 'identical'. Molecular matching for DRB1 also seems to improve results. The value of matching for DQ and DP is not yet established.
- Age of the patient. The favourable influence of young age seems to be more prominent in recipients of unrelated than of sibling transplants.
- Age of the donor. The results of transplant using younger donors seem to be superior to those using older donors. One patient was recently transplanted successfully for CML in transformation using cord blood stem cells [69].
- Cytomegalovirus (CMV) serostatus of the patient. Patients who are seronegative for CMV have a lower transplant-related mortality than CMV-seropositive patients.

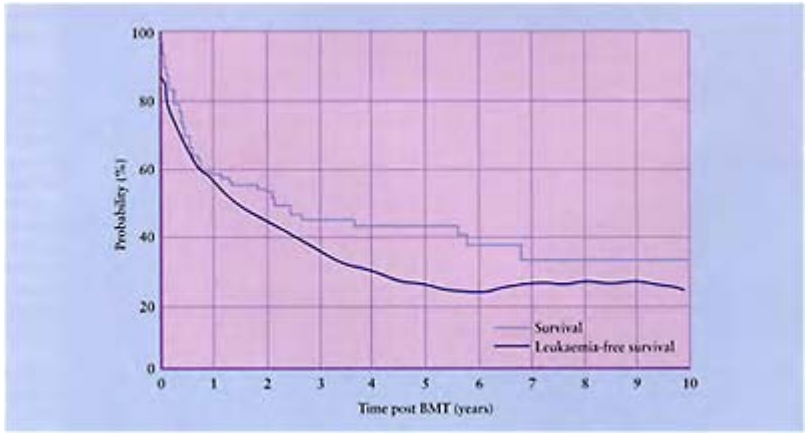

Figure 4.4 Survival and leukaemia-free survival after allogeneic BMT using 'matched' unrelated donors.

Autografting for CML

It is now clear that both marrow and peripheral blood of patients with untreated CML contain stem cells capable of reconstituting haemopoiesis after myeloablative chemotherapy or chemoradiotherapy. In the 1970s, a number of investigators addressed the possibility that such stem cells could be collected and cryopreserved for use as part of the treatment of patients in transformation [70,71]. More recently, investigators have begun to test the possibility that the duration of chronic phase and consequently of survival might be prolonged by administration of high-dose chemotherapy and autografting carried out soon after diagnosis, or at any rate before the onset of transformation [72]. Such an approach might be effective by reducing the size of the stem-cell pool susceptible to transformation; an unexpected result has been the establishment of durable Ph-negative haemopoiesis in some patients. Moreover, CML in chronic phase might be considered the disease *par excellence* in which to test the value of 'purging' the autograft if suitable techniques can be developed.

A number of groups have reported results of treating patients with CML in blast cell transformation with high-dose chemotherapy or chemoradiotherapy followed by transfusion of stem cells collected and cryopreserved earlier in the course of the disease. In general, such patients have shown rapid and predictable engraftment with restoration of haemopoiesis typical of chronic-phase disease. The median time for recrudescence of blast cell transformation is about three months and the median survival about six months [70]. It may be concluded, therefore, that the clinical benefit for patients treated in this manner is only modest. The clinical results may, however, be improved to some extent by repeating the autograft while patients remain in second chronic phase [73], or by

autografting with Ph-negative cells collected during the recovery phase after intensive chemotherapy [74].

Of potentially greater interest is the possibility of autografting patients in chronic phase. The Hammersmith group reported results of autografting 23 patients with blood-derived stem cells on one or more occasions [75]. Survival in these autografted patients was superior to survival of comparable patients treated by conventional methods, but the study was not prospective or randomized. It was notable, however, that after autografting some patients recovered haemopoieisis that was partially Ph-negative, and Ph-negativity in one unique patient has persisted without further treatment for more than ten years [76]. The tantalizing observation in this patient suggests that the autograft procedure *per se* altered the balance between Ph-positive and Ph-negative stem cells in a manner that reversed the proliferative advantage conventionally attributed to the Ph-positive population [77]. There are, of course, other possible interpretations.

Much attention has focused recently on the possibility of collecting autologous progenitor cells for the autograft procedure that are predominantly Ph-negative. There are two possible approaches: *in vivo* or *in vitro* manipulation. The *in vivo* approach has been pioneered by groups in Uppsala and Genoa. The Uppsala group has treated a series of newly diagnosed patients with sequential courses of IFN-α and combinations of cytotoxic drugs [78]. When Ph-negativity is achieved, marrow cells are harvested and cryopreserved. Subsequently patients are subjected to a formal autograft procedure. The Genoa approach is somewhat more complicated [74]. Patients with CML in chronic phase receive a combination of cytotoxic drugs in high doses, comprising idarubicin, cytosine arabinoside and etoposide (ICE). During the recovery period, nucleated cells are harvested from the peripheral blood by leukapheresis. In some cases such cells are predominantly or entirely Ph-negative. These are then cryopreserved and used at a later date for the autograft procedure. Using both approaches, preliminary results suggest that autografting with Ph-negative cells may induce prolonged periods of Ph-negative haemopoiesis and may be associated with prolonged survival. The true value of these approaches must be tested in controlled studies. Table 4.7 summarizes approaches used for *in vivo* manipulation to collect Ph-negative cells.

Table 4.7 Techniques used for in vivo *manipulation to favour collection of Ph-negative cells: initial treatment of the patient*

Interferon-α
Cytotoxic drugs alone or in combination: idarubicin, cytarabine, etoposide
Hydroxyurea
Cyclophosphamide

The use of *in vitro* techniques is based on the notion that the blood or marrow of newly diagnosed patients comprises a mixture of Ph-positive and Ph-negative 'stem' or progenitor cells, even though this is difficult to demonstrate with conventional assays of committed progenitor cells. Thus, treating the blood or marrow *in vitro* with any technique that inhibits survival of Ph-positive progenitors should give surviving Ph-

negative progenitors a further proliferative advantage after autografting. The Vancouver group pioneered in this area [79]. They incubated marrow cells in liquid culture for a ten-day period during which numbers of Ph-positive cells declined to a greater extent than Ph-negative cells, and they then used the surviving cells for autografting. Durable Ph-negativity was achieved in some patients.

In other studies, patients have been subjected to autograft procedures after blood or marrow cells were

Table 4.8 Techniques used for in vitro *manipulation to favour collection of ph-negative cells. Agents for incubation of blood- or marrow-derived stem cells* in vitro

Liquid culture for 10 days
Cytotoxic drugs
Monoclonal antibodies
Antisense oligodeoxynucleotides
Tyrosine kinase inhibitors

incubated *in vitro* with cytotoxic drugs or antisense oligodeoxynucleotides. At present, it cannot be safely concluded that any of the patients treated in these pilot studies have achieved clinical benefit. Currently, perhaps the most promising approach to *in vitro* 'purging' of CML blood or marrow involves the use of specific tyrosine kinase inhibitors [80]. Table 4.8 summarizes approaches used for *in vitro* manipulation to collect Ph-negative cells.

An integrated approach to the management of CML

The introduction in the last decade of interferon-α and bone-marrow transplantation for CML has greatly complicated the approach to management of the newly diagnosed patient. It is no longer possible merely to treat a new patient with busulphan and to await the inevitable transformation with equanimity. Instead, the relative merits of hydroxyurea, IFN-α, allografting and autografting must be discussed with the patient at the time of diagnosis, and the clinician must be prepared to deal with situations in which the optimal approach cannot be defined and in which what seemed at one stage to be reasonable advice appears later to be less promising.

One possible integrated approach to management is based on the assumption that all patients under the age of 60 years who have HLA-identical siblings should be offered the chance of BMT while still in chronic phase. However, the risk of mortality for the older patient (i.e. aged 40–60 years) is such that a short trial of IFN-α is warranted before resorting to BMT. In the younger patient (under 40 years), no such trial of IFN-α is necessary.

For the present, BMT using matched volunteer donors must still be regarded as experimental, but for very young patients (aged under 20 years) with a well-matched unrelated donor, a transplant soon after diagnosis is a reasonable option. For patients aged between 20 and 45 years, an initial trial of IFN-α is warranted. For the present, patients aged older than 45 or 50 years lacking HLA-identical sibling donors cannot safely be treated by BMT. Figure 4.5 suggests the treatment approaches which can be followed in a patient newly diagnosed with CML.

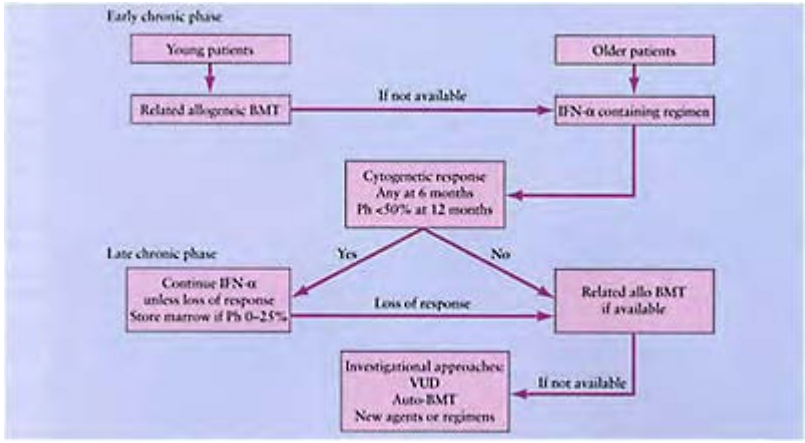

Figure 4.5 Treatment for a patient newly diagnosed with chronic myeloid leukaemia. Auto-BMT=autologous bone marrow transplantation; VUD=Volunteer unrelated donor.

Future prospects

Although it seems reasonably well established that the majority of patients transplanted in chronic phase who survive the transplant will be cured, little progress has been made in recent years in increasing the safety of the procedure. GvHD remains a major and sometimes lethal problem and attempts to prevent it have been associated with a significantly increased risk of relapse. It is probable that more selective removal of cells that mediate GvHD will prove possible but it remains to be seen whether any of the modifications now under study (e.g. selective removal of CD8 cells) will reduce the risk of relapse. Conversely, there is no evidence that any improvement in the prevention of GvHD by pharmacological methods is imminent. It is likely that further progress in prevention of GvHD will await better understanding of its mechanism of induction; such progress could, for example, be based on attempts to induce specific tolerance to donor transplantation antigens, as has been achieved by reconstituting mice with mixtures of T-cell-depleted syngeneic cells and allogeneic marrow cells.

The possibility of increasing the proportion of patients who can be treated by allogeneic BMT looks promising. Although the average size of individual families is not likely to increase in the foreseeable future, it is likely that techniques will be perfected that permit volunteer donors who are not completely HLA identical with the patient to be used with impunity. At the same time, a major challenge will be to minimize the risk of transplant-related complications and mortality for patients in the age range 50–65 years.

The possibility of curing CML by autografting deserves mention although it still seems remote. It does, however, seem likely that methods can be perfected that will permit collection of progenitor cells that are predominantly Ph-negative, and that use of such cells for autografting may in the future significantly prolong survival.

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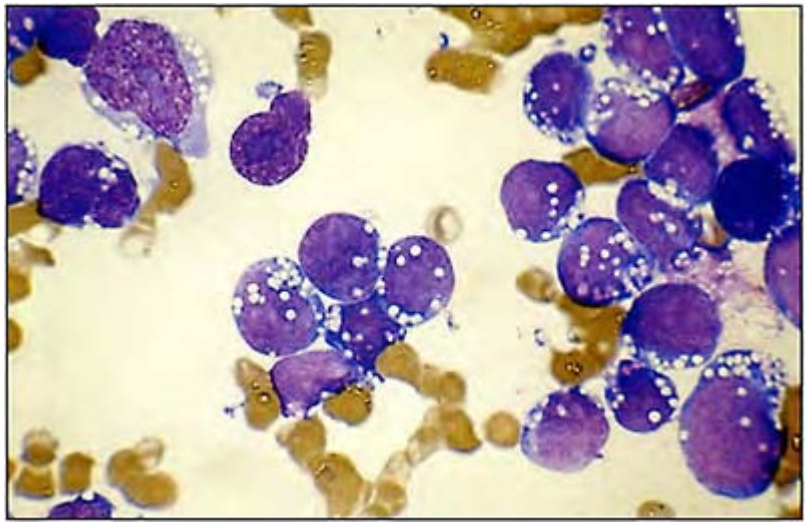

Chapter 5
Adult acute lymphoblastic luekaemia

John Barrett

Introduction

Differences between childhood and adult ALL

Adult acute lymphoblastic leukaemia (ALL) differs from childhood ALL both in the underlying nature of the leukaemia and in its response to treatment. Poor prognosis disease and increased susceptibility to harmful effects of treatment combine to make the outcome for ALL in adults worse than that for ALL in childhood (for a review, see [1]).

ALL subtypes

The subtypes of ALL found in childhood and adults differ significantly. The childhood ‘common’ pre-B phenotype, associated with a high chance of cure with low-intensity

chemotherapy, reaches a peak incidence between the ages of 3 and 11 years, thereafter declining progressively to less than 5% of cases in individuals over 20 years [2,3]. Conversely, the very poor prognosis ALL, associated with the Ph^+ chromosome, occurs in less than 5% of children but has a rising incidence with increasing age, accounting for 40% of all adult B-ALL [4,5]. Other karyotypes associated with a low cure rate, such as the t(4;11), seen in infants and occasionally in younger children, occurs more frequently in adults. Similarly, the poor prognosis ALL with myeloid markers is found in the rare cases of infants with ALL, but with increased frequency in adults [6]. Recently, it has been observed that ALLs expressing the multi-drug resistance (MDR) gene occur more commonly in adults [7]. Apart from a small increase in the frequency for young adults developing B-ALL, T-cell and B-cell ALL do not have a marked predilection for any particular age group [8]. Figure 5.1 illustrates the relative frequencies of the ALL subtypes in different age groups.

Tolerance to treatment

Involvement of the central nervous system with leukaemia is less common in adults [9]. However, adults treated for central nervous system (CNS) involvement with leukaemia tolerate cranial irradiation less well than children. Adult ALL patients tolerate chemotherapy less well than children, and overall take longer to enter remission than children [8]. Cytopenias are more severe and prolonged, possibly because of a lower capacity of adult haematopoietic stem cells to regenerate. With advancing age, hepatic,

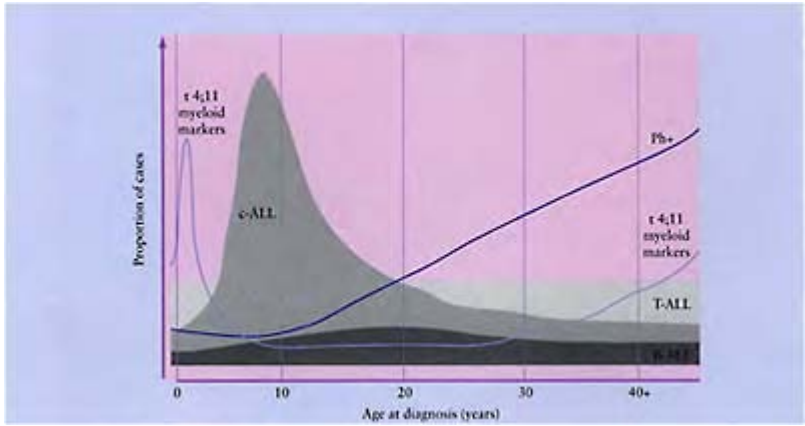

Figure 5.1 Frequency of ALL subtypes with age (not to scale).

renal and cardiotoxicity become more common, and overall regimen-related mortality, whether from chemotherapy or transplant, increases with age, especially beyond the age of 20 years. Neurological sequelae from vincristine are more severe and prolonged in adults, and in elderly patients vincristine can induce permanent neuropathy. Cytosine arabinoside (Ara-C) in high doses can cause cerebellar damage with increasing frequency

with age. In contrast, adults have less toxicity from methotrexate because they metabolize it more efficiently than do children [10].

Adult ALL subtypes and prognostic features

In general, the prognostic features described in childhood ALL also apply to adults. The topic is well reviewed in by Copelan and McGuire [1] and by Hoelzer [11]. Features indicating a poor prognosis are a high presenting leukocyte count (greater than 50×10^9/litre), presence of a mediastinal mass and CNS leukaemia. A study of the morphology, surface marker phenotype and karyotype is essential for a complete assessment of prognosis. Subtypes in adults associated with a better than average response to initial treatment and survival are true common ALL ($CD10^+$, $CD19^+$, cytoplasmic Ig^+), which is less common in adults, ALL L3 with B-ALL markers ($SmIg^+$ $CD19^+$), and T-ALL ($CD2^+$, $CD3^+$, $CD7^+$ TdT^+). These latter subtypes, previously considered to indicate a dismal prognosis in adults, have in recent years become better managed with intensive cyclical chemotherapy: In T-cell ALL, for example, Linker *et al.* [12] reported remission rates of 95% and leukaemia-free survivals (LFS) of 59%. Similarly, the outlook for B-cell ALL in young adults has changed from being an incurable condition, to having a relatively favourable outcome with a 75% chance of attaining remission and a 49% disease-free survival (DFS) [13].

It is important to recognize certain very poor prognosis features conferring a much lower than average durable response to initial therapy. The presence of chromosome translocations t(9;22), t(4;11), t(19;–), and t(11;–) confer a very poor prognosis [14,15]. Although remissions can be induced in about 70% of cases, the commonest translocation t(9;22) (Ph^+ ALL) is essentially incurable by chemotherapy alone, with survivals of less than 10% [5,16]. Finally the presence of myeloid markers (CD33 or CD34) is an independent prognostic feature found in some pre-B, B- and T-ALL in about 20% of adult ALLs. Although some data are contradictory, the presence of a myeloid marker in adult ALL is often associated with a poor prognosis [6]. It should be appreciated that low-risk characteristics in adult ALL are the exception. Unfortunately, most adults wth ALL have high-risk or very high-risk disease. After treatment starts, new treatment-related prognostic factors emerge: a poor outcome is seen in patients taking more than 5–6 weeks to achieve remission, patients failing to enter remission, or relapsing rapidly—especially within a year from diagnosis.

Prognostic implications for bone-marrow transplantation (BMT)

In general, the prognostic features intrinsic to the disease, described above, apply to chemotherapy alone. After BMT, the disease type plays a much smaller role in relapse and survival outcome (see below).

Treatment of adult ALL

The formula of remission induction, consolidation and maintenance, so successfully applied to childhood ALL, has been found to be far less effective for adults with ALL. Progress in recent years has come largely from the efforts of the BFM (Berlin, Frankfurt and Münster) Cooperative Group, who have reshaped adult ALL treatment into two stages: remission induction followed by intensive post-induction therapy [17]. Post-induction therapy takes the form of repeated cycles of high-dose combination chemotherapy, or consolidation, followed by allogeneic or autologous BMT. Whether myeloablation and transplant or less intensive chemotherapy is used, the strategy is similar: namely, to deliver high-dose therapy as soon as possible after remission induction and over a period of a few months to abolish residual disease. There is no proven place for maintenance therapy in adult ALL [1]. Current debate revolves around the best choice of post-remission therapy and the treatment most likely to achieve a cure for individual risk categories of adult ALL.

CNS prophylaxis

Initially, the prophylaxis of CNS leukaemic meningitis was modelled on the paediatric plan of cranial irradiation in conjunction with six intrathecal methotrexate treatments. Because adults tolerate cranial irradiation poorly and the risk from CNS leukaemia is lower than in children, some schedules use combination intrathecal treatment with methotrexate, Ara-C and dexamethasone without radiation. Because both high-dose methotrexate and Ara-C achieve high CNS concentrations, they are used both as post-remission intensification and as CNS prophylaxis. There have been too few comparative studies to determine which approach is best [17].

Recent results of chemotherapy treatment

About 70–90% of adults with ALL achieve a remission with modern chemotherapy regimens. From recent published series it is estimated that up to 40% of patients entering remission will be cured by chemotherapy [11,12,18–22]. Patients with good prognostic features (which include low presenting leukocyte count, T-ALL, childhood-type ALL and younger age) have a better outcome [1]. Some caution should be exercized in evaluating data from published ALL trials: the DFS data usually apply to patients already in remission; sometimes high-risk subtypes (such as Ph^+ ALL); and older patients are excluded from the series. Overall therefore, with a 20% failure to enter remission and further exclusions from study, it is more realistic to assume that only 20% of adult patients presenting with ALL can be cured by standard therapy approaches, despite the general sense of optimism that the treatment of adult ALL is improving [23]. Table 5.1 summarizes results of large chemotherapy-based treatments in adult ALL published since 1989. LFS ranges between 20% and 42%. Differences in results are largely related to the selection criteria used.

Remission induction

Remission is induced with a combination of vincristine, prednisolone and an anthracycline with or without asparaginase. Additional agents do not improve outcome as more patients die from regimenrelated causes [22,23]. Improved outcomes may be achieved with more intensive therapy in association with haematopoietic growth factors [24–26]. Post-induction high-dose chemotherapy, aimed at overcoming drug resistance has contributed to the improved outlook for adults with ALL. High-dose Ara-C, methotrexate, cyclophosphamide, etoposide and anthracyclines are used in three or four cycles in different combinations, together with further vincristine and prednisolone. Repeated cycles of intensive therapy carry a risk of mortality even in remission patients. The paucity of randomized trials makes it impossible to identify any particular schedule as superior. However, with modern treatment, 70–90% of adults with ALL can be expected to achieve a remission.

Table 5.1 Recent results of chemotherapy in adult ALL

Centre	Patients *(n)*	Complete remission rate (%)	Leukaemia-free survival (%)	Median (years)
Single centre				
San Francisco (UCAL) [12]	109	88	42	5
MD Anderson [9]	105	90	32	3
Multiple centre				
MRC UK ALL IX [19]	266	87	22	8
SWOG [21]	168	68	21	3
GIMEMA Italy [22]	358	79	25	6
French Adult ALL Cooperative Group [20]	572	78	32	3
GMATT [11]	182	83	46	5

Allogeneic bone-marrow transplantation

Bone-marrow transplantation was first used as a form of intensive salvage treatment for patients with relapsed or resistant disease. Inevitably, the early results from this approach were not encouraging: Thomas *et al.* [27] reported that out of 100 acute leukaemia patients, 11 (six with ALL) survived long-term following allogeneic BMT. The results indicated that some patients with otherwise incurable ALL could achieve long-term

disease-free survival and be cured of their disease. Subsequently, allogeneic BMT has been used to treat adult ALL, the patients usually being in first or second remission. The curative effect of allogeneic BMT derives partly from the antileukaemic effect of myeloablative therapy and partly from a graft-versus-leukaemia (GvL) effect—an alloimmune response of donor immune cells to the recipient's residual leukaemia [28]. However, the GvL effect in ALL may be less powerful than in other haematological malignancies and has only been demonstrated in conjunction with graft-versus-host disease (GvHD). Perhaps because of the relatively weak GvL effect, relapse rates after allogeneic BMT are higher than in comparably staged patients with myeloid leukaemias [29].

BMT for ALL in first remission

Patients are usually selected for transplantation after one or more consolidation treatments and after some form of CNS prophylaxis has been given. Earlier analysis from large numbers of patients reported to registry data bases found a LFS of about 45–50% [30]. Since these analyses, over 1000 adults with ALL have received BMTs from human leukocyte antigen (HLA)-matched siblings worldwide, and there is good evidence that results of BMT have improved. For example, recent data from the European Bone Marrow Transplant Group (EBMT) support a LFS in the order of 55% as being a realistic figure [31]: over the last decade, while relapse rates have remained constant (in the region of 25%), treatment-related mortality fell from 48±12% to 23±6%. Figure 5.2 illustrates the improvement in outcome after allogeneic BMT for ALL derived from multicentre data from Europe.

Similar improvement in transplant outcome comes from analyses by the International Bone Marrow Transplant Registry (IBMTR): between 1989 and 1995, LFS has risen from 45% to 54% for adults and

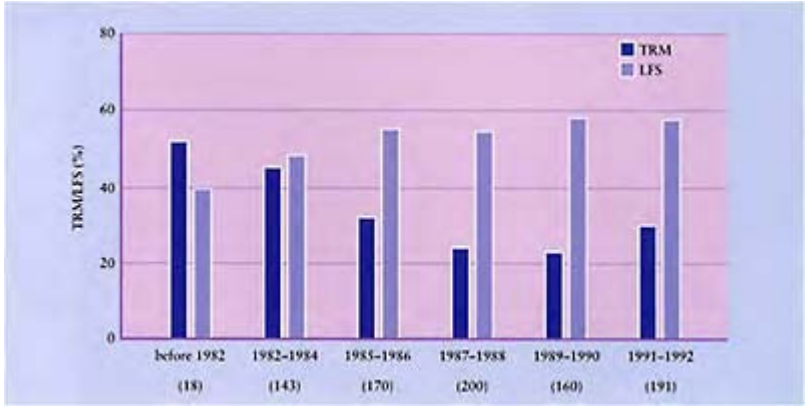

Figure 5.2 EBMT results for ALL BMT [31] (figures in brackets indicate number of patients).

children with ALL transplanted in first remission [30,32]. Recent reports of BMT for adult ALL from single and multiple centres are detailed in Table 5.2 [33–38]. Leukaemia-free survivals range between 54% and 71% and relapse rates between 10% and 26%. These variations are best ascribed to chance and to differences in patient risk factors rather than indicating the superiority of one or other transplant approach.

BMT in second or subsequent remission

Over 60% of adults with ALL relapse despite modern intensive chemotherapy [8]. Retreatment with chemotherapy achieves remissions, usually brief, in about 50% of patients. The long-term survival for adults with relapsed ALL is very poor—probably less than 10% are salvaged by further chemotherapy [39]. In contrast, data from the IBMTR indicate that adults given a marrow transplant from an HLA identical sibling for ALL in second remission have a DFS of about 25%, with a relapse rate of about 50% [30]. Although results of salvage treatment for adult ALL have also improved in recent years, the changes are less dramatic. High transplant-related mortality (TRM) and relapse remain major limitations to success. Recent results of allogeneic BMT for adults with ALL in second remission or more advanced disease are summarized in Table 5.3 [31,32,35,36].

Risk factors for BMT in first and second remission

Leukaemia-free survival is determined by the curative potential of the treatment, measured as relapse risk, and the TRM. Factors influencing outcome can be separated into features of the disease or the patient's general condition, and treatment-related factors, potentially alterable by the transplanter. However, the capacity to change the outcome by modifying the BMT procedure is limited: as a general rule, intensification of the preparative regimen to reduce relapse results in a higher TRM. Conversely, modifications, such as avoiding total body irradiation in the preparative regimen to reduce TRM, allow more relapses.

Several retrospective analyses have identified risk factors for LFS and relapse post-BMT [30,39–42]. In first-remission transplants, patient age is the most important factor affecting LFS. Age impacts both on relapse risk and TRM, which both worsen with increasing age. The presenting leukocyte count also defines the risk of relapse, with a higher relapse rate

Table 5.2 Allogeneic BMT for ALL in first remission—recent results

Data source	Patients *(n)*	Relapse (%)	Disease-free survival (%)	Median age (range)
IBMTR [32]	1005	25±4 SD	54±4 SD	Predominantly adults
EBMT				
1986–1992	757	26		Predominantly adults

[31]				
1992 only	92	26	65	
City of Hope [34]	53	10	61	23 years (1–38 years)
France [38]	41	12	71	
Minnesota [35]	104	56	29	
Baltimore [36]	18	20	42	24 years (5–36 years)
France [37]	29	10	62	Over 15 years
Neimegen [33]	22	NA	63±17	15–51 years
NA=not available				

Table 5.3 Allogeneic BMT for ALL in second remission—recent results

Data source	Patients *(n)*	Relapse (%)	Disease-free survival (%)	Median age (range)
IBMTR (1989–1995) [32]	1074	46	40	Adults and children
EBMT [31]	554	28		Adults and children
Baltimore [36]	36	26	43	Adults
Minneapolis [35]	104	56	29	Adults

observed with higher presenting leukocyte counts. A multicentre study by the IBMTR identified T-ALL as having an unfavourable effect on relapse independently of the leukocyte count. Other factors affecting relapse after chemotherapy (e.g. ALL with chromosomal translocations, null ALL or biphenotypic ALL) have not been demonstrated to affect BMT outcome.

For BMT in second remission, increasing age has an adverse prognostic impact, as does the general category of high-risk disease, based on a conglomerate of poor prognosis features [40]. However, most of the individual disease characteristics at presentation lose predictive significance after second remission. Of greatest importance are factors associated with the pace of progression of the leukaemia defined as ‘fast’ or ‘slow’ disease [39,43]: fast disease describes ALL relapsing within 18 months of remission, usually during maintenance chemotherapy. Slow disease is defined as ALL relapsing beyond 18–24 months from remission, usually after chemotherapy has been stopped. The largest analysis concerns 609 adults and children reported to the IBMTR [30]: in adults, risk factors for relapse after BMT were older age and a brief first remission, absence of

GvHD and the use of cyclosporin rather than methotrexate post-transplant. Older age was the main reason for increased treatment failure.

BMT for Philadelphia chromosome-positive ALL

While approximately 70% of adults with Ph^+ ALL achieve remission, almost all relapse [44]. Several recent analyses have shown that BMT can cure at least a proportion of patients with Ph^+ ALL [45–48]. An analysis by the IBMTR of 67 patients receiving HLA-identical sibling transplants for Ph^+ ALL showed a 62% relapse rate and 31% LFS at two years [47]. Interestingly, relapse and survival did not differ significantly between elective grafts in first remission, and salvage BMT for relapsed or primary resistant disease.

An analysis of 33 matched-pair first remission patients with and without the Ph chromosome showed comparable relapse but higher TRM in patients with the Ph chromosome. These results suggest that Ph^+ ALL is cured in some patients receiving BMT. The higher TRM of the Ph^+ group may reflect the use of intensive induction/consolidation regimens pretransplant in this high-risk leukaemia. The similarity in outcome for BMT performed at any stage of the leukaemic progression suggests that BMT could be performed with equal success as an elective or as a salvage treatment. Furthermore, the data suggest that intensive consolidation prior to BMT does not confer any survival advantage and should be avoided to reduce procedural mortality.

BMT in primary induction failure

It is generally agreed that patients with ALL, who fail to enter complete remssion, have a poor prognosis. An important question is therefore whether a proportion of such incurable patients can be salvaged by BMT from an HLA-identical donor. BMT for partially- or non-responding leukaemia carries an increased risk of persisting leukaemia or relapse, as well as a high TRM related to the prolonged period of poor haematopoietic function prior to BMT. Such considerations usually deter transplant teams from attempting BMT in this group of patients. However, a recent review by the IBMTR indicates that BMT confers about 30% long-term LFS in primary induction failure for ALL [49]. These results suggest that ALL patients who fail to achieve remission should be considered for BMT.

BMT for resistant relapse

Because there are no effective chemotherapy treatments, BMT continues to be attempted in patients who relapse and fail to re-enter remission. The results can be anticipated: there is a high rate of persisting leukaemia or relapse, and a higher TRM, compared with transplants performed in remission. The procedure can, however, result in about 10–20% LFS and results continue to improve. An IBMTR analysis showed that between the periods 1980–1981 and 1988–1989, LFS rose from 11±11% to 23±15% [50]. Many such patients are identifiable as having a poor prognosis earlier in the course of their disease, and only a small proportion should require BMT at this late stage.

BMT using donors other than HLA-identical siblings

Phenotypically matched family donors

The results from the Seattle BMT team indicate an outcome indistinguishable from HLA matched sibling BMT for leukaemia transplants [51]. An IBMTR study of 470 recipients of phenotypically matched donor-recipient pairs indicates a LFS of 28–59%—not significantly different from the LFS of 52–59% for a group of similar recipients receiving BMT from an HLA-identical sibling [52]. Thus, an extended search within the patient's family to find phenotypically matched potential donors is justified.

HLA-matched but unrelated donors

With the rapid increase in the size of national donor panels and increasing international cooperation in identifying marrow donors and supplying marrow, feasibility of finding an unrelated donor for patients with ALL has improved [53]. Adequate data on unrelated donor BMT specifically for ALL are not available. However, the results for unrelated donor BMT for leukemias of all types show that outcomes may be quite favourable—there is a higher incidence of severe GvHD, but increased mortality from GvHD is offset by a higher chance of remaining in remission [54].

Mismatched family and unrelated donors

Attempts to use less than fully compatible unrelated donors to transplant patients with poor-risk leukaemia have a high early mortality from graft failure and GvHD [55]. However, the availability of at least haploidentical family members for many potential BMT recipients with high-risk leukaemia has stimulated attempts to carry out mismatched family donor BMT. Recently, considerable progress has been made in such mismatched transplants, largely due to pioneering work in two centres—Perugia, Italy [56,57] and Columbia, South Carolina [58]. With T-cell depleted donor marrow or blood stem cells, the risk from severe acute GvHD has been minimized, and graft rejection has been reduced by adding thiotepa or fludarabine to the preparative regimen and giving large stem-cell doses from granulocyte colony-stimulating factor (G-CSF)-mobilized donors.

Identical twin donors

Data on BMT between identical twins are scarce. In the absence of alloimmune differences between donor and recipient, the antileukaemic effect of the identical twin marrow transplant depends largely on the efficacy of the preparative regimen. The largest database of identical twin BMT for ALL is held by the IBMTR. By comparing the outcome after identical twin BMT with outcome after intensive consolidation chemotherapy in similar patients, the contribution of intensive marrow ablative treatment to leukaemia cure was evaluated. Results from 16 patients with ALL receiving BMT from an identical twin indicate a relapse probability of about 35%, possibly less than that

of comparable patients given conventional chemotherapy but higher than that achieved by allogeneic BMT [59]. Nevertheless, the low TRM (less than 5%) observed in identical twin BMT results in a favourable LFS of about 60%. Unfortunately, no statistically relevant comparisons between these results and other treatments can be made, but clearly survival following identical twin BMT is favourable, both in comparison to intensive chemotherapy and allogeneic BMT.

Autologous BMT

The rationale of autologous BMT (ABMT) is to collect and conserve marrow stem cells taken during remission in order to rescue haematopoiesis following myeloablative antileukaemia treatment as post-remission intensification. Myeloablation schedules may be total body irradiation (TBI)- or chemotherapy-based but are usually comparable in intensity to those used for allografts. An unresolved problem is to determine the importance of purging the transplanted material from contaminating leukaemia cells. Only in the last decade were large series of ABMTs carried out in ALL. Historically, remission marrow was used as the source of stem cells. Combinations of monoclonal antibodies with leukaemia specificity, or chemotherapeutic agents have been used to purge the marrow of leukaemia [71,74]. It is now possible to collect chemotherapy- and G-CSF-mobilized blood stem cells by apheresis. These can be purged of leukaemia by positive selection of $CD34^+$ cells or by negative selection of leukaemia cells (reviewed by Gorin [60]). Because of the high relapse rates encountered after both purged and unpurged ABMT for ALL, other investigators have focused more on post-transplant approaches to eliminating minimal residual disease, using maintenance chemotherapy [61], cytokines such as interferon and interleukin-2 (IL-2) [62–64] or by induction of autologous GvHD [65]. At present, there is no ideal treatment strategy for ABMT in adults with ALL.

Recent results of autologous BMT for adult ALL

There is now considerable experience with ABMT for adult ALL in first remission. A number of multicentre and national studies have analyzed the outcome of patients, randomized to receive autologous BMT in first remission as post-remission consolidation. Individual trials differ in the amount of pretransplant consolidation received, the type of preparative regimen used and whether an attempt to purge the transplant of contaminating leukaemia cells was made. Recent results are characterized by a TRM in the region of 5%, with relapse being the major cause of treatment failure. LFS ranges between 32% and 75% in current studies. This wide range of outcomes can largely be accounted for by differences in patient population. There is some evidence that relapse may be diminished by purging techniques to remove residual leukaemia cells from the transplanted marrow [60,66]. However, because of the diversity and variable efficiency of the purging techniques used and the variability of the patient groups studied, the role of purging in ABMT for ALL has not been established. Several groups have explored the use of post-transplant treatment in an attempt to eliminate residual disease. There has been no benefit shown from the use of IL-2 or combinations of IL-2 with γ-

interferon (IFNγ) [22,62–64] but methotrexate maintenance therapy following unpurged BMT achieved a 53% three-year LFS [61].

Results of ABMT in second remission are universally disappointing, due to a very high relapse rate, whether or not the transplant is purged. LFS in the region of 20% is reported for adults with ALL undergoing ABMT in second or subsequent remission. Results of ABMT for ALL in first and second remission are summarized in Table 5.4 [22,31,61,67–69,72,73].

With its extremely high relapse risk, the patient with Ph^+ ALL and no HLA-matched donor poses a special problem. ABMT has been increasingly attempted in this disease, in an attempt to intensify post-remission treatment. ABMT for Ph^+ ALL is characterized by a very high risk of relapse. Nevertheless, recent evidence suggests that ABMT is superior to chemotherapy, with several patients becoming long-term disease-free survivors with undetectable minimal residual disease by sensitive polymerase chain reaction (PCR) techniques [68,70].

Treatment approaches and specific problems associated with BMT for ALL

Pretransplant intensification

For first-remission BMT, it is usually considered appropriate to give at least one intensive consolidation treatment prior to transplant, and to avoid the use of CNS irradiation in the induction/consolidation period, since the application of TBI within three months of administering cranial irradiation can be associated with serious CNS damage. As an alternative, six intrathecal methotrexate administrations, with or without one or two courses of high-dose methotrexate, have been used to protect patients selected for BMT in first remission from CNS relapse. These approaches have not, however, been subjected to proper evaluation, and individual BMT centres tend to adopt their own rules. There is no proven advantage in delaying BMT by introducing additional consolidations or in giving cranial radiotherapy.

Table5.4 Recent results of autologous BMT for ALL

Transplant group	Patients (n)	Purging	Post-transplant treatment	Disease-free survival (%)	Median age
First complete remission					
IBMTR (1989–1995) [32]	102	Variable	–	43	3 years
Simonsson *et al.* [67]	21	CD10, 19, 7	–	65	16 months

Morishima et *al.* [68]	8	CD10	–	75	5 years
Gilmore et *al.* [69]	27	CD10, 19, 7	–	32	3.5 years
Powles et *al.* [61]	50	None	Mathotrexate	53	5 years
Fiere *et al.* [20]	95	Variable	IL-2	39	5 years
Second complete remission					
IBMTR (1989–1995) [32]	228	Variable	–	25	3 years
Simonsson et *al.* [67]	29	CD10, 19, 7	–	31	18.5 months
Morishima *et al.* [68]	9	CD10	–	20	3 years
Baumgarten et *al.* [63]	22	None	IFN-γ and IL-2	18	–
Soiffer et *al.* [71]	2.1	CD10, 9, 6	–	20	–
Uckun *et al.* [74]	14	CD10, 19, 20	–	20	–
Cahn *et al.* [72]	26*	Monoclonalantibodies/ mafosfamide	–	28	4 years

*16 second complete remission, 6 first complete remission, 4 beyond second complete remission.

Extramedullary relapse

The efficacy of BMT in preventing extramedullary relapse is evidenced by the relatively low rate of isolated CNS relapse reported in BMT series. The standard single or multiple fraction TBI schedules therefore appear to be adequate in preventing CNS disease. Testicular relapse has been reported after BMT, and may be largely preventable by additional testicular irradiation at the time of BMT [39]. Many BMT groups treating patients in second or subsequent remission or relapse give an additional 5 Gy irradiation to the testes. This, however, reduces testosterone production, otherwise conserved after TBI alone.

Preparative regimens

Despite considerable inventiveness and the application of a number of different drug combinations in the preparative regimen, there is no clear evidence that any preparative regimen is superior to the well-established cyclophosphamide/TBI schedule. Non-randomized studies, however, indicate that high-dose etoposide with or without cyclophosphamide, in conjunction with TBI, may achieve better leukaemia control. For example, Chao *et al.* [34] report 71% LFS and a 10% relapse rate for adult ALL using an etoposide-based preparative regimen. It appears that TBI provides an important part of the antileukaemia effect. Compared with TBI treatments, chemotherapy-only regimens using busulphan and cyclophosphamide are prone to increase the risk of relapse [73,75].

GvHD prevention

Because of the high relapse rate after BMT for ALL, the use of T-cell depletion in HLA-matched sibling BMT is not recommended. However, while the occurrence of GvHD confers protection against relapse, the benefit in terms of survival from preventing GvHD tends to balance out the increased relapse risk. The compromised approach is therefore to use the currently most effective GvHD regimen of cyclosporin and 'short' methotrexate.

Transplant-related mortality

A characteristic of BMT for adults with ALL is a high treatment-related mortality due more to a general increase in risk from a number of factors rather than to any one cause. Patients may be more susceptible to reactivation of DNA viruses, especially cyto-megalovirus (CMV), because of the prolonged immunosuppression from preceding chemotherapy. The main determinant of outcome is, however, the age of the recipient at the time of transplant. Patients over the age of 30 years have a distinctly worse outcome than younger adults. However, although some data indicate further significant deterioration of outcome with age, the differences in outcome between 30 and 55 years are relatively small.

Relapse after BMT

In considering further treatment for patients relapsing after BMT, the interval between the first BMT and relapse is an important criterion: patients relapsing within a year from BMT have a low probability of entering a further remission and becoming long-term survivors, irrespective of the treatment used. Patients relapsing after a longer period have a better chance of survival. Only 11% of patients achieve long-term LFS following second transplants for leukaemia relapsing after BMT [76]. There are insufficient data to determine whether a true isolated CNS relapse responds to a second transplant. These patients should probably first be treated with triple intrathecal therapy. Younger patients may tolerate cranial radiation.

As with salvage transplants for other leukaemias, second BMT for relapsed ALL has only a low (10–20%) probability of achieving a prolonged survival. However, in ALL

relapsing after BMT, chemotherapy alone can induce remission and give survival with a good quality of life for prolonged but not indefinite periods [77,78]. If remission is achieved, a second BMT can still offer the chance of cure. There is a high mortality from the second preparative regimen, in the region of 70% in the first three months, followed by a continuing risk of relapse [76]. Improved results may only be achieved by reducing the intensity of the chemotherapy used and enhancing the immunotherapeutic effect of the graft with a peripheral blood stem-cell transplant [79], or by delayed addition of donor lymphocytes [80]. Another promising salvage approach is the use of matched or mismatched related donors for treatment of relapse following autologous BMT [81].

In recent years, there has been increasing use of donor lymphocyte transfusions (DLT) to treat relapsed leukemia. DLT to treat ALL, which has relapsed after BMT, are usually unsuccessful [82]. However, some patients have responded and achieved long-term remissions [82–86]. Most responders with ALL develop acute GvHD, which brings about the main procedure-related morbidity. It is possible that better results could be obtained by combining DLT with interferon-α or IL-2. Slavin *et al.* were the first to report the efficacy of DLT+IL-2 to treat a boy with relapsed ALL who became a long-term survivor with no persisting GvHD [86]. At present, the optimum approach to the treatment of relapsed ALL is unclear. Because of its lower toxicity, DLT either given as initial therapy for low-grade relapse or following re-induction chemotherapy for more aggressive relapse, is a reasonable approach. Patients failing this treatment can be offered a second transplant with modifications to the GvHD prophylaxis to bias the immune system towards a GvL response. A suggested treatment strategy for relapsed ALL is shown in Figure 5.3.

Treatment decisions in high-risk ALL

Treatment decisions in adult ALL are particularly difficult because comparative studies of chemotherapy, autologous and allogeneic BMT have not provided clear guidelines for treatment. For a number of reasons prospective randomized trials are not easily applied to BMT [87]. Furthermore, large definitive multicentre studies and retrospective comparative database analyses become outmoded by the time their results are analyzed and published, because of the improvements continually occurring in therapy. Additionally, the recurring phenomenon that single centre studies show superior outcomes to multi-institutional data, adds further confusion to determining the true expectations from different treatments. The main treatment dilemmas posed in adult ALL are discussed below, followed by a treatment strategy.

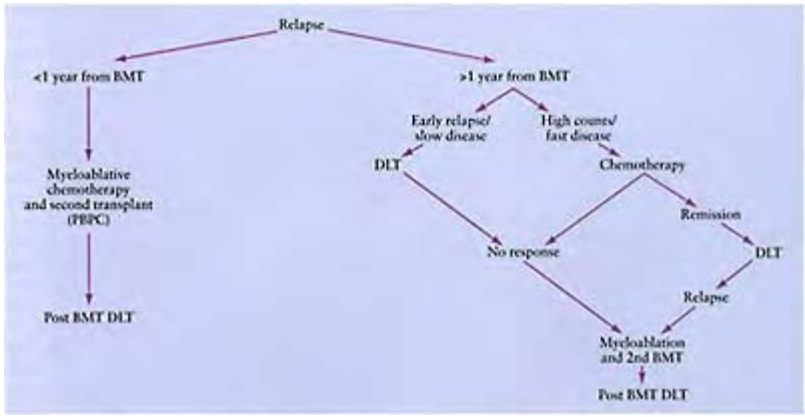

Figure 5.3 Treatment strategy for ALL relapsing after BMT.

- *Chemotherapy:* Low-intensity re-induction with, for example, vincristine/prednisolone ± anthracycline or VAD (vincristine, Adriamycin, dexamethasone).
- *Second BMT:* Preparative regimen. Chemotherapy only for patients who received TBI for first BMT, G-CSF-mobilized peripheral blood progenitor cells preferable to marrow. Consider short GvHD prophylaxis with cyclosporin only.
- *Donor lymphocyte transfusions:* At least 10^8 l $CD3^+$ cells/kg for matched related BMT. Consider low-dose IL-2 (160 U/kg) subcutaneous injection for 5 days after each DLT.
- *Early relapse/fast disease (see text):* Patients in molecular or early marrow relapse with no haematological decompensation and no evidence of rapid progression.
- *High counts/fast disease:* Patients with rapid haematological decompensation and rising blast count in the blood.

Treatment dilemmas

Should patients with an HLA-matched sibling donor receive an allogeneic BMT, autologous BMT or chemotherapy?

For adult ALL patients entering first remission, there is a treatment dilemma: provided they are reasonably fit and under the age of, at most, 55 years, those with an HLA-identical sibling have the option of an allogeneic BMT. The dilemma for these individuals is the contrasting outcome that allogeneic BMT offers in comparison with either continued chemotherapy or ABMT: while the allograft has the most likely chance of curing the leukaemia, it also has the highest risk of early mortality. Delaying the transplant until the leukaemia has relapsed significantly increases the chance of treatment

failure from a subsequent BMT, or in the worst case, disease progression or complications make transplantation out of the question. Thus, the optimum time for the high-risk but potentially curative allograft is early in first remission. Although some prognostic data are available, they are not significantly detailed to provide much help in individual cases. To date, all comparisons between allografting, autografting and chemotherapy are characterized by significant superiority of the antileukaemic effect of the allograft, but only small and usually non-significant differences in LFS [20,38,88–91] (Table 5.5).

The situation is compounded by the fact that treatment advances are occurring in the fields of allografting, autografting and chemotherapy. Available data from prospective randomized trials, comparative database analyses and single-arm studies therefore never provide a clear answer in the individual patient's case. One method of generating sufficient case

Table5.5 Comparative studies of allogeneic BMT, autologous BMT and intensive chemotherapy for adult ALL in first complete remission

Disease-free survival						
Authors	Chemotherapy	P	Allograft	P	Autograft	Comments
Fiere *et al.* [20]	96	116			95	Prospective randomized multicentre
	32(27–37)	43(38–48)	NS	39(34–44)		
Attal *et al.* [38]	–		41	0.001	64	Prospective randomized multicentre
			71 (54–83)		28 (15–49)	
Proctor *et al.* [89]	19		15		13	Prospective randomized multicentre
	21		34		48	
Bernasconi *et al.* [88]	15	NS	16	HS	25	Prospective randomized single centre
	34	NS	45	NS	42	
Horowitz *et al.* (IBMTR) [90]	484		484		–	Retrospective matched database study
	32 (27–37)		34 (28–40)			

Gorin (EBMT) [60]	–	NS	326 47 (44–50)	0.06	300 40 (36–44)	Retrospective unmatched comparison
*NS=not significant						

numbers to improve the statistical power of the comparison is to use comparative database analysis [87]. BMT outcome determined from the large multicentre databases of the EBMT or IBMTR can be compared with that of patients patients receiving chemotherapy in multicentre leukaemia trials. By this method, the smaller BMT patient cohort can be very closely matched with patients selected from the larger chemotherapy database. Since only patients surviving relapse-free long enough to receive BMT will be included in the BMT database, a correction for this time-to-treatment bias is applied. With adequate matching and time-to-treatment adjustment, statistically valid retrospective comparisons of outcome variables can be made. One such study compared IBMTR data for 251 adults with ALL transplanted in first remission, with a German multicentre chemotherapy study of patients entering remission [90]. The results showed a significantly lower relapse probability following BMT (32% versus 96%), but this benefit was offset by a high treatment-related mortality resulting in no significant difference in DFS between chemotherapy (47%) and BMT (51%). No prognostic factor predicted better outcome for one treatment versus the other. Furthermore, an updated analysis failed to show any difference in outcome for survivors beyond three years from transplant [91], These results suggest the limited conclusion that BMT currently offers no major advantage over the best available chemotherapy for similar patients with adult ALL. Although this study has been much cited, the conclusion that BMT is not generally superior to chemotherapy in adult ALL has been challenged [1] and recent improved results for allogeneic BMT in adult ALL have now to be taken into account. An alternative strategy adopted by the Royal Marsden Hospital Group is to use a ‘total therapy’ approach. All patients with adult ALL achieving remission receive high-dose melphalan followed by a G-CSF-mobilized peripheral blood stem-cell autograft. Patients who relapse receive an allograft from a matched or closely matched donor. Initial results show no procedural mortality and a projected DFS of 68% [92].

Should patients with no HLA-matched sibling receive an autograft or chemotherapy?

For the patient in first remission without an HLA-matched donor, the options are to continue with non-myeloablative chemotherapy, or myeloablation and ABMT. There are insufficient data at present to determine whether ABMT or continued intensified consolidation is the optimum choice in the individual case. Results from several multicentre studies are now available. They show no significant differences in LFS for the chemotherapy and ABMT arms [20,38]. Many of these trials, however, suffer from the problem that patient and physician preference cause large discrepancies between the therapies randomly assigned and the actual treatment received. Thus, some patients randomized to receive BMT chose chemotherapy and vice versa. Consequently, results

based on intention to treat (the statistically unbiased form of analysis) differ significantly and are often inferior to the results actually achieved.

Alternative donor BMT, ABMT or chemotherapy?

A further dimension to the patient's dilemma has opened up with the increasing use of transplants from unrelated donors and mismatched family members. What is the place of these treatments for adults with ALL? Clearly the high risk of treatment failure restricts their use largely to patients who have already failed other treatment approaches—chemotherapy or ABMT. However, some adult ALL subtypes, in particular Ph^+ ALL, have such an abysmal outlook that the risk from non-HLA identical sibling transplants may be considered justifiable even as an elective treatment in first remission. Only one study has compared the options of ABMT versus partially matched allogeneic BMT [93]. The study of 43 allografts and 77 autografts included 20 ALL allografts from unrelated donors and 36 closely matched ALL autografts. The allograft recipients had a higher DFS both for remission and relapsed acute leukaemia (for remission transplants 33% allo-BMT versus 25% for ABMT, for relapse transplants: 12% versus 5%). These results did not reach significance and are therefore only suggestive that the allograft is a better treatment.

Relapsed ALL: BMT or chemotherapy?

A comparative analysis of 280 BMT with 400 matched chemotherapy patients entered into Paediatric Oncology Group (POG) relapse protocols for childhood ALL showed an unequivocal advantage in LFS for BMT, both for fast and slow disease, and for patients with high-risk features, such as high presenting leukocyte count [94]. No similar data exist for adult ALL, but these results suggest that BMT from a matched sibling donor is the treatment of choice for adults with ALL achieving a second remission.

A treatment strategy for adult ALL

It is clear from the preceding discussion that it is not possible at present to define an ideal and universally acceptable treatment approach for adult ALL. Faced with these almost insurmountable obstacles to rational decision-making, how should the physician advise the patient? One option is to submit patients to randomized prospective studies comparing outcomes. However, in the light of the limitations of these studies, and despite pressure from national study groups, the physician should not feel an automatic obligation to enter all patients into randomized trials, especially if there are specific features that suggest a better outcome with a particular treatment option. For example, the rare adult with a typical childhood ALL with other good prognositc features might be better to continue with chemotherapy, rather than risk an allogeneic BMT, while a patient with Ph^+ ALL might be better to elect to have a BMT from an unrelated donor in first remission. The following guidelines are intended to suggest some of the approaches to transplant decision-making. In the end, however, it is the patient who must make the

decision. A suggested approach to treatment selection in adult ALL is discussed below and outlined in Figure 5.4.

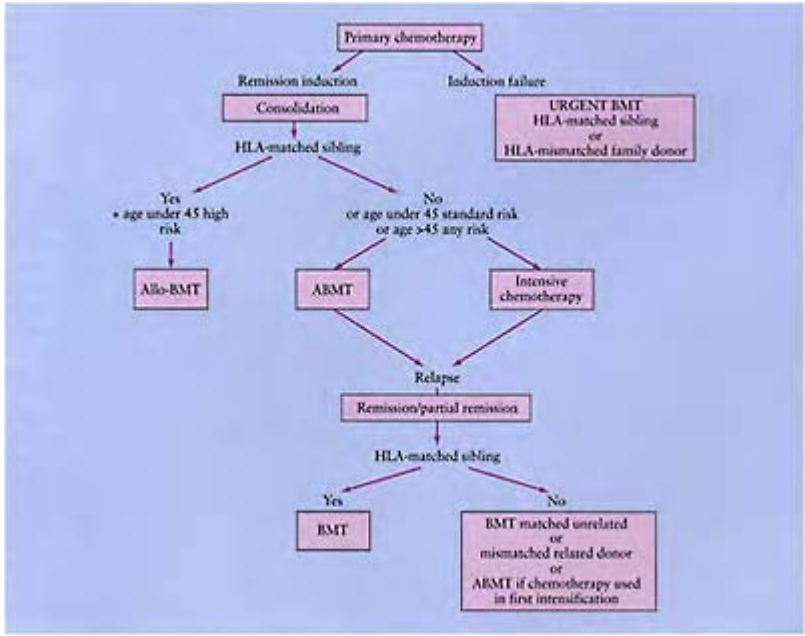

Figure 5.4 A treatment strategy for adult ALL.

HLA-matched sibling BMT in first remission

A BMT from an HLA-matched sibling is the treatment of choice for the group of adults under 50 years with very high risk ALL: Ph^+ ALL, t4;11 and ALL with very high leukocyte counts (over 100000/mm^3 at presentation). In contrast, because of increasingly favourable results with chemotherapy treatments, a case can be made for avoiding allogeneic BMT in patients with low-risk features: T-ALL with low counts, some B-ALLs, adolescents and younger adults with features of good risk (common) ALL of childhood, and also older patients with a high risk from TRM. In the intermediate group, there is insufficient evidence to be dogmatic about the role of allogeneic BMT. The current bias remains in favour of allogeneic BMT when an HLA-matched sibling is available because, while they do not differ significantly, all multicentre prospective comparative trials published to date show a 5–10% survival advantage over the chemotherapy group for allograft recipients.

HLA-matched sibling BMT in second or subsequent remission

Data from comparisons of chemotherapy with BMT in childhood ALL indicate an unequivocal advantage of allogeneic BMT over chemotherapy approaches. This is the basis for recommending allogeneic BMT for adult patients who relapse and achieve a further remission.

Primary induction failure

Allogeneic BMT from a matched or a mismatched donor is appropriate for patients who have primary induction failure with adequate chemotherapy (vincristine, prednisolone and an anthracycline). Patients not in remission at six weeks should be considered for prompt allogeneic BMT. Because of the inevitable delay in identifying and locating an unrelated donor, the only practical approach is to use the best available family donor. HLA-identical sibling BMT gives a 20% chance of LFS. Mismatched BMT should only be considered in younger adults in good general condition.

Salvage allogeneic BMT

Patients in relapse and with resistant disease have a low probability of long-term LFS. Nevertheless, a proportion may be rescued by either matched or mismatched BMT. Because of the compounding poor prognosis effect of poor prognosis disease with a high risk of TRM, mismatched BMT should be reserved for younger adults (under the age of 30 years), who are more likely to survive the procedure.

Unrelated donor BMT

Current results support the use of unrelated donor BMT for ALL, especially in second remission and in some high-risk patients achieving first remission. However, the time required to identify the donor and prepare them for the transplant places an important practical constraint on the use of unrelated donors.

Identical twin transplants

Data from the IBMTR support the use of an identical twin donor for all high-risk adult ALL. While the treatment-related mortality is low, the relapse incidence of 40% is similar to that observed after HLA-matched sibling BMT. Thus, BMT from the identical twin should be strongly considered on the rare occasions where it is possible.

Autologous BMT

ABMT is limited to the population of patients with ALL who achieve a first or second remission which enables a stem-cell collection to be made. While the relapse rate from ABMT is high, the low treatment-related mortality continues to make the procedure an attractive alternative, both to continuing chemotherapy, or to an allotransplant from a donor other than an HLA-identical sibling. In the current climate of opinion, it would be wrong to be dogmatic about the place of ABMT in adult ALL. The physician is faced

with the choice of either entering the patient into a randomized study, or selecting for ABMT the patients at highest risk for relapse. Again, this category includes Ph^+ ALL and t4;11 ALL. A case can also be made for ABMT in T-ALL and B-ALL, both rapidly proliferating yet chemosensitive leukaemias which appear to benefit most from dose-escalation strategies.

In patients with a high risk of relapse, the choice may also have to be made between ABMT or allo-BMT from an unrelated or less than fully matched family donor. As mentioned above, the mismatched allograft has a small but statistically insignificant treatment advantage.

Chemotherapy

There remains a significant group of adults with ALL in whom chemotherapy remains the best treatment option either because their disease is likely to be cured by chemotherapy alone, or because other treatments are contraindicated. This category includes good-risk (common) ALL in first remission in young adults, most T-ALL and B-ALL, patients older than 55–60 years, and those with comorbidities which would increase unacceptably the transplant-related mortality.

Conclusions

Adult ALL remains a poor-risk leukaemia with imperfect treatment options. However, improved definition of prognostic subgroups has helped delivery of the optimum treatment for individual patients. Given the continuing poor outcome for this disease, single-centre attempts to improve outcome by experimental treatments have equal importance to large randomized studies. What remains important is to collect data on every patient treated for ALL and to report the information to large registries, so as to allow every case to contribute to our knowledge. Improvements in transplant-related mortality should, in the future, make allogeneic BMT a more frequently used option.

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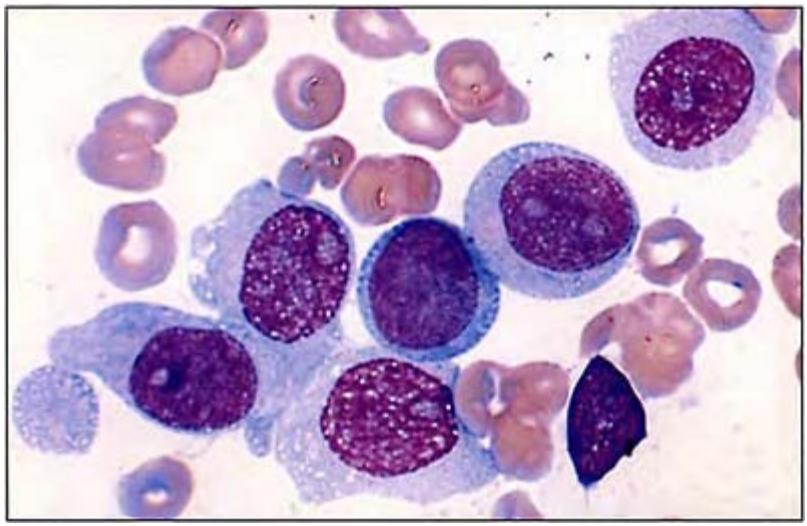

Chapter 6
Paediatric leukaemias

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Introduction

Bone-marrow transplantation has been an especially successful therapy for a number of haematological malignancies of childhood. Series describing the results of transplantation are consistently superior in children compared to adults. This is mainly due to the lower incidence and severity of graft-versus-host disease (GvHD) in younger patients.

The role of HLA-DR identical sibling transplant is well established in a number of diseases but remains controversial in others. Lack of suitably matched sibling donors is the greatest impediment to the use of this therapy in the majority of children in whom it would be beneficial. In order to increase availability of transplantation, alternative sources of haematopoietic stem cells have been studied. These include use of allogeneic donors other than matched siblings, both related and unrelated, cord blood and the use of autologous bone-marrow transplant (ABMT), both purged and unpurged.

As the proportion of children with haematological malignancies cured by transplantation increases, late effects of this therapy are of growing concern. Children actively undergoing the process of growth and development are especially susceptible to the toxic effects of therapy and are at risk for evolving organ dysfunction over time.

This chapter will contrast transplantation with chemotherapy in the childhood haematological malignancies in which it has been utilized. The role of transplantation using alternative sources of haematopoietic stem cells in various haematological malignancies for children who lack HLA-DR identical siblings will be discussed. Finally, late sequelae of transplantation in children will be reviewed.

Acute myelogenous leukaemia (AML)

Allogeneic transplantation

The Seattle transplant team initially reported that transplantation could salvage patients with relapsed or refractory AML in 1975 [1]. In 1979, the group followed this report with a study of transplantation in first complete remission (CR) AML (including six children) [2]. The preparative regimen employed was cyclophosphamide and total body irradiation (TBI). Four of the children included in this early report have become long-term disease-free survivors [3]. Since this report, BMT has played an important role in the therapy of children with AML. Tables 6.1 and 6.2 summarize the results of larger paediatric AML trials utilizing BMT in first CR.

The efficacy of BMT must be judged in comparison to the best available chemotherapy. The success of chemotherapy in the treatment of AML has vastly improved over the past 15 years, with more aggressive treatment protocols and improved supportive care.

The Children's Cancer Group (CCG) has prospectively studied the outcome of children with AML in first CR treated with transplantation compared to chemotherapy in a series of sequential studies outlined in Table 6.2. Assignment to the transplant arm in these studies has been made and analysed based on availability of a suitably matched family donor (6/6 sibling in the 251 and 213 studies and 5 or 6/6 matched family member in the 2891 study). Disease-free survival (DFS) of the transplantation arm was superior to chemotherapy in each of these studies, although it took prolonged follow-up to be appreciated in the CCG-251 [10] study, and statistical significance has not yet been achieved in the CCG-213 study [11].

It is interesting to compare the outcome of BMT in the CCG-251 study and the CCG-213 study [14]. In both studies, a TBI cyclophosphamide preparative regimen was utilized. The GvHD prophylaxis on the CCG-251 study was 'long' methotrexate, but in the subsequent CCG-213 study cyclosporin (CSA) and 'short' methotrexate were substituted. The two-drug GvHD prophylaxis was marginally superior in preventing grade II or greater GvHD, as expected, and patients on CCG-213 experienced less treatment-related mortality (TRM). However, the event-free survival (EFS) curves of the two studies were identical as there was a higher relapse rate in the 213 study. This suggests the importance of a graft-versus-leukemia effect (GvL) in the efficacy of allogeneic BMT in AML. Therefore, in the successor 2891 study, GvHD prophylaxis was 'long' methotrexate.

In addition to the BMT question, the CCG-2891 study also posed a question about dose intensity of induction therapy, comparing standard timing of courses of a five-drug combination with the same drugs given in an intensively timed schedule (i.e. obligatory

second course after a 6-day rest regardless of marrow status). Of interest, the CR rate did not

Table 6.1 Results of paediatric trials of allogeneic bone-marrow transplantation AML—first remission

Study group	Time of patient entry	Patients (n)	Conditioning	DFS/EFS†	Reference
FHCRC*	1976–83	38	Cy/TBI	64	[4]
Minnesota**	1976–80	38	Cy/TBI	61	[5]
MSKCC***	1979–85	24	TBI/Cy	66	[6]
SFGM†	1979–92	23	Bu/Cy 120	46	[7]
		19	Bu/Cy 200	82	
		32	Cy/TBI	80	
AIEOP/GITMO††	1980–90	59	TBI/Cy	58	[8]
			(7 patients Bu/Cy)		
POG†††	1988–93	89	Bu/Cy	52	[9]

*Fred Hutchinson Cancer Research Centre (Seattle). **University of Minnesota (Minneapolis). ***Memorial Sloan-kettering Cancer Centre (NewYork). †Société Français de Greff de Moelle. ††Associazione Italiana de Emotologiae Oncologia Pediatrica/Gruppo Italiano per il Trapianto di Midollo Osseo. †††Pediatric Oncology Group. Cy=cyclophosphamide; TBI=total body irradiation; Bu=busulfan. †DFS=Disease-free survival; EFS=event-free survival. □□

differ between the two arms. However, the survival and DFS, regardless of post-induction therapy (BMT, ABMT, consolidation chemotherapy), was significantly better in patients treated with intensively timed therapy (P=0.0002). This highlights an important principle in the interpretation of BMT trials: the ultimate outcome of patients may be dependent on the therapy preceding BMT.

Most investigators recommend BMT in first CR for children with family matches. The exception is the German Berlin-Frankfurt-Münster (BFM) Study Group who have conducted a series of chemotherapy-based protocols for the therapy of AML in children [15–17]. These protocols have resulted in excellent outcome for children with AML. The EFS (event-free survival after CR) of the AML-BFM-83 study of 173 children was 61% at six years [16]. This is superior or equivalent to the EFS seen in most BMT trials done in first remission (Tables 6.1 and 6.2). The exception is the CCG-2891 study, where EFS of the 140 patients who underwent BMT was 70% at three years (77% for those who received intensively timed induction therapy) [12,13].

There are children with AML who should not be treated with BMT in first remission. The BFM group argues there are two risk groups in patients treated on their protocols

[16] (Tables 6.3 and 6.4). The stratification is based on French-American-British (FAB) morphology, presence of Auer rods and eosinophilia. The lower risk group (FAB M1 with Auer rods, M2 white blood cells (WBC)<20000, M3, M4 >3% eosinophils, M6) has an estimated EFS at six years>80%, whereas the high-risk group (FAB M1 Auer rods absent, M2 WBC>20000, M4 eosinophils <3%, M5 and M7) has an estimated EFS at six years of <45%. They argue that BMT in first CR should be reserved for the high-risk group. There is also consensus among many investigations, in agreement with the BFM recommendation, that combined therapy with *all-trans*-retinoic acid and chemotherapy is the treatment of choice for acute promyelocytic leukaemia (APL, FAB M3) [18]. An estimated EFS of >70% has been achieved in most studies.

Table 6.2 Consecutive Phase III Children's Cancer Group (CCG) AML trials including BMT

		Patients (n)					DFS (from remission)			
Trial	Time of patient entry	Assessable	Remission	Induction treatment	Post-remission treatment	Patients *(n)*	3 years	5 years	P value	Reference
CCG-251	1979–83	490	381	DNM (ADR)/ARA-C*	BMT (CY/TBI)	85	48%	45%	0.04	
					versus			33%		[10]
					multidrug maintenance	287	36%			
CCG-213		591	439	DNM/ARA-c	BMT (TBI/CY)	113	50%	46%	0.06	
				versus	versus			38%		[11]
				'DENVER'**	HDARA-C ± multidrug maintenance	298	38%			
CCG-2891	1989–93	589	407	Standard time	BMT (BU/CY)	140	70%	0.0001	0.01	
				DCTER***	versus					[12,13]
				versus	ABMT (BU/CY)	160	40%			
				intensive time	versus					
				DCTER	HDAra-C	150	50%			

*Daunorubicin (initially doxorubin) 3(2) doses and 7(5) day continuous infusion (Ara-C) 2–4 courses, **Five-day regimen consisting of etoposide, thioguanine, dexamethosone, daunorubicin and Ara-C. ***Four-day continuous infusion Ara-C, daunorubicin with and dexamethasone standard time repeated depending on response intensive time day 0–4 and 10–14. CY=cyclophosphamide; TBI= body irradiation; HDAra-C=high-dose cytosine arabinoside; ABMT=autologous bone-marrow transplantation.

Table 6.3 Children with AML who should not receive BMT in CR1 [16]

- FAB morphology M1 with Auer rods and WBC<20 000 at diagnosis.
- FAB morphology M3.
- M4>3% eosinophils.
- M6.
- EFS>80% at 6 years.

Table 6.4 Children with AML who should receive BMT in first CR [16]

- FAB morphology M1 with Auer rods absent and WBC>20000 at diagnosis.
- M4<3% eosinophils.
- M5 and M7.
- EFS at 6 years <45%.

Another group of AML patients who have been noted to fare well with chemotherapy are children with Down's syndrome [19]. Transplantation is not indicated for these patients in first remission.

Autologous BMT (ABMT)

The role of autologous marrow rescue after myeloablative therapy has been studied as post-induction consolidation for children with AML in first CR. The Pediatric Oncology Group (POG), Associazione Italiana de Ematologia Oncologia Pediatrica (AIEOP) and CCG have conducted and reported on prospective randomized trials comparing autologous 4-hydroperoxy-cyclophosphamide (4HC) or mafosfamide purged marrow transplants with intensive consolidation therapy [9,12,13,20]. In these three studies, ABMT was not superior. There were 117 patients in the POG study randomized to intensive chemotherapy, and 115 to autologous transplant. The three-year leukaemia-free survivals were 36% and 38% [9]. There were 37 patients randomized to chemotherapy and 35 randomized to ABMT in the Italian study [20]. DFS at five years was 27% and 21%. The CCG-2891 study is summarized in Table 6.2. There were 150 patients randomized to intensive chemotherapy and 160 to autologous transplant [12,13]. The three-year DFS was 50% and 40%. Survival was significantly better in the chemotherapy arm (P=0.03).

The inferior or equivalent outcome of ABMT, compared to allogeneic transplant may be the result of two factors: contamination of autologous marrow with residual leukaemia and loss of a graft-versus-leukaemia effect. Because of the concern about contamination of autologous marrow with residual leukaemia cells, there has been a bias in most trials to

employ *ex vivo* marrow purging [21]. However, there are no randomized trials demonstrating the efficacy of marrow purging. The principle upon which purging is based is that there is a differential sensitivity and specificity between leukaemia and normal stem cells. The most widely employed purging methods are *in vitro* treatment of marrow with chemotherapy such as 4HC or monoclonal antibodies. The best experimental data that support the necessity for purging come from retroviral gene-transfer studies [22]. These demonstrated that autologous remission after BMT with unpurged marrow contributed to disease recurrence, as leukaemic cells at relapse contained the genetic marker that had been transfected *ex vivo*. Purging studies using transfected autologous marrow offer an effective method to evaluate efficacy of existing and new methods of purging. Peripheral blood stem cells (PBSC) have also been proposed as an alternative approach to provide autologous rescue, as peripheral blood may contain fewer contaminating leukaemic cells [23]. PBSC can be successfully harvested from children with malignancies [24].

The second theoretical problem with autologous rescue is the lack of a GvL effect. Studies of the immune modulator interleukin-2 (IL-2), which promotes activated killer cell function *in vitro*, suggest this agent may decrease the risk of relapse after ABMT in patients treated after first relapse [25]. Prospective randomized studies of ABMT, or intensive consolidation therapy, followed by immune modulators such as IL-2, may determine the usefulness of this strategy in the future.

First relapse and beyond

The prognosis for patients who relapse with AML is extremely poor. It is unlikely that a patient will be cured with chemotherapy. BMT is indicated for these patients. The major impediment, however, is lack of a suitable donor. ABMT in second CR can salvage some children [26–29]. Two larger studies of ABMT in children with AML in second CR were reported by the European Bone Marrow Transplant (EBMT) registry and AIEOP. The EBMT report included 41 children who achieved a DFS of 44% at three years [28]. The AIEOP reported on 31 children with a DFS of 59% at 59 months [29].

Partially matched related allogeneic BMT has also been used to salvage children who have relapsed. The numbers in individual series are small, but combined, perhaps 30–40% of children transplanted for refractory malignancies can be salvaged using a partially matched related donor [30–32]. Most of these studies employ some method of T-cell depletion to control the incidence of life-threatening GvHD.

As more volunteer donors are available on computerized registries, the availability of matched or partially matched unrelated donors increases [33,34]. In a summary of the Fred Hutchinson Cancer Research Center (FHCRC), experience of children who underwent transplants using unrelated donors, 13 children with AML were included. Six remained in remission a minimum of two years from transplant (six with matched donors, seven with donors disparate for one HLA antigen). Analysis of the entire group (88 children) indicated there was less transplant-related mortality in the patients who received matched marrow.

Placental blood is a source of haematopoietic stem cells that is rapidly becoming available through computerized registries. A recent report of the outcome of 25 children undergoing transplant with unrelated cord blood as a source of haematopoietic stem cells

included five children with advanced AML. Two of these patients survived in remission for longer than 238 days after transplant [35]. The choice of the source of haematopoietic stem cells for children with advanced AML depends on the availability of one of these donors for a given patient. At this time, it is not clear which of these options is the best.

Relapse after BMT

Rarely will a patient who relapses after BMT be cured by chemotherapy [36]. There are a few patients who can be salvaged with a second BMT [37,38]. The factors which determine the success of a second BMT are time of relapse after initial BMT, and age. A second strategy that has been used in patients who relapse after transplant is the infusion of donor lymphocytes to effect a graft-versus-leukaemia reaction. This strategy has been most successful for patients with CML but may have a role in some patients with AML [39].

Acute lymphoblastic leukaemia (ALL)

BMT as initial therapy

The outcome of children with ALL treated with chemotherapy is a great success story of modern cancer therapy. Multiple trials conducted worldwide in the 1980s have achieved four- and five-year EFS near 70% (Table 6.5). Thus, BMT is not indicated as initial

Table 6.5 Large modern chemotherapy trials in childhood ALL

Study group	Time of patient entry	Patients *(n)*	Event-free survival	Reference
CCG-105*	1983–89	1675	5 years (68%)	[40]
ALL BFM 86**	1986–90	998	5 years (72%)	[41]
St. Jude XI***	1984–88	358	5 years (71%)	[42]
Dana Farber†	1985–87	220	7 years (78%)	[43]
UKALL X††	1985–90	1612	5 years (68%)	[44]
ALinC 14†††	1986–91	1854	4 years (72%)	[45]

*Children's Cancer Group. **Berlin-Frankfurt-Münster Group. ***St. Jude Children's Research Hospital. †Dana Farber Cancer Institute/Children's Hospital (Boston).††UK Medical Research Trial. †††Pediatric Oncology Group.

Table 6.6 Indications for BMT in CRI of childhood ALL

- Hyperleukocytosis at presentation.
- Infants and adolescents.
- Specific cytogenetic abnormalities including t(4:11); t(9:22) [Philadelphia-positive]; t(1:19); t(8:14); any 11q23 abnormality.
- Slow response to induction therapy.

therapy in the majority of children with ALL. The current strategy in paediatric ALL therapy is to identify subsets of patients based on risk factors at initial presentation, and tailor therapy to maximize chance of cure but to avoid unnecessary treatment-related sequelae. BMT may have a role in first CR as therapy for those patients identified as having poor prognostic risk factors at initial presentation.

Hyperleukocytosis at presentation and age at diagnosis (infants and adolescents) have been and continue to be powerful poor prognostic indicators in ALL. Specific cytogenetic abnormalities have been identified to portend a poor prognosis. Specifically these include: t(4;11) and perhaps any 11q23 abnormality, t(9;22) (Philadelphia chromosome, Ph^+), t(1;19) and t(8;14) [46]. Slow response to induction therapy has also been identified as a poor prognostic factor [47] (Table 6.6).

There are only a few published series of children treated with BMT in first CR for ALL with poor prognostic features. EFS ranges from between 34% and 84% in the larger series outlined in Table 6.7. The MRC UKALL X trial compared BMT with a chemotherapy regimen for patients presenting with WBC>100000/μl [49]. Patients with HLA-DR matched siblings were eligible for BMT. There was no difference in the DFS between patients treated with chemotherapy or BMT, but the relapse rate was higher in the chemotherapy group. This is the only report of a direct comparison between BMT and chemotherapy in children with poor prognosis ALL in first CR.

BMT in second CR or greater

In 1981, a report by Johnson *et al.* from the FHCRC comparing the outcome of children with ALL in second CR treated with BMT and chemotherapy suggested there was a role for BMT in children with relapsed ALL [51]. Table 6.8 presents the outcome of large series in children. There is general agreement that BMT has a role in the treatment of children with ALL in second CR. However, controversy remains concerning which children should be considered for this treatment, as a significant number

Table 6.7 Bone-marrow transplant for ALL in first remission reports of outcome in children

Study group	Time of patient entry	High-risk indicator	Patients *(n)*	DFS/EFS†	Relapse	Reference
BMTR*	1978–90	t(9;22)	33 (13 Patients <16years)	2 years (34%)	42%	[48]
UKALL X**	1985–90	White blood cells >100000/µlitre	34 (1–15 years) 144	5 years BMT 69% NS Chemotherapy 52%	12% 41% P = 0.001	[49]
SFGM***	1980–87	White blood cells >100000/µlitre t(4;11), t(9;22), t(8;14) Induction failure	32 (1.5–16 years)	5 years (84%)	3.5%	[50]

International Bone Marrow Transplant Registry. **UK Medical Research Council Working Party on Childhood Leukaemia. ***Société Française de la Greffe de Moelle. ~Diesease-free survival/event-free survival. BMT=bone-marrow transplant; NS=not significant.

Table 6.8: Results of allogeneic transplant in children with ALL in second remission

Study group	Time of patient entry	Patients (n)	Conditioning	DFS/EFS (%)‡‡	Reference
FHCRC*	1973–85	57	CY/TBI	40	[52]
MSKCC**	1979–85	31	TBI/CY	5 years 64	[6]
Leiden (case-control study)***	1982–91	25 BMT 97 Chemotherapy	Multiple	4 years 44 24 NS	[53]
AIEOP/GITMO†	1980–90	57 BMT	Multiple	5 years 41 22 NS‡‡‡	[54]
Paris††	1983–93	42	Multiple	4 years 53	[55]

IBMTR (case-control study)[†††]	1983–91	255 BMT 255 Chemotherapy	Multiple	5 years	40 17	0.001	[56]
BFM[‡]	1985–91	51	Multiple	5 years 52			[57]

*Fred Hutchinson Cancer Research Center. **Memorial Sloan-Kettering Cancer Center. ***University Hospital Leiden. Hospital. [†††]International Bone Marrow Transplant Registry. [‡]Berli Frankfurt-Münster Group. [‡‡]Disease-free survival (DFS) [†]Associazione Italiana de Ematologiae Oncologia Pediatrica/Gruppo Italiano per il Trapianto de Midollo Osseo. [††]St Louis event-free survival (EFS). [‡‡‡]Early relapse (<30 months) BMT better P=0.002. CY=cyclophosphamide; TBI=total body irradiation. NS=not significant.

of children who relapse late can be salvaged with chemotherapy.

There are no prospective trials directly comparing BMT with chemotherapy. There are two case-control studies. The largest of these was carried out using data from the International Bone Marrow Transplant Registry (IBMTR), compared to data on the outcome of children treated with salvage chemotherapy on POG trials. This study demonstrated a superior outcome for children treated with BMT [56]. The second casecontrol study was smaller, and although there was no statistical difference between the two treatment groups for leukaemia-free survival, there was a greater relapse rate in children treated with chemotherapy [53].

A multicentre AIEOP/GITMO study conducted between 1980 and 1990 in children with ALL in second CR compared the outcome of 57 children who underwent BMT with 230 children treated with chemotherapy [54]. In this study, outcome with BMT was superior to chemotherapy in those children who relapsed early (<30 months from first CR). This result contrasts with that of the IBMTR study in which early and late relapse patients benefited from BMT.

The BFM group has developed a salvage protocol for children who relapse on front line BFM ALL protocols. They reported the outcome of 326 children treated on this protocol and identified a group of 101 children who relapsed more than six months off therapy in bone marrow and in an extramedullary site who achieved a seven-year EFS of 60% [58]. They conclude that chemotherapy is a more appropriate therapy for this cohort, considering the early treatment-related mortality associated with BMT and the long-term sequelae. In a subsequent paper, the BFM group evaluated the outcome of 130 patients with isolated extramedullary relapse and determined that salvage chemotherapy was superior to BMT (allogeneic and autologous) as the five-year EFS was 47% for those treated with chemotherapy compared 36% for those treated with BMT [59].

The majority of children who would clearly benefit from a matched allogeneic BMT will not have a suitably matched sibling donor. Thus, the role of alternative donors must be addressed. Table 6.9 outlines the results of BMT series in children with ALL using either mismatched family donors or unrelated donors identified through registries. Disease-free survivals between 30% and 47% are reported, with 10% reported in a very high risk subset.

Table 6.9 Results of unrelated donor or mismatched family donors transplants for ALL in children

Study group	Time of patient entry	Patients (n)/ disease status	Type donor	TCD††	Conditioning	DFS /EFS†††	Reference
Kentucky*	1987–92	32 total 21<15 years Relapse CR2 or 3	mm family† (1–3 Ag mm)	Yes	TBI/VP-16 Ara-C/CY	38% median follow up 3.7 years	[60]
SFGM**	1991–93	19 CR2	mm family (1–3 Ag mm) URDS††	Yes	TBI/Ara-C/Mel	30% median follow up 25 months	[61]
FHCRC***	1985–93	15 CR1 or 2 28 Relapse or CR3	URD (0–1 Ag mm)	No	CY/TBI	3 years (47%) 3 years (10%)	[34]

*University of Kentucky (Louisville). **Société Française de Greffe de Moelle. ***Fred Hutchinson Cancer Research Center. †Mismatched family (mm) donor. ††Unrelated donor/T-cell depleted. †††Disease-free survival (DFS)/event-free survival (EFS). CR=complete remission (2=second, 3=third); AG=Antigen; TBI=total body irradiation; Ara-C=cytosine arabinoside; CY=cyclophosphamide; Mel=melphalan.

Cord blood from unrelated donors is another source of haematopoietic stem cells that is becoming more accessible as cord blood registries are being developed. An early report from Kurtzberg *et al.* of 25 children who underwent BMT with unrelated cord blood rescue includes 12 with ALL [35]. Five children have engrafted and survive leukaemia-free 338 to 998 days post-transplant. Thus, these alternative sources of allogeneic stem cells are viable options for children who lack HLA identical siblings to serve as donors. As in AML, the best choice is not clear and will largely depend on which source of haematopoietic stem cells is available for a particular child. It is less clear in ALL which children should be treated with these alternative sources of stem cells, since salvage therapy is more effective in these patients (especially those relapsing late) than it is in children with relapsed AML.

ABMT

As the outcome for the majority of children with ALL is excellent, ABMT has not usually been considered in first CR. Most studies of ABMT in second CR and greater report DFS between 26% and 33% (Table 6.10). A case-control study comparing ABMT

to BFM salvage chemotherapy suggested there was no advantage to ABMT (EFS of chemotherapy 32%, of ABMT 26%) [64]. The exception is the Dana Farber study. Children with ALL who relapse longer than 24 months from initial diagnosis and in second CR or greater, experienced a DFS of 53% [65]. The conditioning regimen included teniposide, cytarabine, cyclophosphamide and TBI. Autologous marrow was treated with monoclonal antibodies. The results for patients who relapsed less than 24 months from initial CR were not as good.

The Minnesota group attempted to determine the contribution of *in vivo* minimal residual disease in treatment failure after ABMT [66]. This analysis determined treatment failure and was related to the leukaemic burden in the bone marrow prior to ABMT as measured by leukaemic progenitor assays. The study demonstrated that failure of the conditioning regimen to eradicate residual leukaemia, rather than re-infusion of inadequately purged marrow was the major factor responsible for relapse after ABMT in their patients.

Table 6.10 Results of pediatric trials of ABMT

Study group	Of	Patients (n)/ disease status	Purging	Conditioning	DFS/ EFS†††	Reference
EBMT*	1981–90	64 CR1	±	Multiple	4 years (46%)	[62]
		175 CR2	±	Multiple	6 years (33%)	
AIEOP/ GITMO* *	1987–92	27 CR2	±	Multiple	4 years (26%)	[63]
BFM (case-control study)***	1983–94	52 (ABMT) CR2	±	Multiple	9 years (26%)	[64]
DFCI†	1980–91	52 Chem therapy†† 51 CR 2–4	+	VM26/Ara-C/ CY/TBI	9 years (32%) 3 years (53%)	[65]

*European Bone Marrow Transplant Working Party. **Associazione Italiana de Ematologiae Oncologia Pediatrica/Gruppo Italiano per il Trapianto di Midollo Osseo. ***Berlin-Frankfort-Münster. †Dana Farber Cancer Institute. ††BFM relapse protocol. †††Disease-free survival (DFS)/event-free survival (EFS). CR=complete remission (1=first etc.); Ara-C=cytosine arabinoside; CY=cyclophosphamide; TBI=total, body irradiation; ABMT= autologous bone-marrow transplant.

This is in contrast to the study of Brenner in AML which demonstrated contaminating leukaemic cells in autologous marrow were implicated in relapse [22]. The relative importance of these factors may be different in AML and ALL.

A study of ABMT, reported by Tiley *et al.*, for high-risk ALL in first CR is noteworthy [67]. Thirty-eight patients, including 17 children, were treated with ABMT. The conditioning regimen included TBI and melphalan. After recovering from the transplant, patients were treated with two years of maintenance chemotherapy with 6-mercaptopurine and methotrexate. The projected DFS of this study is 50%. This suggests that results of ABMT could be improved by subsequent antileukaemic therapy, perhaps using chemotherapy, immunotoxins or immune modulators after ABMT.

Chronic myelogenous leukaemia (CML)

Adult-type CML accounts for 2–3% of the leukaemias seen in children. The biology of this disease in children is similar to that in adults [68], and is discussed in Chapter 4. Likewise, treatment of children with CML follows similar principles to those employed in adults. Because this disease is rare in children, there are few trials reported that focus exclusively on children.

The initial therapy of children with CML is usually hydroxyurea. A randomized adult trial comparing hydroxyurea with busulfan determined that median survival was greater in the hydroxyurea-treated patients [69]. Moreover, hydroxyurea has a better toxicity profile for patients who will ultimately undergo BMT. Specifically, busulfan can produce organ fibrosis, particularly marrow fibrosis which can impede post-BMT marrow engraftment [70].

Interferon-α, unlike hydroxyurea and busulfan, can produce karyotypic remissions in a subset of patients with CML. A randomized trial of interferon-α and chemotherapy (hydroxyurea initially followed by busulfan for failures) in adults demonstrated that interferon-α produced superior survival [71]. In a paediatric series of 15 children, 4 children treated with interferon-α responded with disappearance of Philadelphia chromosome-positive clones [72]. Two of these patients sustained prolonged karyotypic remissions. Interferon was relatively well tolerated in these children. Interferon therapy prior to BMT did not adversely effect BMT outcome in one study in which this question was evaluated [73].

Allogeneic BMT is the treatment of choice for children with CML. For children with HLA-matched sibling donors, BMT is generally recommended early in chronic phase. Most children will not have a matched sibling, but those with a 5 of 6 matched related donor should be transplanted, as outcome is comparable to matched sibling BMT [74].

BMT is the only established curative therapy for CML, although it remains to be determined whether a subset of patients responsive to interferon-α will ultimately be cured. Therefore, searching for an appropriately matched unrelated donor is justified.

The University of Minnesota transplant team compared the outcome of 11 children with CML who underwent BMT utilizing an unrelated donor with 11 children transplanted using matched sibling donors during the same time period [75]. The three-year DFS was 45% versus 78%. Of note, 10 of the 11 patients transplanted with unrelated donors were in advanced stage (accelerated phase, blast phase or greater than second

chronic phase) compared to 5 of 11 patients transplanted with matched sibling donors. This suggests that earlier identification of an unrelated donor prior to disease progression may have improved the outcome of these patients.

Approximately 10% of patients transplanted in chronic phase will relapse after BMT for CML. Treatment with donor mononuclear cells and interferon-α has been successful in sustaining karyotypic remissions in this situation [76]. This strategy has been reported to be successful in children [77].

Juvenile chronic myelogenous leukaemia (JCML)

Juvenile chronic myelogenous leukaemia is a rare but distinct clinical and pathophysiological haematological malignancy of childhood. This leukaemia presents in early childhood, generally prior to two years of age, with a male predominance. Presenting features include fever, infection, bleeding, pallor and failure to thrive. Consistent physical findings include massive splenomegaly, hepatomegaly, lymphadenopathy and cutaneous lesions. Haematological manifestations include leukocytosis, especially monocytosis, and thrombocytopenia. Frequently, blasts and normoblasts are present in the peripheral blood [78]. A characteristic feature is spontaneous proliferation of peripheral-blood leukaemic progenitor cells specifically sensitive to granulocyte-macrophage colony-forming factor (GM-CSF) [79]. JCML represents a disproportionate amount of the leukaemia seen in children with neurofibromatosis [80].

In most series, JCML has been reported to be universally fatal, although occasional patients have been reported with a less fulminant course [78]. Patients frequently respond to AML induction-type therapy, although these response are not durable [81,82]. A pilot trial of isotretinoin in 10 children with JCML induced complete and partial responses that were durable in 3 children [83].

BMT has been the treatment of choice for JCML since the initial report by Sanders *et al.* from the FHCRC [84,85]. A case report from the University of Graz suggested that a busulfan-etoposide-cyclophosphamide preparative regimen was insufficient to eradicate JCML [86]. A subsequent case report suggested that splenic radiation added to busulfan and cyclophosphamide resulted in successful BMT [87]. If busulfan is used in the preparative regimen, dosing should either be determined by surface area or pharmacokinetic dosing, as younger children are most likely to achieve inadequate levels by weight-based dosing [88]. Two recent reports of single-centre experience indicate the outcome with matched siblings is excellent and unrelated donors can also be successful for some patients [89,90].

Myelodysplastic syndromes (MDS)

Myelodysplastic syndromes are a group of clonal abnormalities with a propensity for transformation into overtly malignant conditions, particularly AML. They are rare haematological malignancies in children. BMT is the only curative therapy for these

conditions. Generally, patients with matched sibling donors should proceed directly to BMT. AML-type induction therapy can induce remission in many patients. Unfortunately, remission duration in these patients is usually short and prolonged survival rates low (<8% at four years) [91].

Because of the low incidence of this disease in children, there are not many large BMT series reported in the literature. A report from the University of Pavia of 8 children transplanted from matched sibling donors reported 6 patients who remained disease-free for more than eight months post-BMT [92]. A report of unrelated donor transplants from Milwaukee included 9 children with MDS. Five of these patients were reported to have survived 27–80 months after BMT [93].

Late complications of BMT

Long-term sequelae in children treated with BMT are an important consideration, especially when choosing BMT rather than an alternative treatment option which may result in less morbidity. Late effects may be the consequence of a combination of the following factors:

- the conditioning regimen;
- therapy preceding BMT especially cranial radiation;
- delayed or incomplete immune reconstitution;
- GvHD and its therapy, especially corticosteroids.

Dysfunction has been documented in multiple organ systems including the central nervous system, lungs, kidneys and the skeletal system [94–97]. The central nervous system effect of TBI is most worrisome in younger patients, especially those previously treated with cranial radiation [94]. The long-term neurotoxicity of busulfan is unknown, but as busulfan crosses the blood-brain barrier, important cerebral sequelae could ensue. The neurotoxicity of busulfan therefore deserves prospective study. Cataract formation is principally due to TBI, especially single-fraction therapy, but corticosteroid therapy also plays a role [98].

Growth and endocrine disturbances

Linear growth is impaired after BMT. Factors associated with reduced growth include younger age, prior cranial radiation, TBI—especially single dose, possibly busulfan, and GvHD [99,100]. The aetiology of this growth disruption may be multifactorial [101]. Growth hormone deficiency has been detected by many investigators. There is a greater risk of growth hormone deficiency in patients who were treated with cranial radiation prior to BMT, and it may be more prevalent in patients treated with single dose rather than fractionated TBI. Not all children with abnormal growth hormone testing respond to growth hormone replacement. Radiation-induced skeletal dysplasia, chronic GvHD and corticosteroid therapy, and thyroid deficiency may also play a role in poor growth. Thyroid dysfunction is common after transplant in patients who have received TBI. A mild compensated primary hypothyroidism has been noted to be transient [102].

Development of normal reproductive function depends on the patient's sex, preparative regimen and age at time of transplant [101,103]. Prepubertal boys treated with cyclophosphamide alone have been documented to progress through normal puberty. Germ-cell damage has been reported in boys treated with cyclophosphamide during or after puberty, manifested by elevated follicle-stimulating hormone (FSH). Two-thirds of these men may have normal semen and some have fathered children.

Boys treated with TBI retain the ability to subsequently produce testosterone and enter puberty spontaneously. The exception is boys who have received testicular radiation and require androgen replacement. Virtually all males treated with TBI develop germ-cell dysfunction manifested by reduced testicular volume and azoospermia. Recovery is rare and happens mainly in males treated with single-fraction TBI. A few men have fathered children after TBI.

Women treated with cyclophosphamide alone for aplastic anemia have been shown to develop normal ovarian function. This contrasts with the outcome of women treated with busulfan and cyclophosphamide who have a high risk of ovarian failure which does not seem to be reversible. The effect of TBI on subsequent ovarian function depends on age at the time of BMT. Fifty percent of prepubertal girls treated with fractionated TBI will develop normally. However, girls more than 12 years of age at the time of BMT will require hormonal replacement. Rarely, ovarian function has recovered and a successful pregnancy has been documented. Further details are given in Chapter 56 and 57.

Second malignancies

The most common second malignancies after BMT are Epstein-Barr virus (EBV)-associated lymphoproliferative disorders [104]. The greatest risk is in patients who receive mismatched T-cell-depleted transplants [105]. In addition to lymphomas, leukaemias have occurred in patients after transplant. Some have been determined to be of donor cell origin. Thyroid carcinoma is a well known complication of thyroid gland irradiation and has been diagnosed after transplant. There is also an increased risk of brain tumours in children with ALL and these have been documented to develop after BMT.

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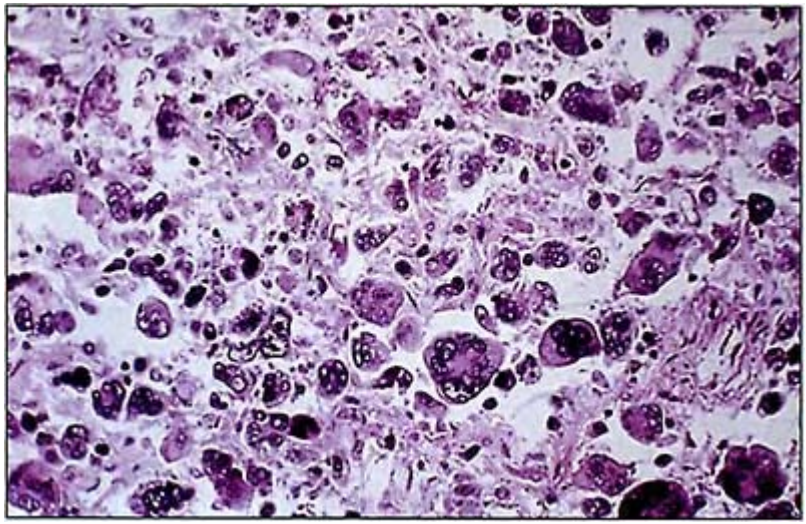

Chapter 7
The myelodysplastic syndromes and acute myeloid leukaemia following myelodysplastic syndrome

Theo de Witte

Introduction

The myelodysplastic syndrome (MDS) describes patients with refractory cytopenias and dysplastic changes in blood and bone marrow which carry an increased risk for undergoing transformation to acute myeloid leukaemia (AML). This syndrome has been variously termed ‘preleukaemia’, ‘refractory anaemia’, ‘oligoblastic leukaemia’ or ‘smouldering leukaemia’. MDS is a clonal expansion of the multipotential haematopoietic progenitor cell [1–3] with, in some cases, involvement of the lymphoid lineage too [4]. MDS occurs mainly in elderly persons around 70 years of age [5], but in a population-based study the annual incidence of MDS was 3.4/1000000 in children

below the age of 15 years [6]. The rising incidence in recent years may reflect increased awareness of the physician and willingness to perform diagnostic procedures in elderly patients [7], but an influence of occupational and/or environmental exposure cannot be excluded. The incidence of therapy-related MDS and AML (t-MDS/t-AML) is increasing owing to better survival following irradiation or chemotherapy for primary malignancies [8,9].

The myelodysplastic syndrome has been classified by the French-American-British (FAB) group into five subcategories [10] (Table 7.1). The FAB classification is based on the percentage of blast cells in marrow or blood, the presence of Auer rods in granulocyte precursors in bone marrow or blood, and the presence of ring sideroblasts, together with morphological evidence of dysplasia.

Clonal cytogenetic abnormalities have been reported in 23–78% of cases of MDS [5,11,12]. In

Table 7.1 French-American-British (FAB) classification of myelodysplastic syndromes.

- Refractory anaemia (RA).
- Refractory anaemia with ring sideroblasts (RARS).
- Refractory anaemia with excess of blasts (RAEB).
- Refractory anaemia with excess of blasts in transformation (RAEBt).
- Chronic myelomonocytic leukaemia (CMML).

contrast to AML, MDS is often associated with chromosome deletions as a primary karyotypic abnormality. Translocations such as t(8;21) and t(15;17), which are characteristic of certain types of AML, are rare [13]. t-MDS/t-AML occurring after chemotherapy with alkylating agents is characterized by cytogenetic abnormalities which are also associated with primary myelodysplasia (abnormalities involving chromosome 5 or 7, and complex abnormalities) [14]. In patients treated with drugs targetting DNA-topoisomerase II, such as etoposide, doxorubicin or mitoxantrone, balanced translocations of chromosome bands 11q23 and 21q22 have been observed quite frequently [15,16].

Prognosis

The clinical course of the myelodysplastic syndromes varies from an indolent form to a rapidly fatal disease. Eventually, MDS may evolve into frank leukaemia. RA or RARS without profound cytopenias are characterized by a low risk of transformation to acute myeloid leukaemia and a median survival usually exceeding 30 months [17]. Median survival of patients with RAEB or RAEBt is less than 12 months despite any treatment given [10]. The median survival of patients with therapy-related MDS is usually very short: 12 to 30 weeks. A rapid evolution to AML occurs in 30–55% of patients with therapy-related MDS within 3–5 months [18].

A modified Bournemouth score for CMML identified patients with a good prognosis and a clinical course similar to that of refractory anaemia, and a less favourable subgroup [19]. Marrow infiltration with more than 5% monoblasts, a neutrophil count of more than 16×10^9/litre and/or a monocyte count of more than 2.6×10^9/litre was associated with a poor prognosis (Table 7.2).

Patients with a normal karyotype have a better prognosis than those with single abnormalities [11,12]. Patients with complex cytogenetic abnormalities have the worst prognosis [11,12,20]. Patients with a normal *in vitro* growth pattern and a normal *in vitro* differentiation in the CFU-GM assay have a median survival of more than 18 months (Table 7.3). These categories of patients constitute a minority amongst patients with MDS [21].

Table 7.2 Poor prognostic features of CMML.

- Marrow infiltration with more than 5% monoblasts.
- Neutrophil count of more than 16×10^9/litre.
- Monocyte count of more than 2.6×10^9/litre.
- Patients with complex cytogenetic abnormalities have the worst prognosis.

Table 7.3 Good prognostic features of CMML.

- Patients with normal karyotypes have a better prognosis than those with single abnormalities.
- Patients with a normal *in vitro* growth pattern and normal *in vitro* differentiation in the CFU-GM assay have a median survival of more than 18 months.

The cornerstone of therapeutic management for most patients with MDS is supportive care [18], mainly in view of the average advanced age in MDS and the poor response to therapy.

Intensive chemotherapy

Conventional, multidrug chemotherapy such as that used to induce complete remission (CR) in *de novo* AML has been demonstrated to be effective in MDS, with CR rates varying from 15% to 64% [18,22–26]. CR rates of patients with MDS or secondary AML (sAML) appear lower than those of patients with *de novo* AML treated with similar chemotherapy regimens. The higher failure rate of remission-induction therapy can be explained partly by the longer duration of hypoplasia after chemotherapy [25–27], and also by a higher intrinsic biological drug resistance of the leukaemic clone [28,29]. Some patients with MDS in CR after combination chemotherapy may achieve prolonged, disease-free survival [23,30], but overall median remission duration appears to be short and usually less than 12 months [25,31]. Prolonged survival rates are disappointingly low and have been reported to be 8% at four years after chemotherapy [24] and 7% after three years [25].

Prognostic factors for outcome after intensive chemotherapy

Patients with the morphological picture of RAEB [23] and RAEBt [30] appear to respond favourably to intensive chemotherapy, with remission rates approaching those of *de novo* AML. Auer rod-positive patients, regardless of karyotype, appear to have a better prognosis than Auer rod-negative patients when treated with intensive chemotherapy [32]. Patients with secondary AML evolved from MDS respond less well to chemotherapy than do those with *de novo* leukaemias, and long-term remissions are rare [13,25,31]. The clinical outcome after intensive chemotherapy for t-MDS/t-AML is usually poor, with only an exceptional patient surviving beyond one year [23,33]. A minority of the patients with specific cytogenetic rearrangements of *de novo* AML, or no cytogenetic abnormalities, who present with AML not preceded by MDS seem to have a high remission rate and prolonged CR duration in many cases [13].

Childhood myelodysplastic syndrome responds poorly to intensive chemotherapy. The proportion of complete remissions in 20 patients with MDS was 35% compared to 74% in 35 children with AML treated with the same protocols [34]. Patients younger than 45–50 years appear to respond better to combination chemotherapy than do older patients. CR rates in patients less than 45–50 years old ranged from 71% to 86% in several studies [22,25,31,35] and the remission rates in the older patients ranged from 25% to 43% [22,25,31,35].

The presence of cytogenetic abnormalities specific for MDS, such as abnormalities of chromosomes 5 or 7, has a major negative impact on the prognosis after combination chemotherapy. Fenaux *et al.* observed a CR rate of 57% in MDS patients with a normal karyotype [30], contrasting with a CR rate of 31% in patients with rearrangements of chromosomes 5 or 7. None of the five patients with multiple chromosomal abnormalities achieved complete remission in a Leukaemia Cooperative Group study of the European Organisation for Research and Treatment in Cancer (EORTC) [36]. Remission duration is extremely short in patients with cytogenetic abnormalities of chromosomes 5 and/or 7, with all patients relapsing within five months [30].

Autologous bone-marrow transplantation (ABMT)

Maintaining remission after remission-induction chemotherapy is a difficult issue. Some patients may achieve prolonged, disease-free survival (DFS) if treated with post-remission chemotherapy, but overall median remission duration is usually less than 12 months [24,25,30]. The experience with ABMT in patients with MDS or sAML is limited, and the literature contains only case reports [54,55], Until now, 114 recipients of autologous marrow grafts with MDS or AML following MDS have been reported to the registries of the EBMTG [37]. The overall survival at two years of the 79 patients transplanted in first CR was 39%, the DFS was 34% and the actuarial relapse rate was 64%. Nineteen patients were transplanted for MDS which had not progressed to AML prior to ABMT. The actuarial DFS at two years after ABMT was 40% and the relapse rate 58%.

Thirty-nine MDS patients had progressed to AML prior to chemotherapy and ABMT. The DFS of these patients was 30% and the relapse rate 68%. Twenty-one patients were transplanted for MDS or AML which had developed after treatment with chemotherapy for other malignancies or autoimmune diseases. Actuarial DFS of these patients was 36% and the relapse rate 60%. Patients younger than 40 years had a significantly (P=0.04) better DFS compared to patients with ages over 40 years. This difference could be explained by the significantly higher relapse rate of 72% in the older age group compared to the 59% in patients younger than 40 years (P=0.05).

Transplant-related mortality and death due to regeneration failure did not appear to occur more often than after ABMT for *de novo* AML. Haematopoietic engraftment was slower despite sufficient numbers of CFU-GM collected per kilogram body weight (5×10^4/kg), similar to the observations in *de novo* AML patients [38]. In the first retrospective analysis of the EBMT on 17 autografted MDS patients, median time to engraftment was 37 days for white blood cells and 75 days for platelets [39].

Laporte *et al.* reported the results of ABMT with mafosfamide-treated marrow in 7 patients with AML following MDS. Haematopoietic engraftment was also slower in these 7 patients, but all patients engrafted except for one who died early of treatment-related causes, before engraftment. Two patients were alive and well at 10 and 28 months following ABMT [38]. Demuynck *et al.* investigated the feasibility of collecting peripheral blood stem cells (PBSC) in 11 patients with myelodysplasia [40]. This resulted in an adequate yield ($>1\times10^6$/kg body weight) of CD34 cells in seven patients [40]. Three patients with a normal to excellent stem-cell harvest were demonstrated to be polyclonal by polymerase chain reaction (PCR) techniques based on X-chromosome inactivation patterns [41]. Six patients received autologous peripheral blood stem-cell transplantation in a prospective study of the EORTC Leukemia Cooperative Group and the EBMTG [42]. Peripheral stem cells were collected during recovery after the first consolidation course using 300 µg of filgrastim (s.c.) daily until completion of PBSC collections. Preliminary data indicate that repopulation was much faster compared with autologous bone-marrow transplantation [40,42].

Allogeneic bone-marrow transplantation with HLA-identical sibling donors

In view of the lack of satisfactory therapies, allogeneic BMT is today the treatment of choice in the majority of young patients with histocompatible siblings. The first reported cases were transplanted more than ten years ago [43,44]. Larger series of patients have been reported from Seattle [45,46] and City of Hope Hospital, Duarte [47]. Eight children with MDS received allogeneic transplants in the Dana Farber Cancer Institute [48]. National and international bone- marrow transplant registries collected data on several hundreds of patients transplanted for myelodysplasia and the first analysis was reported in 1990 [49].

The results of treatment with allogeneic BMT varied considerably depending on the stage of disease at the time of transplantation and various clinical factors, such as the presence of cytogenetic abnormalities [49,50], age [49,50] and the percentage of blasts in the bone marrow at time of transplantation [46,47,50]. For these reasons, various

prognostic factors (Table 7.4) which may influence treatment outcome after allogeneic BMT will be discussed.

Table 7.4 Good prognostic features for allogeneic BMT.

• Myeloablative conditioning regimen.
• Short duration of disease.
• Less than 10% blasts in marrow.
• No cytogenetic abnormalities.
• Lower age at time of transplant.
• Prior Intensive chemotherapy.

Refractory anaemia and refractory anaemia with ring sideroblasts

Patients with RA and RARS and profound cytopenias are generally considered good candidates for allogeneic BMT. Relapses are rare, provided that the pretransplant conditioning includes a bone-marrow ablative regimen, such as total body irradiation (TBI) and high-dose cyclophosphamide. Disease-free survival usually exceeds 50% after allogeneic BMT [45,49]. Patients with the clinical picture of severe aplastic anaemia (SAA), including hypocellular marrow and severe pancytopenia, but with specific cytogenetic changes associated with MDS (such as monosomy 7) should also receive ablative conditioning therapy. Three patients, prepared for transplantation with cyclophosphamide alone, showed either a persisting or rapidly re-emerging abnormal clone [44].

Due to the low numbers of transplanted patients, it is still not possible to assess the impact of most pretransplant variables on the outcome of transplantation. In the Seattle analysis, disease duration appeared to have a significant effect on transplant-related complications but not on disease-free survival in a multivariate analysis [45,46]. For this reason, the aim should be early transplantation before sensitization due to transfusion of blood products and before development of iron overload and opportunistic infections. However, postponement of the transplant may be justified in patients with a relatively good prognosis. These patients are characterized by an absence of profound, and life-threatening cytopenias and an absence of transfusion requirement.

Refractory anaemia with excess of blasts (in transformation)

Twenty patients were transplanted for RAEB and RAEBt in the City of Hope Hospital, Duarte [47]. An increase of marrow blasts to more than 10% appeared to have a negative impact on disease-free survival, since three out of four relapses were in this group. Only

2 of 10 patients with this characteristic survived, as compared with 6 of 10 patients who had less than 10% marrow blasts at the time preparation for BMT was started.

The EBMT analysis showed an actuarial DFS of 74% when the transplant was performed in a patient with RAEB at time of transplantation and 50% when the patient had RAEBt [49]. A later analysis from the EBMT showed a three-year DFS of 32% from 18 patients transplanted for RAEB, and of 27% for 11 patients transplanted for RAEBt. Late relapses occurred especially in patients with RAEB, showing a relapse pattern similar to chronic myeloid leukaemia (CML) after allogeneic BMT. The actuarial relapse rate at three years after BMT was more than 50% in this group [51].

One of the analyses from Seattle [45] showed a similar cumulative relapse risk of 45% in 30 patients transplanted with RAEB or RAEBt. Half of the relapses occurred more than one year after transplantation.

Chronic myelomonocytic leukaemia

Only a few patients with CMML have been treated with allogeneic BMT. The City of Hope report contained data on two patients with CMML. One patient was alive almost three years after BMT; the other died from transplant-related complications [47].

Secondary acute myeloid leukaemia (sAML)

Most European transplant centres will only consider allogeneic bone-marrow transplantation for sAML after remission-induction therapy. Results of allogeneic BMT as primary therapy appeared worse for patients with overt sAML as compared to patients with RAEB or RAEBt. The relapse risk after BMT was invariably very high, and ranged from 65% to 100% [49,50]. Prolonged DFS was around 20% when transplants were for sAML [49]. Some cases of sAML, with hypocellular marrows who are unlikely to respond favourably to intensive chemotherapy, have achieved prolonged disease-free intervals after allogeneic BMT without chemotherapy prior to the conditioning [42]. In these cases, immediate allogeneic BMT may be considered the treatment of choice.

Remission duration for patients treated with AML-type remission-induction chemotherapy is usually short [23,25,30], especially in those patients with cytogenetic abnormalities [30]. These results suggest that allogeneic BMT should be offered to these patients as consolidation therapy in complete or partial remission after intensive chemotherapy. The two-year DFS was 60% for 16 patients transplanted in complete remission after chemotherapy [49]. Patients with a partial response after chemotherapy responded less well and showed a two-year DFS of 18%, while none of those who either relapsed or who were resistant to chemotherapy survived BMT for more than two years.

Therapy-related MDS/AML

The actuarial DFS of 11 patients transplanted for therapy-related MDS/AML was 27% compared to a DFS of 56% in 12 patients transplanted for primary MDS. These two groups were not completely comparable since the numbers of patients with overt AML was 5 in the therapy-related group compared to none in the control group [52]. All 7

patients transplanted for overt AML secondary to therapy for Hodgkin's disease died of multiorgan failure (4 patients) or leukaemia [53].

Four patients with AML secondary to treatment for Hodgkin's disease were transplanted in first complete remission. Two patients were alive and disease-free at the time of reporting [54].

The EBMT [51] compared transplant results of 28 patients with therapy-related RAEBt or AML with the results of 53 patients with *de novo* RAEBt or AML evolved from MDS. The overall DFS was identical, but the relapse rate was slightly higher in the therapy-related group.

Cytogenetic abnormalities

In an earlier Seattle analysis, cytogenetic abnormalities were significantly associated with better DFS compared to patients without a cytogenetic abnormality, largely due to an increased incidence of transplant-related mortality in patients without cytogenetic abnormalities [45]. A larger cohort of patients with a longer follow-up in the most recent analysis from Seattle showed that cytogenetic abnormalities were no longer significantly associated with an improved outcome [46].

The EBMT analysis [51] showed an identical overall DFS when patients with and without chromosomal abnormalities were compared. However, the relapse rate was significantly higher in the subgroup with cytogenetic abnormalities: 45% versus 8% in the group without cytogenetic abnormalities (P=0.03).

A French registry study showed a higher relapse rate for the patients with complex cytogenetic abnormalities, resulting in a low (14%) EFS [50].

Myelofibrosis

Myelofibrosis may result in slow engraftment and graft rejection. None of the 6 patients with grade 3 or 4 myelofibrosis survived in the reported series from Seattle [45]. The survival in patients having extensive marrow fibrosis as compared with those with little or no fibrosis appeared similar in another study [47].

Allogeneic bone-marrow transplantation with alternative donors

Two-thirds of the patients with MDS who may benefit from allogeneic BMT lack a suitable family donor. Better functioning of the worldwide registries of human leukocyte antigen (HLA)-typed volunteer marrow donors has made allogeneic BMT with fully or partially matched unrelated donors a realistic alternative. Reported data are scarce and follow-up limited [46,56]. Recently, Kernan *et al.* [56] reported an 18 ± 14% DFS two years after transplantation for 32 patients with MDS. Arnold *et al.* observed a probability of survival at three years after BMT of 0.30 for the 67 patients reported to the EBMT registry [57]. Anderson *et al.* described the results of 52 patients with MDS and secondary AML transplanted from an unrelated donor [58]. The median age was 33 years

(range 1–53 years). The two-year DFS, relapse and non-relapse mortality rates were 38%, 28% and 48%, respectively. The incidence of non-relapse mortality was higher compared to HLA-identical related recipients, which may be related to a higher incidence of acute and chronic graft-versus-host disease.

Increasing age was significantly associated with increased risk of death from non-relapse causes. The non-relapse mortality was 16% in patients younger than 20 years, 66% and 53% for patients between 21 and 40 years and patients older than 40 years, respectively [58]. Arnold observed a significant increase of transplant-related mortality (TRM) in patients older than 35 years [56]. Patients over 35 years (n=17) of age had a TRM of 0.90, versus 0.50 in the patient group under 35 years (n=50).

Longer disease duration was also associated with an increased transplant-related mortality [58]. The relapse rate was 6% for patients with RA, RAEB or CMML at time of transplantation, which was significantly less than the 56% relapse rate for patients who had progressed to RAEBt or sAML. Fifteen patients had received intensive chemotherapy prior to the transplantation. The results suggested a reduced incidence of relapse after BMT and improved survival, but the limited number of patients and selection biases precluded making firm recommendations about whether patients with RAEBt or sAML should routinely receive induction therapy before transplantation [58].

Conclusions

Allogeneic BMT is the treatment of choice for patients with MDS or sAML, with a high chance of long-term DFS if the transplant is performed at an early stage of the disease or if the patient is transplanted in complete remission after chemotherapy. Transplantation is limited to a minority of relatively young patients (aged below 55 years) with an HLA-identical sibling. This means that less than 10% of the patients with MDS or sAML may benefit from this treatment approach. Nevertheless, the EBMT survey showed that around 100 patients in Europe received allogeneic BMTs for MDS or sAML in 1992 [59].

Allogeneic BMT may also be considered when a closely or fully matched unrelated donor has been identified for a young and fit patient.

Most patients will benefit optimally from allogeneic BMT when the transplant is performed as soon as an HLA-identical family member has been found. Progression to more advanced leukaemia will be associated with a higher failure rate, mainly due to an increased incidence of relapse after BMT. Delay of the transplant may be justified in a minority of RA or RARS patients without cytopenias or complex cytogenetic abnormalities and without leukaemic *in vitro* growth characteristics.

All patients, including those without excess of blasts, should be conditioned with bone-marrow ablative therapy rather than an immune suppressive regime, such as cyclophosphamide alone. Total body irradiation has been included in most BMT conditioning regimens, but preparative regimens based upon oral busulfan may produce similar results [60,61]. The degree of marrow infiltration with leukaemic blasts appears to be prognostic for the chance of relapse after BMT. Whether a more intensive conditioning regimen or intensive polychemotherapy prior to the transplant procedure may yield better results is at present unknown.

The Chronic Leukaemia Working Party of the EBMT is conducting a prospective registration study [62]. Patients with MDS or sAML are to be entered prior to any attempt to induce remission by polychemotherapy or BMT. This study is expected to give comparable groups of patients with HLA-identical siblings who are treated either by BMT as primary treatment or by BMT as consolidation therapy. The second aim of this registration study is to evaluate survival of potential candidates for BMT with MDS or sAML, depending upon the availability of an HLA-identical donor.

However, for the majority of patients, there is no standard therapy other than appropriate supportive care. Relatively young patients below the age of 60 years with poor-risk features can be considered for treatment with combination chemotherapy. Simple scoring systems, such as the Bournemouth [17] and the Düsseldorf [63] scores should be combined with cytogenetic data [11]. The potential benefit of haematopoietic growth factors in reducing the hypoplastic period following chemotherapy, or in inducing proliferation of leukaemic progenitors to render these more sensitive to cycle-specific cytotoxic agents, should be assessed in prospective randomized studies.

Maintaining remission after remission-induction chemotherapy is a difficult issue. Patients not eligible for allogeneic BMT could be treated with post-remission chemotherapy or autologous BMT within the framework of prospective studies. Older patients can be considered for treatment with haematopoietic growth factors alone or in combination with differentiating agents, such as low-dose cytosine arabinoside (Ara-C). This treatment should be delivered in the context of carefully designed and conducted trials including analysis of biological features, such as cytogenetics, oncogene expression and the various drug-resistance mechanisms.

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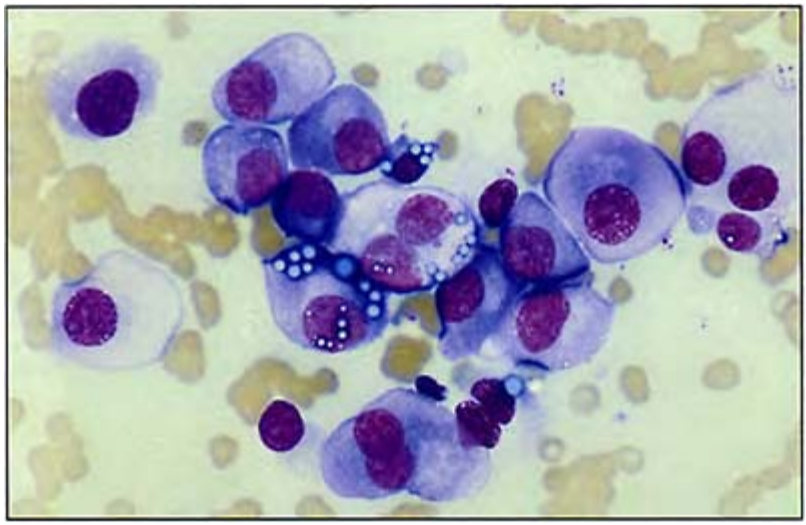

Chapter 8
Multiple myeloma

Noopur Raje and Ray Powls

Introduction

Although numerous studies have documented tumour responsiveness to both chemotherapy and radio-therapy, multiple myeloma remains an incurable disease. The standard treatment for myeloma for more than three decades has remained a combination of oral melphalan and prednisolone [1]). Various other combinations such as ABCM, VMCP/VBAP and MOCCA [2–5] have been used, but with the exception of ABCM no other treatment strategy has shown a benefit over melphalan and prednisolone. A recent meta-analysis of combination chemotherapy versus melphalan and prednisolone by Gregory *et al.* [6] of 3814 patients concluded that both treatments were equally effective. A subgroup analysis in this same study revealed melphalan and prednisolone to be superior for patients with intrinsically good prognosis disease, while combination chemotherapy was beneficial for poor prognosis myeloma. This meta-analysis, however, excluded the important MRC V trial [7] which documented the benefits of ABCM combination chemotherapy over oral melphalan, because the control arm was treated with melphalan alone.

Irrespective of the nature of the frontline treatment in terms of chemotherapy, response is seen in not more than 60–70% of the patients, and median survival does not exceed three years.

A complete remission (CR) in myeloma was documented for the first time in the early 1980s [8], and this was the first step towards curative treatment approaches in myeloma. Eight patients with myeloma and 1 patient with plasma cell leukaemia were treated with escalating doses of melphalan (100–140 mg/m^2). All patients responded, and 3 out of 5 previously untreated patients achieved a biochemical and bone-marrow CR, with disappearance of serum paraprotein and a clearing of the bone marrow of plasma cells. As is true of all haematological malignancies, achieving CR is the first step towards cure. Thus, achieving CR became an important goal in the therapy of myeloma. Data using escalating doses of melphalan accumulated, and a dose-response effect was noted even in patients who were primarily resistant to conventional doses of melphalan. This dose-response effect to high-dose melphalan has lead to its wide use in the treatment of myeloma in the transplant setting.

The last decade has seen the use of high-dose chemoradiotherapy and autologous transplantation in more than a thousand patients. Experience in allogeneic transplantation is more limited. This can be attributed to the fact that the median age of myeloma patients is around 60 years, and therefore the number of patients eligible for such a procedure is limited. With the histocompatibility complex allowing only approximately 1 in 4 eligible patients to have a matched sibling donor, a further restriction on the number of patients undergoing this procedure is imposed.

However, with improvements in supportive care and better graft-versus-host disease (GvHD) prophylaxis, the age limit for allogeneic bone-marrow transplantation (BMT) is becoming less restrictive and more patients are being offered aggressive treatment.

Allogeneic bone-marrow transplantation

Current experience

The first reported BMT for myeloma was carried out by the Seattle team using a syngeneic donor [9], and over recent years the proportion of patients undergoing allografting with curative intent has increased [10–13]. To date, approximately 268 allotransplants have been carried out for myeloma worldwide [14]. This number appears low, given the fact that myeloma remains incurable with other treatment approaches. However, with advances in the field of transplantation and the consequent reduction in transplant-related mortality, more patients will undoubtedly be offered this form of treatment in the future.

Most reported literature in the allogeneic setting comprises registry data and results are now maturing sufficiently to allow certain conclusions to be drawn, though a lot of questions remain unanswered. The European Bone Marrow Transplant (EBMT) Registry initially reported on the outcome in 90 myeloma patients undergoing an allogeneic BMT [11]. The rationale for using this procedure was to provide a graft devoid of tumour cells, and to possibly induce a graft-versus-tumour effect, as seen in certain leukaemias [15,16]. In this study, Gahrton *et al.* [11] concluded that the stage of disease at diagnosis and the

number of treatment regimens tried before transplantation were predictive of the likelihood of complete remission occurring after engraftment. There were trends toward longer survival among patients who were responsive to treatment before BMT, patients with stage I disease at diagnosis, and patients who had received only first-line treatment before transplantation, as compared with those who were not responsive, those with stage II or III disease at diagnosis, and those who had received three or more lines of treatment. However, the differences in these factors were not statistically significant.

Two post-transplantation factors predicted for better long-term survival: complete remission after engraftment (P=0.0001) and grade I GvHD rather than grade II, III or IV. The problem of GvHD was no greater than after transplantation for other disorders, and a slightly poorer survival rate was noted in patients who had no GvHD as compared to grade I GvHD, increasing the probability of a possible graft-versus-myeloma effect.

In a recent update of the registry data, Gahrton *et al.* [17] have analyzed 162 allogeneic matched sibling-donor transplants who were treated between 1983 and 1993. The median age of these patients was 43 years (range: 23 to 59 years). This was a heterogeneous patient population with varying numbers and types of pre-BMT treatments. Forty-six patients had received three or more lines of treatment prior to BMT. Conditioning for BMT was total body irradiation (TBI) plus cyclophosphamide alone in 55 patients, TBI plus cyclophosphamide and melphalan in 27 patients, and TBI plus other drug combinations in 35 patients. Forty-seven patients received non-radiation containing conditioning, of which 25 received busulfan and cyclophosphamide. Treatment for prevention of GvHD varied. Twenty-one different combinations of T-cell depletion, methotrexate, cyclosporin, prednisone and cyclophosphamide were used. Cyclosporin plus methotrexate was the single most common combination and was used in 74 patients. Of the 121 evaluable patients, 72 were in CR following BMT. The median time from BMT to CR was three months, and in 90% of the patients who achieved CR, the paraprotein had disappeared 12 months post-transplant.

Outcome after BMT was highly dependent on factors before conditioning. Forty-nine of 64 evaluable patients receiving first-line treatment prior to BMT were assessable for CR, and 36 out of these 49 patients achieved CR, while only 19 out of 41 patients receiving second-line treatment and 16 out of 29 evaluable patients on third-line treatment entered CR (P= 0.008). Other factors which were important predictors of response included stage at diagnosis and patient sex. Stage I disease patients and female sex experienced a significantly better CR rate. Fifty-seven of 76 patients who had evidence of bone disease prior to BMT showed no evidence of change or healing following transplant. One hundred and thirty-four patients were evaluated for GvHD. Thirty-seven percent of the patients had no evidence of GvHD. Forty-three had grade I, 29 had grade II, 6 had grade III and 6 more had grade IV GvHD. Use of T-cell depletion as a preventive measure for GvHD significantly reduced the incidence of Grade II to IV GvHD (P=0.02).

The overall median duration of survival after BMT was 17 months. The four-year survival rate was 32% and the seven-year survival rate 28% (Figure 8.1). Female sex, stage I disease at diagnosis, patients who had undergone only one line of treatment prior to BMT, patients in CR at the time of conditioning and those who achieved CR following BMT predicted for improved survival in univariate analysis.

Patients with grade III or IV GvHD had significantly poorer survivals than those with grade I or II GvHD. No difference in survival was noted between patients who did not develop GvHD and those who had grade I or II GvHD. Multivariate analysis revealed grade III or IV GvHD to be adverse prognostic factors for survival. Patients who achieved CR following BMT had a significant survival advantage, with the five-year survival being 52% and the seven-year survival 47% (Figure 8.2).

The overall relapse rate was 45% at 60 months. The relapse-free survival of patients who entered CR was 34% at 6 years. Transplant-related mortality (TRM) was fairly high in this series, with 76 of 103 patients dying due to regimen-related toxicity. Unfortunately, only 9 patients remain in continuing CR at more than four years after allografting.

The Seattle group [18] presented their series of 60 myeloma patients conditioned with busulfan and cyclophosphamide. Forty percent of patients died of transplant-related causes. CR was achieved in 37%, and the two-year event-free survival and overall survival of the CR patients were 48% and 68%,

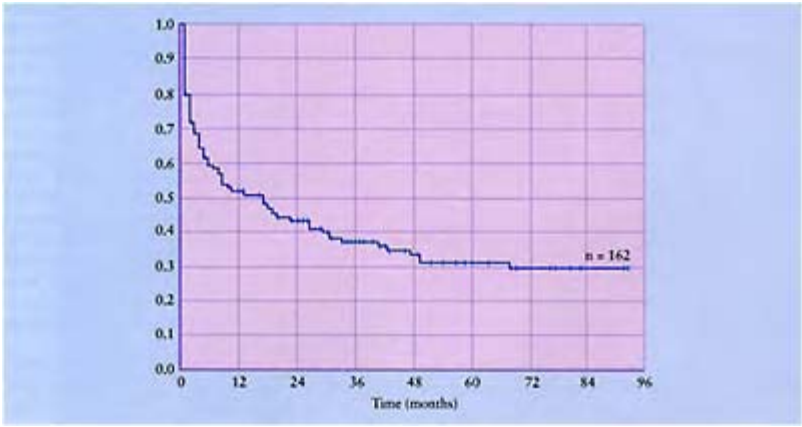

Figure 8.1 Actuarial survival in months in 162 patients after allogeneic bone-marrow transplantation for multiple myeloma [17].

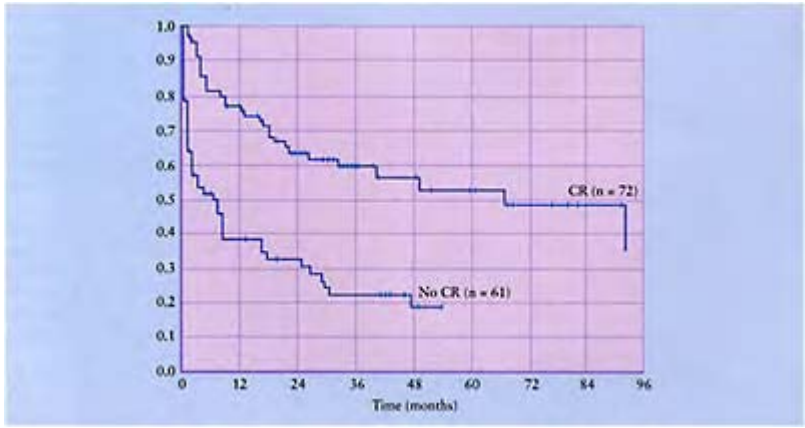

Figure 8.2 Actuarial survival after BMT according to whether patients were in complete remission (CR) after engraftment. There is a significantly better survival for patients who entered complete remission after engraftment than for those who did not (P=001). [17].

respectively. Syngeneic BMT has been performed infrequently, but the Seattle group report 2 of 7 patients remaining disease-free at 9 and 15 years after BMT [9,19,20].

Anderson *et al.* [21] presented their series of 40 patients with plasma cell dyscrasias who received monoclonal antibody-purged BMT for myeloma. Fourteen of these 40 patients underwent an allograft, including 1 syngeneic transplant. Ablative treatment included TBI and cyclophosphamide in 11 allografts and in the syngeneic transplant. Two patients received a combination of busulfan and cyclophosphamide. These 2 patients had received prior radiotherapy which precluded the use of TBI. Allogeneic marrow that had been treated with anti-CD6 monoclonal antibody and rabbit complement to deplete T-cells was reinfused fresh. Seven of the 14 patients achieved CR and 2 patients died of toxicity. With a 24-month median follow-up, 9 of the 14 patients are alive and 8 patients remain free from progression at >8 to>34 months post-BMT.

Vesole *et al.* [22], in their series of 35 patients from the University of Arkansas, demonstrated a CR rate of 34%, with a two-year event-free and overall survival of 22% and 44%, respectively. All their patients received conditioning with busulfan and cyclophosphamide and their TRM was 26%.

In 1994, the International Bone Marrow Transplant Registry (IBMTR) presented their series of 257 patients reported to the IBMTR between 1981 and 1992 by 63 centres worldwide, at the American Society of Haematology [13]. Eighty-seven percent of the transplants were from HLA-identical sibling donors, 4% from syngeneic donors and 9% from alternative donors. Thirty-three percent of the patients received conditioning with

busulfan and cyclophosphamide, while 65% received TBI and either cyclophosphamide or melphalan. Seventy-two allograft recipients survive a median of 30 months (range: 3 months to 10 years) post-transplant. Probabilities of survival (95% confidence interval) were 53±7% at six months, 37± 7% at two years, 27±7% at four years and 24±7% at five years. Eleven patients are alive more than five years post-transplant; 7 are in remission. Probability of survival was increased in persons with performance scores of greater than 80% and with sensitive disease pretransplant.

No consensus has as yet been reached concerning the optimum conditioning regimen. Both the EBMTR and the IBMTR data revealed the superiority of TBI plus cyclophosphamide-containing conditioning over busulfan and cyclophosphamide. These two regimens have recently been compared by the Nordic Bone Marrow Transplant Action Group [23] in a prospective randomized trial of other haematological disorders. Although no significant difference in overall survival was found, patients with more advanced disease had significantly poorer survival using chemotherapy alone. Veno-occlusive disease and haemorrhagic cystitis were also more frequently noted in the chemotherapy alone arm.

The combination of busulfan and cyclophosphamide has been extensively studied by the Seattle group [24]. Twelve out of 15 assessable patients (n= 20) achieved CR, and the probability of survival at three years was 36%. Schiller *et al.* [25] used this combination for transplantation in advanced myeloma. Regimen-related toxicity consisted of 5 early deaths out of 23 patients, 3 of which were a result of veno-occlusive disease. Fatal and non-fatal hepatic and renal toxicities were related to busulfan, with most complications occurring at the 16 mg/kg dose. They therefore concluded that toxicity precluded using a total dose of busulfan beyond 14 mg/kg.

New approaches

Allogeneic peripheral stem cells are being used [26,27] in transplantation for all haematological malignancies with a view to securing early haematopoeitic and immune reconstitution and thereby reducing the incidence of transplant-related complications. However, the possibility of an increased incidence of chronic GvHD exists for recipients of allo-peripheral blood stem cells) [28,29]. Results of these trials are very preliminary and longer follow-up is required before any firm conclusions can be drawn.

Persistence of the underlying disease remains a major obstacle limiting the success of ablative therapy followed by allogeneic BMT. A graft-versus-myeloma effect has recently been documented by Tricot *et al.* [30], and the existence of a graft-versus-leukaemia effect is well established. Tricot *et al.* [30] treated a 40-year-old patient with myeloma refractory to standard chemotherapy and autologous transplantation with a matched unrelated T-cell-depleted transplant, after conditioning with fractionated TBI, thiotepa and cyclophosphamide. This procedure resulted in a transient and incomplete response with evidence of rapidly progressive disease within 2.5 months after BMT. The patient then received a small number of donor peripheral blood mononuclear cells ($CD3^+$ cells 1.2×10^6/kg) without any further cytotoxic therapy. A CR was attained, which lasted for 14 months. The procedure was associated with severe acute, and subsequently, limited chronic GvHD. This is the first reported direct evidence for a graft-versus-myeloma effect.

More recently, Alyea *et al.* [31] presented their data on 7 patients with relapsed disease following allogeneic BMT who received $CD4^+$ donor lymphocyte infusions. All patients had previously undergone CD6 T-cell-depleted allotransplantation and all had received interferon post-BMT. Six out of 7 patients had persistent evidence of disease after BMT and all had progressive disease at the time of donor lymphocyte infusion. One patient died of rapidly progressive disease three weeks after immunotherapy and was not evaluable for response. The median follow-up for the remaining 6 patients is 39 weeks, and 4 out of 6 patients showed a response to donor lymphocytes, thereby demonstrating a graft-versus-myeloma effect.

Approaches such as this may be useful in patients with early relapse, or evidence of minimal residual disease following BMT and should be studied in more patients.

Donor immunization with myeloma idiotype represents a new strategy for enhancing the specific antitumour effect of allogeneic marrow grafts [32,33]. The idiotypic determinants of the immunoglobulin synthesized by a clonal B-cell cancer are unique and can serve as tumour-specific antigen. In a pilot study, Kwak *et al.* [34] observed that purified autologous idiotype protein could be made into an immunogenic vaccine by chemical conjugation to a carrier protein in patients with lymphoma. Following this pilot study, the authors immunized an HLA-matched sibling marrow donor with myeloma IgG isolated from the plasma of the transplant recipient, conjugated to a carrier and emulsified in an adjuvant. That a myeloma idiotype-specific T-cell response was successfully transferred to the recipient was proven by:

- A lymphoproliferative response.
- A parallel response in the carrier protein.
- Recovery of a recepient $CD4^+$ T-cell line with unique specificity for the myeloma idiotype.
- Demonstration by *in situ* hybridization that the cell line was of donor origin.

Such an approach provides a novel strategy for enhancing the specific antitumour effect of allogeneic grafts.

Summary

The results of the above clinical trials are summarized in Table 8.1 and help us to draw a few conclusions:

1. Complete remission by biochemical and haematological criteria can be achieved by as many as 40% of the patients.
2. Early mortality which is treatment-related is about 40%. This high mortality can be, however, attributed to the fact that most of our results are based on registry data which have a high incidence of patient heterogeneity. In selected series [12,35] this figure is much lower.
3. The event-free survival and overall survival at two years are approximately 35% and 35–40%, respectively, and the curves show a tendency towards plateauing beyond this time period, making the possibility of a cure in this otherwise fatal disorder very real.

The optimum timing of an allogeneic transplant is difficult to assess. Recent reports [12,35] have stressed the benefits of allogeneic transplantation early in the course of the

disease, with improved outcome in patients with chemosensitive disease. These have further been confirmed by the IBMTR data [13] and the EBMTR data [17]. The better outcome of patients transplanted earlier is the result of better tolerance of treatment, better tumour control, and fewer transplant-related complications such as GvHD and interstitial pneumonitis.

The prognostic factor analysis by Gahrton *et al.* [17] does not give any conclusive help in deciding which patients should be candidates for an allograft. Their data suggest that female sex, stage I disease, patients having received only one line of treatment pretransplant and those who enter CR have a favourable outcome. One could, however, argue that these same factors would predict a favourable outcome with other approaches such as autologous transplantation where the procedure-related mortality is under 5%. Therefore it may be justifiable to recommend an allogeneic BMT for patients who have failed first-line treatment or who have resistant disease.

Table 8.1 Result of allogeneic BMT for multiple myeloma

Study	Patients (*n*)	TRM (%)	CR (%)	OS (%)	EFS (%)
Gahrton *et al.* (1995) [17]	162	~70	60	47 (7 years)*	34 (6 years)*
Bensinger *et al.* (1993) [18]	60	40	37	68 (2 years)*	48 (2 years)*
Anderson et al. (1993) [21]	14	15	50	64 (2 years)	57 (8–34 months)
Vesole *et al.* (1993) [22]	35	26	34	44 (2 years)	22 (2 years)

TRM=transplant-related mortality. CR=complete remission. OS=overall survival. EFS=event-free survival.
*Results for CR patients.

Allogeneic BMT should be considered in patients with proven poor prognostic factors including a raised β_2-microglobulin, unfavourable cytogenetics and high plasma cell labelling index. Ideally, a controlled randomized trial should be carried out to ascertain whether or not an allotransplant is superior to an autograft. The design of such a study is, however, extremely difficult and therefore matched pair analysis between these two procedures should be undertaken.

Autologous transplantation

Current experience

Experience in the field of autografting is comparatively extensive. More than 1000 patients have been autografted and there have been anecdotal reports of patients having undergone this procedure at the age of 75 years. The relative safety of this procedur accompanied by the low TRM (<5%) allows most specialist centres to autograft patients up to the age of 70 years.

The initial encouraging results with high-dose melphalan (HDM) [8] led to a larger evaluation of this treatment approach [36,37]. In the follow-up study, the use of melphalan (140 mg/m2) as a single intravenous injection was tested in 63 previously untreated myeloma patients and was associated with a high response rate. Seventy-eight percent of the patients responded, with 27% achieving a CR. In this study, complete and partial remissions lasted for a median of 19 months but relapse was seen in almost all patients. HDM resulted in a predictable period of myelosuppression. The median time to recover white cells to 1×10^9/litre was 28 days in patients who had not received previous treatment. Myelosuppression was associated with a significant infection risk and 8 early deaths occurred due to infection and bleeding. Long-term follow-up data on these 63 patients [38] have revealed an overall response rate of 82%, with 32% achieving CR. The median duration of response was 18 months, and 6 patients remain alive and free from disease progression at >60 to >84 months. With a median follow-up of 74 months (range: 63–100 months), 23 patients are alive with a median survival duration of 47 months, and 35% of patients are expected to be alive at nine years. An improvement in quality of life was documented in the majority of patients (pain grade: 89%; and performance status: 92%).

At approximately the same time as the HDM treatment strategy evolved, Barlogie *et al.* [39] introduced the concept of infusional chemotherapy for treatment of myeloma. This was based on the rationale of continuous exposure of the myeloma cell to chemotherapy due to the slow growth fraction of these cells [40]. Barlogie used continuous infusions of vincristine and doxorubicin together with oral dexamethasone (VAD). We modified this regime slightly and used methylprednisolone (VAMP) instead of dexamethasone in our patients [41]. A combination of infusional chemotherapy and high-dose treatment has been crucial in the development of further intensive treatment programmes requiring the use of haemopoietic stem-cell support [37,42,43]. The increased susceptibility to infection and high initial mortality led to the use of autologous bone-marrow transplants (ABMT) following high-dose therapy. Besides ameliorating haematological toxicity, this approach allowed further intensification of HDM. The prerequisite for a bone-marrow harvest, however, was a low level of marrow infiltration by disease. Therefore, treatment regimens such as VAMP and VAD were used initially to reduce tumour burden prior to transplant.

Autologous bone-marrow transplantation

Barlogie *et al.* [42] treated 7 patients with high-dose melphalan (HDM) followed by ABMT as supportive treatment. Bone marrow had been harvested during prior remissions, and contained 2–7% plasma cells. However, remissions were short and no patient given ABMT survived for more than nine months. An attempt was subsequently made to induce more frequent and durable remissions by adding TBI (8.5 Gy in five fractions) to HDM 140 mg/m2 [44], Seven patients with advanced refractory disease were treated. Bone marrow had been collected either during previous remissions or after failure to respond to initial therapy, and contained 6–30% plasma cells. There were 2 transplant-related deaths and median remission duration was 15 months. The marked cytoreduction in patients with previously refractory disease proved that apparent drug resistance could be overcome by dose escalation.

Data on HDM and TBI was further updated in 1990 [45] with special reference to prognostic factors. Of 55 patients, 14 were in first remission and 20 in later remission. Twenty-one had resistant disease, of whom 7 were refractory to primary treatment and 14 had resistant relapse. Of the latter patients, none entered CR and there were 5 early deaths, with an overall median survival of seven months. Of the remaining 41 patients, 27% attained CR and the projected survival at four years was 82%. High β2-microglobulin and non-IgG myeloma were identified as poor prognostic factors.

Our group, along with St Bartholomew's Hospital [37,43], used the approach of induction chemotherapy with VAMP to reduce tumour burden followed by HDM (200 mg/m2) and rescue with ABMT in a group of previously untreated patients. Fifty patients received treatment. The overall response rate was 74%, and 50% of patients achieved CR as defined by haematological and biochemical criteria. These remissions were associated with a good quality of life as measured by performance status, pain grade and the reversal of humoral immunosuppression. Six patients died during the VAMP phase of treatment and there was 1 death related to HDM.

In 1994, our ABMT results were updated [46] and included 53 previously untreated patients who received HDM and ABMT. Following ABMT, all but 1 patient responded, with 40 patients achieving CR (75%). There was 1 treatment-related death. The median duration of response had not been reached at 20 months, and it was significantly longer for patients in CR than for those in partial remission (PR) (P= 0.025). At a median follow-up of 31 months, 12 patients were dead and 40 alive, with an estimated probability of survival at 54 months of 63%. Multivariate analysis found haemoglobin >10g/dlitre and stage A disease as favourable indicators for survival.

Attal *et al.* [47] tested the feasibility of a three-phase approach which included induction chemotherapy with either VAD or VMCP followed by HDM with TBI supported by unpurged ABMT and interferon-α maintenance treatment in previously untreated myeloma patients. Thirty-five consecutive patients under the age of 65 years were enrolled. Eighty-nine percent of these received an ABMT. Forty-three percent of patients achieved a CR. Low β_2-microglobulin was the only predictive factor for achieving CR. The 33-month post-ABMT probability of progression-free survival was 85% for patients achieving CR, versus 24% for patients in PR. The 42-month post-diagnosis probability of survival was 81%. Interferon α was started soon after ABMT and was well tolerated.

Purged autologous bone-marrow transplants

Although a high proportion of patients achieve remission with ABMT, relapse is inevitable. Most of these relapses are probably due to residual *in vivo* myeloma secondary to failure of the conditioning regimen. There is, however, the theoretical possibility that the malignant clone is reinfused via the autologous bone-marrow return. To circumvent this problem, marrow purging using monoclonal antibodies either with complement [21] or as a part of an immunotoxin involving momordin [48] has been applied in myeloma patients undergoing ABMT. Reece *et al.* [49] have used autologous marrow purged with 4-hydroxyperoxy-cyclophosphamide (4-HC). They treated 17 of 24 patients with *ex vivo* marrow purging with 4-HC followed by cryopreservation. These 14 patients received conditioning with busulfan, cyclophosphamide and melphalan followed by rescue with 4-

HC purged marrow. Two patients died of hepatic veno-occlusive disease and 1 patient succumbed to fungal infection. The remaining 11 patients achieved responses (CR: 6; and partial response: 5) associated with a normal performance status. Seven patients have had progressive myeloma at a median of 17 months (range: 5–30 months) after BMT while 4 patients remain continuously free of progression at a median of 20 months (range: 6–42 months) following transplant.

Anderson *et al.* [21] reported treating 26 patients with chemoradiotherapy followed by ABMT using *in vitro* lysis with monoclonal antibodies and complement. With a 24-month median follow-up for survival after ABMT, 16 of 26 patients are alive and free from progression at >2 to >55 months post-BMT. At present there are limited data available concerning purged ABMT in myeloma, and although results are encouraging, the procedure is expensive and has not gained popularity because newer alternative approaches are becoming available.

Peripheral blood stem-cell transplants

An alternative approach to purged ABMT is the use of autologous peripheral blood stem-cell (PBSC) transplants on the theoretical basis that this source of stem cells will be less contaminated with tumour cells. The existence of circulating stem cells capable of producing sustained engraftment was demonstrated during the 1960s and 1970s in a variety of animal species. For a review, see McCarthy and Goldman [50]. PBSC transplant was an attractive option in myeloma patients due to the fact that patients with marrow infiltration and those with prior radiotherapy to the spine and pelvis could still be considered for this form of treatment.

The first report of PBSC transplant in myeloma came from Henon *et al.* in 1988 [51]. PBSC were collected in 2 patients during the recovery phase after HDM (140 mg/m2). A total of 10×10^4 and 30×10^4 CFU-GM/kg were obtained. No plasma cells were identified by cytology or immunofluorescence. Both patients were autografted after a further course of HDM and fractionated TBI, but the patient with the lower number of harvested PBSC also received bone marrow. The second received PBSC alone. Engraftment was prompt and he remained in CR two months after PBSC transplant. PBSC transplant has since been carried out in all phases of the disease and many studies have reported the combined results of ABMT and PBSC transplant.

Harousseau *et al.* [52] treated 97 patients with high-risk myeloma with a first course of HDM. Eight toxic deaths were noted and only 38 of the 69 patients who responded were able to receive the second phase of the treatment, which was a transplant procedure.

Among the 35 patients undergoing autologous transplantation, 4 received PBSC transplants. The median survival of the 35 patients undergoing autologous transplantation was 41 months, with a median remission duration of 28 months without a visible plateau. Jagannath *et al.* [53] attempted PBSC collections in 75 previously treated patients after the administration of high-dose cyclophosphamide (6 g/m^2) with or without granulocyte-macrophage colony-stimulating factor (GM-CSF). Sixty patients subsequently received high-dose treatment followed by rescue with both autologous bone marrow and PBSC; 38 patients received GM-CSF post-transplant. Among 72 patients undergoing apheresis, good mobilization was achieved when prior chemotherapy did not exceed one year and when GM-CSF was used post high-dose cyclophosphamide. These same variables also predicted for rapid engraftment. The cumulative response rate for all 75 patients was 68% with 12 month event-free and overall survival projections of about 85%. The exact place of PBSC transplant could not, however, be ascertained from this study because of the combined use of ABMT and PBSC transplant and the use of growth factors in the post-transplant setting.

Fermand *et al.* [54,55] initially assessed feasibility and subsequently carried out a phase II study of results of PBSC transplant after high-dose chemoradiotherapy. They treated 63 patients with high tumour mass multiple myeloma with high-dose chemotherapy. This comprised etoposide, carmustine, melphalan and cyclophosphamide in the last 26 patients plus TBI which was delivered in a single fraction of 10 Gy in 31 patients or in six fractions of 2 Gy at day −3 to −1 in 32 patients. Blood stem-cell collection was performed at the time of diagnosis in 30 patients, while 33 had been previously treated. Mobilization included semi-intensive chemotherapy containing cyclophosphamide, doxorubicin, vincristine and steroids. This treatment resulted in a short period of cytopenia and PBSC were collected on haematological recovery by three to five leukapheresis procedures performed on consecutive days. Twenty-three patients received maintenance treatment with interferon-α for a median of six months. Seven patients died from early toxicity. At six months post-engraftment, 40 (71%) of the surviving patients had minimal residual disease and 11 (20%) were in apparent CR. During follow-up, 25 out of the 63 patients relapsed and 16 of these died. The median overall and event-free survivals after transplantation were 59 and 43 months, respectively. The presentation serum β_2-microglobulin (greater or less than 2.8 mg/litre) and duration of previous chemotherapy (greater or less than 6 months) were the only significant prognostic factors.

We treated 73 myeloma patients [56] with high-dose melphalan followed by rescue with PBSC transplant. All patients received induction treatment with C-VAMP (infusional vincristine and doxorubicin plus weekly bolus cyclophosphamide and oral methylprednisolone). Forty-nine of these patients were previously treated while 24 were newly diagnosed. Our mobilization schedule included recombinant human Growth Colony Stimulating Factor (rh G-CSF) (Amgen) alone followed by leukapheresis on two consecutive days. PBSC collection was carried out on an out-patient basis. All patients received maintenance treatment with interferon-α following transplant. This was based on results of our previous randomized trial comparing maintenance interferon treatment versus no treatment [57,58]. The overall CR rate was 33% in this series, with 54% of the previously untreated patients achieving CR versus 22% in the pretreated group ($P<0.01$). The overall survival in the whole group was 79.2% at two years with a median follow-up

of 7.75 months and the progression-free survival was 58%. Engraftment was significantly faster in the previously untreated patients.

Several studies have reported on the limited value of myeloablative treatment in the late stages of the disease [59–61]. Ten to 25% of patients have died of transplant-related causes and median remission durations have not exceeded a year.

Tricot *et al.* [62] have identified favourable variables for rapid engraftment in 225 patients with myeloma. All PBSC were collected after high-dose cyclophosphamide and haematopoietic growth factor mobilization. A highly significant correlation was observed between the number of $CD34^+$ cells per kilogram infused and prompt recovery of both granulocytes (P=0.0001) and platelets (P=0.0001). Exposure to even less than 6 months of alkylating agents was associated with significantly delayed engraftment post-transplant. The threshold dose of CD34 cells necessary for prompt engraftment was $>2.0\times10^6$/kg for patients with less than two years of treatment prior to transplantation, whereas $>5\times 10^6$/kg CD34 cells were required to ensure rapid engraftment in patients exposed to longer durations of treatment.

Bearing in mind the problems with mobilization and engraftment and the long-term outcome for patients transplanted in the late stages of the disease, it followed that the logical approach would be to treat patients as early as possible with myeloablative treatment followed by a transplant procedure of some sort. The French Registry [63] has recently reported on 133 autologous stem-cell transplants performed after first remission induction in myeloma. The source of stem cell was marrow (n=81), blood (n=51) or marrow plus blood (n=1). Thirty-seven percent of the patients entered CR and 46% achieved a PR. There were 17 failures and 5 toxic deaths. With a median follow-up of 35 months, the median remission duration was 33 months and the median time to treatment failure was 22 months. The median survival was 46 months overall, 54 months for the 103 responding patients and 30 months for the 30 non-responders. A multivariate analysis revealed the quality of response after transplantation as the most important prognostic variable for outcome.

Our data [64] of 195 previously untreated patients under the age of 70 years have examined outcome following autologous transplantation. All patients received induction chemotherapy with the intention of going on to high-dose treatment. One hundred and forty-one patients received high-dose treatment, with 112 patients receiving HDM plus an autograft (bone marrow or blood stem cells), and 29 received a modified high-dose treatment of melphalan alone (n=23) or busulfan (n=6). A total of 57 patients also received interferon maintenance following HDM and graft. The CR rate for the whole group was 53%, while 74% of those receiving HDM and an autograft entered CR. Not all patients who were intended to receive HDM plus autograft did so because of increasing age (P=0.001) and a raised creatinine (P=0.05).

Median overall survivals from first treatment for the whole group and for the HDM/autograft group (from transplant) are 4.5 years (Figure 8.3) and 6.6 years, respectively. The median overall survival of the patients in the HDM/autograft group receiving interferon has not occurred at eight years. The median progression-free survival for the whole group is 25 months (from first treatment) (Figure 8.4), 27 months for the HDM/autograft group and 44 months (from HDM) for patients receiving maintenance interferon.

In a series of 496 consecutive myeloma patients enrolled in clinical trials of tandem transplants with PBSC support, Vesole *et al.* [65] have reported 95% as having completed the first autologous transplant with HDM and 73% as having completed the second transplant with either HDM alone or TBI plus HDM or a combination of alkylating agents, depending on response status prior to the second transplant. Thirty-one patients up to the age of 60 years received an allograft as the second transplant. The median interval from first to second transplant was five months. Treatment-related mortality during the first year after transplantation was 7%, and CR was obtained in 36% of patients. The median durations of event-free survival and overall survival after transplant were 26 and 41 months, respectively. Low β_2-microglobulin and C-reactive protein were significant parameters associated with prolonged event-free and overall survival. Cytogenetic analysis revealed the presence of 11q abnormalities and/or complete or partial deletion of chromosome 13 as negative prognostic variables with respect to event-free and overall survival. Attaining CR and undergoing two transplants within six months both significantly extended event-free and overall survival.

Harousseau *et al.* [66] made a comparative analysis of ABMT versus PBSC transplant in myeloma patients by a retrospective analysis of 132 transplant patients treated at 18 French centres. The ABMT group (*n*=

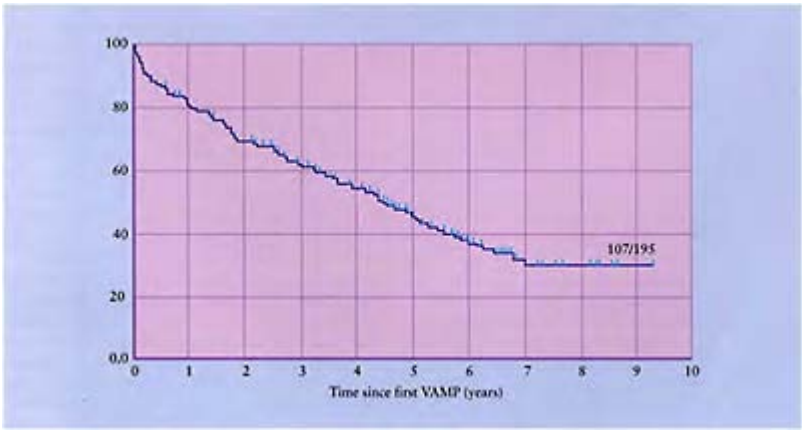

Figure 8.3 Overall survival after VAMP treatment for multiple myeloma in years since first treatment [64].

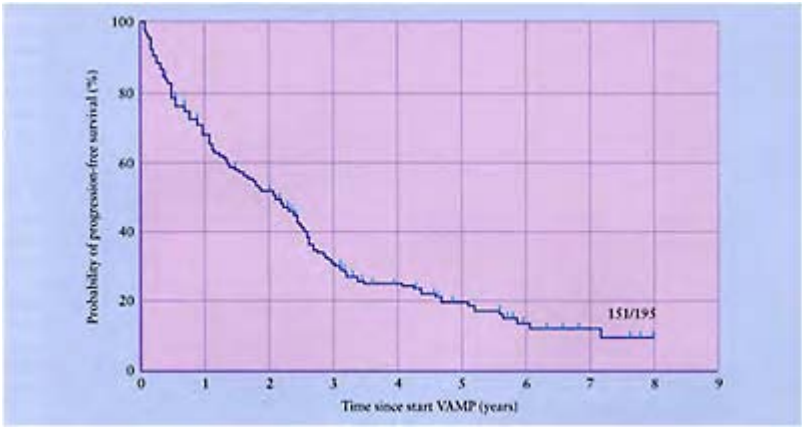

Figure 8.4 Progression-free survival in multiple myeloma in years since start of VAMP chemotherapy [64].

81) and the PBSC transplant group differed in median age, duration of chemotherapy prior to transplant, the interval between stem-cell collection and transplant and the conditioning regimen. The PBSC transplant group had a significant advantage with respect to neutrophil recovery (13 days versus 20 days, $P< 0.001$) but no difference was noted in platelet recovery. There was no significant difference between the two groups regarding response rate. The actuarial relapse-free survival, time to treatment failure and overall survival were not significantly different.

The above data suggested that an autologous transplant irrespective of the stem-cell source was of benefit to patients early in the course of the disease. The studies did not, however, throw any light on the benefits of autografting over conventional chemotherapy for similar stage disease. Blade *et al.* [67] have published data on 77 of 487 patients who could have been candidates for early intensification therapy but instead were treated with conventional chemotherapy. Their median age was 56 years. Thirty-six patients were initially treated with melphalan and prednisolone and 41 with VCMP/VBAP. The median response duration to initial chemotherapy was 22 months, and the actuarial probability of being in continuing first response at five years was 14%. After a median follow-up time of 58 months, 59 patients have died, 1 was lost to follow-up and 17 are alive 69 to 119 months after initial chemotherapy. The median survival time from initiation of treatment was 60 months and 52 months from the time when autotransplantation would be considered. Because of similarities in survival trends of these patients treated with conventional chemotherapy and those who received intensive treatment, it became crucial to compare these two treatment options in a prospective randomized trial and the Intergroupe Français du Myeloma began a trial to address this issue [68].

Two hundred previously untreated patients under the age of 65 years who had myeloma were randomly assigned at the time of diagnosis to receive either conventional chemotherapy or high-dose therapy and ABMT. All analyses are on an intention-to-treat basis. The patients enrolled in the conventional-dose arm received a median of 18 cycles

of VMCP and VBAP. Seventy-four patients enrolled in the high-dose arm underwent transplantation. The median time from diagnosis to transplantation was 5.5 months. This was usually after four to six alternating courses of VMCP and VBAP. High-dose treatment comprised HDM and TBI. Interferon was started in both groups, three times weekly, from cycle 9 in the conventional-dose group and on haematological recovery in the transplant group. The response rate among the patients who received high-dose treatment was 81% (including CR in 22% and very good PR in 16%), whereas it was 57% (CR in 5% and very good PR in 9%) of the patients treated with conventional chemotherapy ($P< 0.001$). The probability of event-free survival at five years was 28% in the high-dose group and 10% in the conventional-dose group ($P=0.01$) (Figure 8.5). The overall estimated survival at five years was 52% and 12% in the high-dose and conventional-dose group, respectively ($P=0.03$). Treatment-related mortality was similar in both groups. In a multivariate analysis of all 200 patients, event-free survival was significantly related to the level of β_2-microglobulin ($P<0.001$) and the treatment assignment ($P=0.01$). Overall survival was related to the level of β_2-microglobulin only ($P= 0.01$).

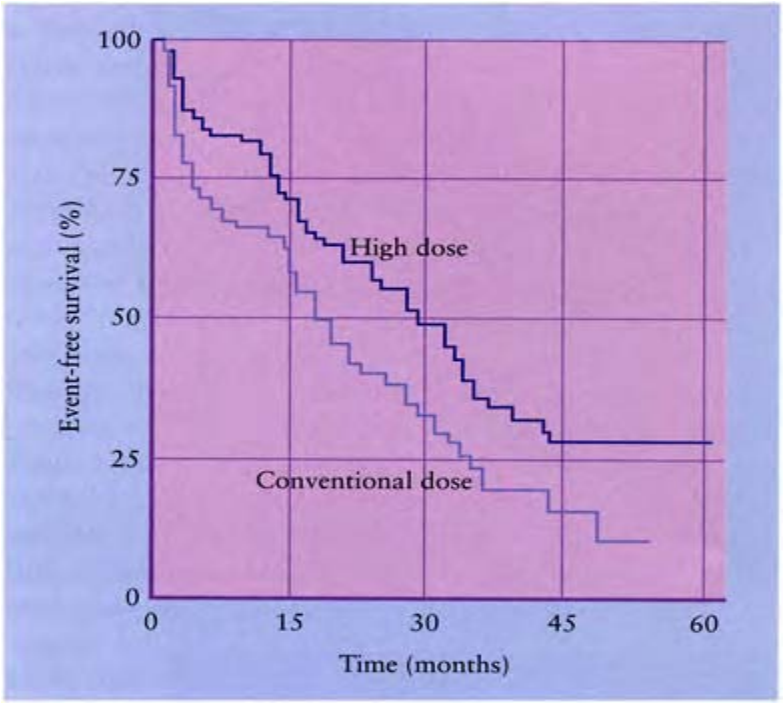

Figure 8.5 Event-free survival in months for patients with multiple myeloma treated with either conventional or with high-dose therapy [68].

A study by the Southwest Oncology Group [69] has also shown a similar advantage for patients treated with intensive therapy upfront. The Medical Research Council Myeloma VII trial [70] addresses the same issue. Patients under the age of 65 years are eligible and it will compare the less-intensive ABCM with infusional C-VAMP followed by high-dose therapy and either ABMT or PBSCT. All patients who respond will receive maintenance treatment with interferon.

Various different high-dose regimens have been used for autografting, but by far the most commonly used protocols have used melphalan alone at a dose of 200 mg/m2 or melphalan at a dose of 140 mg/m2 plus TBI. Busulfan plus cyclophosphamide has also been used frequently. Dimopoulos *et al.* [71] used a new conditioning regimen, including thiotepa (750 mg/m2), busulfan (10 mg/kg) and cyclophosphamide (120mg/kg) followed by either ABMT or PBSC transplant. Sixty-five percent of all patients responded to treatment. Thirteen percent of the patients died of regimen-related toxicity but, by and large, the treatment was well tolerated and response duration and survival outcomes were comparable with other studies.

Adkins *et al.* [72] investigated the feasibility of escalating doses of dacarbazine (DTIC) in combination with high-dose cyclophosphamide, carmustine and etoposide (CBV) in patients with refractory myeloma and lymphoma in a phase I trial. TRM was 18% but the feasibility of combining high doses of DTIC with CBV was demonstrated. Dose-limiting hypotension was transient and reversible when DTIC was administered at 3900 mg/m^2 with CBV. We have used busulfan alone [73] at a dose of 16 mg/kg in a group of patients with renal impairment and short remission duration following HDM. The TRM was high (3 of 15), but 46% of patients responded thereby demonstrating the efficacy of busulfan in heavily pretreated patients, and that its administration in patients with renal impairment was safe.

Summary

From all these studies, it is clear that autologous transplantation in myeloma is currently the superior mode of treatment compared to conventional chemotherapy and results are summarized in Table 8.2. This form of intensive treatment results in biochemical and haematological CR in as many as 30–50% of

Table 8.2 Result of autologous transplantation for multiple myeloma

Study	Patients *(n)*	TRM (%)	CR (%)	OS (median)	EFS (median)
Fermand *et al.* (1993) [55]	63	11	20	59 months	43 months
Harousseau *et al.* (1995) [63]	133	3.7	37	46 months	33 months
Vesole *et al.* (1996)* [65]	496	7	36	41 months	26 months

Attal *et al.* (1996)* [68]	100	2	30	52% (5 years)	27 months
Powles *et al.** [64]	195	4.9	53	54 months	25 months

*Includes patients who were intended for an autograft but did not necessarily receive it. TRM=tranplant-related mortality, CR=complete remission. OS=overall survival. EFS=event free survival.

patients. Transplant- related mortality is less than 5% in most series. The event-free survival and the overall survival approximate 30 months and four to five years post-transplant. It is also apparent that patients who benefit most are those who are treated early in the course of the disease. In spite of these encouraging results, there is no evidence of the survival curves plateauing off and patients with myeloma continue to relapse.

New approaches

Positive stem-cell selection

Reinfusion of the malignant clone is a major concern in all autografting procedures. PBSC transplants are being used preferentially over ABMT because of the advantages of rapid engraftment, less morbidity, cost benefit and the possibility of less tumour cell contamination in blood harvests. Several authors [74–76] have, however, identified myeloma cells in PBSC harvests as well as in peripheral blood of patients. Corradini *et al.* [75] have demonstrated the presence of both pre- and post-switch B-cells in both bone-marrow and blood stem-cell harvests by a polymerase chain reaction (PCR)-based strategy, using clone-specific sequences derived from the rearrangement of Ig heavy-chain *(IgH)* genes. The complementarity-determining regions (CDR) of *IgH* genes are used to generate tumour-specific primers and probes. Their data suggest that blood-derived stem-cell harvests may have a lower rate of contamination compared to bone marrow. Contrarily, growth factors such as G-CSF and GM-CSF used in most mobilization schedules for PBSC harvesting have been implicated in the mobilization of tumour cells [77].

The fear of introducing tumour cells at the time of graft has led to trials involving positive stem-cell selection. Recent studies show that haematopoietic cells positively selected for CD34, an antigen expressed on early haematopoietic cells, can support haematopoietic recovery following myeloablative chemotherapy. Schiller *et al.* [78] have reported a multi-institutional study of purified CD34-selected peripheral blood progenitor-cell transplantation in 37 patients with advanced myeloma. The positive selection for haematopoietic progenitor cells was carried out using the CEPRATE system (Cellpro, Bothell, WA) which is a continuous flow column selection system that relies on the high affinity between avidin and biotin to select antibody-labelled haematopoietic progenitor cells. After CD34 selection, a greater than 2.7–4.5 log reduction in myeloma-cell contamination was achieved in the harvests. Engraftment was prompt in patients treated with CD34-selected grafts and blood and platelet requirements were minimal. A dose of 2×10^6/kg $CD34^+$ cells was considered adequate for prompt and sustained

engraftment. The one-year progression-free survival in this study was 67%±19% but longer follow-up is required before any firm conclusions concerning this approach can be drawn.

Gazitt *et al.* [79] purified $CD34^+$ Lin− Thy^+ stem cells from mobilized PBSC harvests of 10 myeloma patients by sequentially using counterflow elutriation centrifugation, treatment with phenylalanine methylester, and flow sorting with five-parameter gating. Stem-cell purification led to an overall enrichment of about 50-fold in all 10 patients. Quantitative PCR amplification of patient-specific CDR III DNA sequences showed depletion of clonal B cells by 2.7 to 7.3 logs, with the highest log reductions seen in samples initially containing the most tumour cells. Depletion of myeloma cells by such methods offers an important tool for investigating whether relapses post autografting can be reduced.

Retroviral gene marking

There have been contradicting reports concerning whether or not the CD34 antigen is expressed on myeloma cells [76,80]. CD34-enriched bone marrow and peripheral blood have been retrovirally marked by Dunbar *et al.* [81]. The main aims of their study were to investigate the efficiency of retroviral gene transfer to CD34-enriched haematopoietic cells, to compare the use of peripheral blood versus bone marrow as targets for retroviral gene transfer, to study the kinetics of reconstitution after autologous transplantation, to study engraftment potential, and finally to determine if marked tumour cells were detectable post-transplantation. Eleven patients with myeloma or breast cancer received cyclophosphamide and filgrastim-mobilized peripheral blood cells CD34-enriched and transduced with a retroviral marking vector containing the neomycin resistance gene, and CD34-enriched bone-marrow cells transduced with a second marking vector also containing a neomycin resistance gene. After high-dose conditioning therapy, both transduced cell populations were reinfused, and patients were followed over time for the presence of the marker gene and any adverse effects relating to the gene transfer procedure. In all 10 evaluable patients the marker gene was detectable at the time of engraftment, and 3 of 9 patients showed persistence of the marker gene for greater than 18 months post-transplantation. The source of the marking was both the transduced peripheral blood graft and the bone-marrow graft, with a suggestion of better long-term marking originating from the peripheral blood graft. There was no obvious toxicity, and patients did not develop detectable replication-competent helper virus at any time post-transplant. The *Neo* signal has not been detectable in residual $CD38^+$ plasma cells isolated from 2 patients post-transplant. Obviously, such interventions are critical and will be useful in designing future therapeutic trials in autografting procedures.

Immune modulation

There are a number of possible approaches with immune modulation in conjunction with autologous transplantation [82]. PBSC harvests can be incubated with interleukin-2 (IL-2). These harvests, once reinfused, engraft promptly and contain cells that recognize and destroy tumour cells including the autologous tumour cells in the harvest. Seven-day cultures of bone marrow with IL-2 have led to effective purging in diseases including

myeloma, chronic lymphocytic leukaemia (CLL) and chronic myeloid leukaemia (CML). A more specific approach will include patients with secretory myelomas, who will undergo plasmapheresis and have their idiotype protein isolated, conjugated to keyhole limpet haemocyanin which will be used for *in vivo* immunization with SAF1 adjuvant on days −21 and −14. Patients will then have their bone marrow harvested. This will be cultured *in vitro* with the idiotype as a tumour antigen for seven days, with IL-2. Patients will, in the meantime, have treatment with HDM. After transplant, they will be boosted *in vivo* at 1, 2, 3 and 4 months with the particular tumour antigen vaccine.

Another interesting approach currently being assessed in CLL is the demonstration that CD34-positive cells in long-term bone-marrow cultures will generate *de novo* T-cells and natural killer cells. These T-cells and natural killer cells have autoreactive properties and are also able to lyse autologous tumour, which in turn leads to effective purging and graft-versus-leukaemia generation.

Post-transplantation maintenance therapy

The use of interferon-α in the post-transplant setting has been tested by our group in a randomized trial [57,58], Interest in the use of interferon in myeloma was evoked after Mellstedt *et al.* [83] demonstrated its efficacy as a single agent in previously untreated myeloma. Following induction with conventional chemotherapy, two European studies [84,85] have demonstrated that interferon can prolong partial response, but a beneficial effect appears limited to patients who acieve an objective response with induction treatment. When Mandelli [84] reported the results of the first trial of maintenance interferon, the greatest benefit occurred in the patients with the lowest tumour burden at the start of maintenance therapy. Based on these results, we concluded that an ideal group of patients in whom to test the role of maintenance therapy would be those with evidence of minimal residual disease. Since high-dose therapy has resulted in very high CR rates when the majority of patients have a low tumour burden, we tested this approach following ABMT. Eighty-four patients with myeloma were randomized to receive interferon-α, 3 mega-units/m^2 s.c. three times weekly, or no treatment following induction chemotherapy followed by high-dose treatment. At a median follow-up of 52 months, the median progression-free survival was 27 months in the control arm, and 46 months in the interferon-treated patients (P<0.025). For the 65 patients who achieved CR, there was a significant prolongation of remission (P=0.02) for those receiving interferon treatment, and 33% of these patients remain in remission six years after high-dose treatment (updated data).

Most studies to date have looked at the effect of interferon maintenance following conventional treatment [3,86]. Our study is the first to compare the use of interferon maintenance following high-dose treatment.

Attal *et al.* [47] reported a 33-month post-ABMT progression-free survival of 85% in CR patients in a non-randomized study. Their results, although encouraging, concerned a small number of patients with a short follow-up.

Data from the French registry [63], although unable to confirm our findings in a phase II non-randomized trial, did demonstrate a favourable trend for CR patients receiving interferon maintenance therapy following high-dose treatment. We have shown a significant progression-free survival advantage in our interferon-treated patients in our

own analysis of consecutive previously untreated patients [64]. We compared the robustness of PBSC transplant with respect to interferon maintenance, versus ABMT [87] and have demonstrated that PBSC transplant is as robust in tolerating treatment with interferon.

Obviously, more data are required to confirm the efficacy of interferon following high-dose treatment, and the results of the MRC VII trial [70] which uses interferon maintenance in all responding patients (high-dose group and conventional chemotherapy) will be important in confirming its role, at least in the high-dose group.

Relapse after autologous transplantation

With the increasing use of high-dose therapy and autografting without any evidence of cure, relapse following transplantation is becoming a common problem and poses a therapeutic dilemma for the clinician. We have demonstrated the feasibility of repeated administration of HDM [88]. Twenty-nine patients who had relapsed at a median of 20 months following first HDM, received a second course of HDM after induction chemotherapy. The median remission duration in these patients was 17 months. The period of neutropenia was similar during first and second high-dose procedures, but the duration of thrombocytopenia was longer in patients receiving melphalan for a second time. There was one treatment-related death due to thrombocytopenic haemorrhage.

More recently, Tricot *et al.* [89] have studied 94 patients who relapsed following autologous transplantation, in order to evaluate the efficacy of further therapy. Post-transplant salvage treatment consisted of either standard dose therapy (n=53) or a transplant procedure with an intensive preparative regime in the others [autograft (31) and allograft (10)]. With a median follow-up of 11 months, the projected overall survival of all patients at 18 months was 59%. A multivariate analysis revealed pre-salvage β_2-microglobulin and late relapse after the preceding transplant as independent significant variables for overall survival. Transplantation performed as primary salvage therapy was associated with significantly prolonged survival. However, after inclusion of 15 patients who had initially received salvage chemotherapy followed by subsequent transplantation, the survival advantage for the transplant group was no longer apparent. These data also confirm that a second transplant is feasible and may produce long-term survival in patients with good prognostic factors.

Attal *et al.* [68], in their randomized trial, have compared the effect of salvage treatment in both the conventional-dose treatment group and the high-dose group. In the conventional-dose group, 45 of 50 patients who relapsed received treatment, of which 9 received high-dose therapy. With a median follow-up among the surviving patients of 11 months from the time of relapse, the probability of survival for two years after relapse was 25%. In the high-dose group, 46 patients relapsed and 41 received salvage treatment. Eight of these patients were treated with a second high-dose regimen. With a median follow-up among the surviving patients of 15 months from the time of relapse, the probability of relapse was 35%, a number not significantly different from that in the conventional-dose group.

These studies demonstrate the feasibility of further intensive treatment following relapse after an autograft. They do not, however, demonstrate the advantages of such an

approach. Because such interventions involve considerable toxicity, studies involving prognostic factor analysis to identify patients who will benefit from these treatment approaches are essential, and should be followed by matched comparisons with patients treated with conventional-dose treatment in order to define their exact role.

Conclusions

With nearly 1000 transplants, including allogeneic and autologous transplants, the role of high-dose treatment is becoming clearer in myeloma. As a haematological malignancy, attaining CR is crucial in developing curative strategies for this otherwise fatal condition. High-dose therapy has resulted in CR rates of between 25% and 75%. Obviously, the meaning of CR depends on the definition, and how closely one looks for evidence of residual disease. Most of the patients who achieve CR show molecular evidence of disease. Most studies, whether in autografting or allografting, have identified attainment of CR based on haematological and biochemical criteria, as a favourable prognostic indicator for survival, and therefore this should be the goal in all future trials. Further dose intensification and trials of new high-dose combinations may improve our myeloablative strategies by removing minimal residual disease which may be contributing towards relapse. Newer approaches including interferon maintenance, immune modulation with IL-2, positive stem-cell selection and retroviral gene marking, in the setting of transplantation, are exciting and will be areas of future interest.

Autografting has been crucial in decreasing the procedure-related mortality of high-dose therapy and it is therefore justifiable to offer this mode of treatment to all myeloma patients under the age of 70 years as front-line treatment. The problems with allografting are not as straightforward. This is mainly due to patient heterogeneity in terms of stage, disease responsiveness and the number of treatments received pretransplant. The procedure-related mortality is approximately 30–40% in reported series, and efforts to reduce this are crucial in permitting this treatment to be offered upfront.

Allogeneic peripheral blood stem-cell transplants, T-cell depletion of donor marrow, improved treatment of GvHD and treating patients early on in their disease may be ways of circumventing this problem. Patients under the age of 55 years with a matched sibling donor should be considered for an allograft if there are associated poor prognostic indicators including elevated β_2-microglobulin and advanced stage disease. Patients with stage I disease may first be offered the option of an autograft, with an allograft reserved for relapse. Data collected on a homogeneous set of patients as may be the case in autografting will give us better selection criteria for allografting. Moreover, such studies will help us to identify patients who will not benefit from this approach, for example, chemoresistant and multiply relapsed patients, who should then be considered for novel therapeutic approaches.

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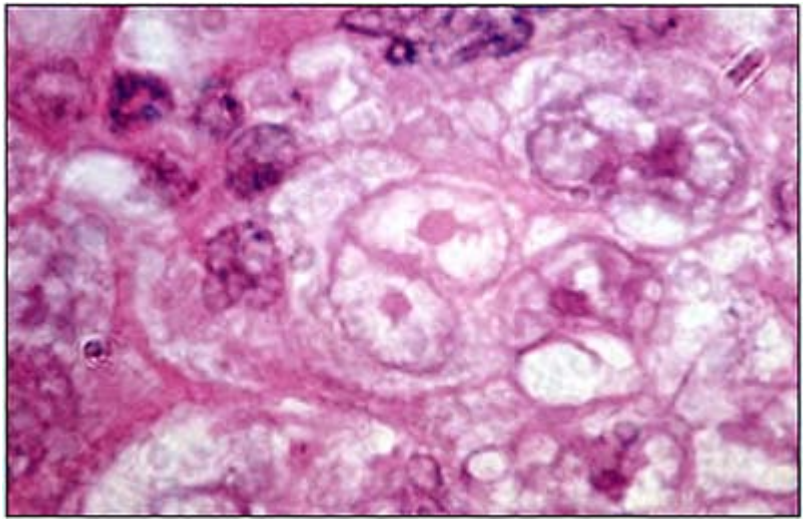

Chapter 9
Hodgkin's disease

Andrew J.Peniket and David C.Linch

Introduction

In 1832, Thomas Hodgkin produced a treatise entitled 'On some morbid appearances of the absorbent glands and spleen' which gave an account of 7 patients who had lymph-node enlargement without evidence of current infection [1]. With time, the histology of such lymph-node enlargements was elucidated, and the presence of the so-called Reed-Sternberg cells and their mononuclear counterparts was identified as a characteristic feature of the malignant disease that had become known as Hodgkin's disease [2–4].

Compared to many malignancies, Hodgkin's disease is rare, with an annual incidence in the UK of aproximately 2.5 per 100000. The disease is infrequent in childhood but incidence rises rapidly during adolescence, reaching a peak in the third decade and then remaining constant at a slightly lower level for the remainder of life [5]. Earlier epidemiological surveys had suggested that there was a marked bimodal age distribution [6] and the second peak in the elderly is still discernible in more recent US incidence data [7]. The apparent fall in the incidence of Hodgkin's disease in the elderly in the UK is probably accounted for by the fact that many such cases classified in the past as

Hodgkin's disease are now considered to be non-Hodgkin's lymphomas. This change in classification is particularly likely to have occurred in patients within the mixed cellularity and lymphocyte-depleted subgroups. In an analysis of patients entered into the British National Lymphoma Investigation (BNLI) registry up to 1979, 8% of cases were deemed to have lymphocyte-depleted Hodgkin's disease as defined in the Rye modification of the Lukes and Butler classification [8], but no cases have been defined as lymphocyte-depleted in the last five years. Some cases previously diagnosed as lymphocyte-depleted Hodgkin's disease are now considered to be nodular sclerosis grade II disease and others are now classified as anaplastic large cell-lymphomas. In several European series, the incidence of lymphocyte-depleted Hodgkin's disease is now similarly below 1%. It is also noteworthy that in the US data, there is no second peak in the elderly in patients with nodular sclerotic disease [9]. The unique age distribution of Hodgkin's disease means that this disease is a major form of malignant disease in young adults. In *Cancer in South East England 1992* (Thames Cancer Registry) for individuals aged between 15 and 35 years of age, Hodgkin's disease was the second most prevalent type of cancer in men and the fourth commonest in women [10].

Untreated Hodgkin's disease was almost invariably fatal, but with the advent of high-dose radiotherapy in the 1940s, between 20% and 40% of afflicted patients had prolonged survival [11] although it was not established for many years that such patients were actually cured [12]. Combination chemotherapy introduced in the 1960s resulted in the cure of many patients with anatomically advanced disease, and the overall long-term survival rate rose to approximately 70% [13]. Since then there have been further modest improvements in initial therapy, but a siginificant proportion of patients still require effective salvage therapy and, in view of the young age of many patients, there has been long-standing interest in employing high-dose strategies in this condition.

In 1959, McFarland and colleagues published a series of 5 patients with resistant lymphomas, 3 of whom had Hodgkin's disease, treated by dose escalation and autologous bone marrow support [14]. The intensity of the chemotherapy used would not by today's standards be considered highly myelo-suppressive, but the principle of attempting dose escalation in Hodgkin's disease was well established. In 1960, Beilby *et al.* described a patient with relapsed Hodgkin's disease who received one of the first successful allogeneic bone-marrow transplants, in so far as stable partial engraftment was established [15]. In 1995, 918 autologous blood or marrow transplants in patients with Hodgkin's disease were reported to the European Blood and Marrow Transplant (EBMT) Registry and 16 allogeneic transplants were performed [16].

Conventional therapy of Hodgkin's disease

Initial treatment

Pathological staging with a laparotomy is now infrequently performed in Europe in Hodgkin's disease. Patients with clinical stage IA and IIA disease are usually treated initially with local radiotherapy. The large majority of patients achieve complete remission and depending on the extent and bulk of the disease and the nature of the radiation fields employed, relapse rates will vary between 10% and 50% [17]. Overall,

relapse occurs in about one-quarter of patients. Most of these patients will then be treated with combination chemotherapy, and the results of this treatment are very similar to those of chemotherapy *ab initio* in patients with disease that was more advanced at presentation. There is currently considerable interest in the use of combined modality therapy as initial treatment, even in relatively good prognosis disease [18–20]. Less intensive or shorter duration chemotherapy can then be used than is the case with standard combination chemotherapy used for advanced or relapsed disease. Such strategies undoubtedly reduce the relapse rate but there is no evidence that their use improves survival or will reduce the use of very intensive salvage regimens requiring autologous stem-cell support in those occasional patients who relapse after initial radiotherapy and then fail standard combination chemotherapy.

For many years, the standard combination chemotherapy for advanced Hodgkin's disease was MOPP (mechlorethamine, vincristine, prednisone and procarbazine) which was initially developed by DeVita at the National Cancer Institute in the USA [21,22]. Variants of the MOPP regimen include MVPP (vinblastine in place of vincristine with a lower risk of neurotoxicity) [23], ChlVPP (as for MVPP but with chlorambucil in place of mechlorethamine resulting also in less nausea and vomiting) [24] and LOPP (as for ChlVPP but with vincristine rather than vinblastine) [25]. Of patients with advanced disease (stages IB, IIB, III and IV) about 50% will be cured by MOPP-type therapy. ABVD (doxorubicin, bleomycin, vinblastine and dacarbizine) was initially introduced by Bonadonna *et al.* for use in patients relapsing after MOPP therapy [26]. Complete remissions were obtained in almost 60% but only one third of these were durable. ABVD/radiotherapy was then compared with MOPP/radiotherapy in patients with stage IIB, III and IV disease, and although there was a trend towards improved results with ABVD/radiotherapy, this was not significant. A larger study restricted to patients with stage IIB and III did, however, show significantly superior outcomes with ABVD/radiotherapy. Subsequently, MOPP was compared to MOPP alternating with ABVD as front-line therapy in patients with stage IV disease, the alternating therapy introducing multiple drugs with theoretical non-cross resistance in line with the Goldie Coldman hypothesis [27]. The alternating regimen produced a superior relapse-free survival (65% at eight years) although the results obtained in the MOPP only group were worse than expected (36%) [26]. Several other trials have however confirmed the superiority of alternating regimens [28–30]. The (ECOG) study reported by Canellos *et al.* [28] was a three-arm trial: MOPP, MOPP/ABVD and ABVD. Both the MOPP/ABVD and the ABVD were superior to MOPP and there was no statistical significance between MOPP/ABVD and ABVD. This led to ABVD being accepted as standard therapy in some centres because it is less likely to cause sterility and is less leukaemogenic than MOPP. However, the confidence intervals in the ECOG trial were quite large and it is possible that the alternating arm is superior at a clinically relevant level. Furthermore, a recent British National Lymphoma Investigation (BNLI)/Central Lymphoma Group Trial has found the disease-free survival to be significantly better with the ChlVPP/PABLOE alternating regimen than with PABLOE (prednisone, adriamycin, bleomycin, vincristine and etoposide). It may thus be prudent to consider alternating therapy to be the gold standard at the present time, with about 60% of patients with advanced disease remaining disease-free.

Following on from MOPP/ABVD, the Milan group developed a hybrid of the two regimens which delivered all eight drugs in each cycle. The ten-year results of the randomized trial comparing the alternating and hybrid regimens showed similar results for both arms with 90% of patients reaching complete remission and around 70% remaining free of disease progression [31].

Other multiagent regimens appear to have been even more successful. Most notable is the Stanford V regimen which is a 12-week regimen of seven drugs (adriamycin, vinblastine, mustine, vincristine, bleomycin, etoposide, prednisone) followed by radiotherapy to sites of initially bulky disease. In the first 94 previously untreated patients who received this regimen, the actuarial progression-free survival at six years was 89% with an overall survival of 93% [32]. These results require confirmation in large randomized trials, but if upheld, suggest there could be a significant fall in the requirement for high-dose salvage regimens.

Salvage therapy

Patients who have had a good response to initial therapy but who have not quite achieved a complete remission (CR) can usually be converted to a durable CR by involved field radiotherapy, providing the residual disease is of small bulk (<1.5 cm nodal disease and <3 cm mediastinal mass) [33]. Patients relapsing after a long CR can often be rescued by further standard-dose chemotherapy. Data from the Milan Institute showed that in patients relapsing after more than one year, retreatment using the same chemotherapy regimen that induced the first CR resulted in a second CR in 75% of patients with a median relapse-free survival of 42 months [34]. Tannir *et al.* showed that for patients relapsing after CR of more than one-year duration, 79% attained CR with further conventional therapy and 45% of these CR patients did not relapse at up to 22 years [35]. It must be noted, however, that the overall projected survival for the group of patients relapsing after a long CR was only 24% due to a large number of deaths not due directly to the Hodgkin's disease. By contrast, patients who fail to reach a CR or relapse within one year of initial chemotherapy fare poorly with conventional salvage regimens. Less than 25% of early MOPP failures are cured with ABVD [34], ABDIC [35,36] or B-CAVe [37] and in the early relapse patients studied by Longo *et al.*, the actuarial overall survival at 20 years was only 11% [38]. Furthermore, although patients initially treated with front-line non-cross-resistant regimens such as MOPP/ABVD benefit from a higher cure rate, those who relapse have fewer conventional salvage options. The Milan group report that only 10–15% in early relapse after MOPP/ABVD or MOPP/ABV were salvageable with CEM (clomustine, etoposide and predmustine) [39]. Patients relapsing after several lines of chemotherapy also have a poor outcome and it was in these poor prognosis settings that high-dose therapy and autologous transplantaion was introduced in earnest in the early 1980s.

High-dose therapy

Indications for high-dose therapy

Precise indications for high-dose therapy can only be defined by randomized trials but there is a marked paucity of such studies. The primary reason for carrying out a transplant procedure has therefore been that the patient is deemed to have a poor prognosis if conventional chemotherapy is continued, although in many series the criteria for defining poor prognosis status are not given. At University College London Hospitals (UCLH), the criteria for transplantation (Table 9.1) [40] were based on an analysis of 600 patients registered with the BNLI who were treated initially with MOPP chemotherapy. Patients were considered suitable for transplantation if their anticipated life expectancy at five years from the time of treatment failure was less than 35%. Today this might be a considered to be a very conservative guideline but in the early and mid-1980s, the procedure-related mortality from autologous bone-marrow transplantation was in the order of 10–15%, which inspired a cautious approach.

*Table 9.1 University College London Hospitals' criteria for transplantation in Hodgkin's disease.**

- Patients failing to obtain complete remission on MOPP-type chemotherapy:
  high-grade histology**
  low-grade histology*** and erthrocyte sedimentation rate>59
- Patients failing two treatment modalities
- All patients failing alternating or hybrid regimens

*Based on an analysis of 600 registered with the BNLI who were treated initially with MOPP chemotharapy.
**High-grade histology: cellularity, nodular sclerosis II, lymphocyte-depleted.
***Low-grade histology: Lymphocyte predominant, nodular sclerosis I.

A number of aspects of these selection criteria merit further comment. Firstly, not all patients who failed to attain CR with MOPP were deemed to be of sufficiently poor prognosis, but only those patients who had other poor prognostic features at presentation. In an analysis from the National Cancer Institute in the USA, however, of the 51 of 439 patients who failed to achieve CR with conventional therapy, the median survival was only 16 months [38] with no projected survivors beyond 10 years. It should be noted that major uncertainties can arise in such patients if they have had a 'good response' to initial therapy but they have residual computed tomography abnormalities. Jones *et al.* only offered high-dose treatment for this group of patients with radiological partial remissions, if their disease subsequently progressed [41], but in most series it is not clear how this problem was addressed.

Secondly, all patients failing two or more courses of chemotherapy were considered eligible for high-dose salvage therapy although there is a small group of patients with chronic relapsing Hodgkin's disease who have a very protracted course and reasonable survival rates at five years from second-line treatment failure with standard-dose salvage

therapy [42]. Desch and colleagues considered patients described in the literature who had attained a CR on MOPP or ABVD which had lasted for less than one year and had then been treated with different forms of salvage therapy, and they concluded that high-dose therapy was best given after second-line chemotherapy failure [43].

Thirdly, all patients relapsing within a year of alternating therapy were considered to be candidates for high-dose therapy although at that time there were insufficient long-term data on patients treated with such regimens within the BNLI database to base this judgement on hard facts. Increasingly, such patients have received high-dose therapy even if their relapse occurs after one year, and Gause and Longo recently concluded that 'the accumulated data seem to favour high-dose therapy with autologous transplantation as first treatment option for patients who are induction failures and for those who achieve a CR of any length and then relapse' [44]. This approach means that the natural history of treatment failure with conventional dose salvage therapy can not be accurately ascertained. This is well illustrated by the BNLI trial in advanced Hodgkin's disease between 1983 and 1989. In the arm receiving alternating LOPP/EVAP+radiotherapy, during the first two years of the trial, only 6 of the 32 patients (18%) failing this initial therapy received high-dose therapy as the next line of therapy, but in the last two years of the trial, 18 of 29 failures (62%) were treated in this way (unpublished data). Not everyone necessarily agrees that there is no place for conventional-dose salvage regimens not requiring stem-cell support, particularly those that are more intensive than conventional front-line therapy. Preundschuh and colleagues have reported results using the DexaBEAM (BEAM=BCNU, etoposide, cytarabine, melphalan) salvage regimen that are similar to those reported for high-dose therapy, although it should be noted that repeated courses of DexaBEAM are very intensive and represent considerable dose escalation [45]. Fourthly, the analysis of the BNLI data indicated that the high-dose therapy selection criteria developed for patients treated initially with chemotherapy were also appropriate for patients with limited stage disease at presentation who received radiotherapy and then received chemotherapy at the time of treatment failure [40].

The encouraging results obtained with autologous transplantation in the salvage setting, and the generally better results achieved when disease bulk was low, raised the possibility of using high-dose therapy in first CR in those patients at high risk of relapse. The problem with this strategy is that it is actually difficult to predict accurately which patients will relapse. Gribben and colleagues concluded that no suitable group of patients in first CR could be identified, because once remisssion was attained, most of the poor prognostic factors at presentation dissappeared [40]. This referred to an analysis of patients treated with MOPP rather than alternating therapy, based on overall survival rather than progression-free survival, and using conservative thresholds for high-dose therapy. Carella and colleagues analyzed the presenting features in 386 patients with Hodgkin's disease and identified a group of patients who achieved CR but had a very high risk of relapse. These patients had 4 of 6 of the following: a bulky out mediastinum, a nodal mass >10 cm, age over 30 years, B symptoms, lung involvement or involvement of more than one extranodal site [46]. This interesting prognostic stratification, however, lacks validation from an independent large series of patients. The International Prognostic Factors project on advanced Hodgkin's disease has looked at 4937 patients from Europe and North America who all received initial therapy containing an anthracycline. The worst prognostic group who had a progression rate of approximately 50% accounted for

less than 10% of all patients [47]. This small group of patients might certainly be candidates for early intensive therapies but it should be noted that progression occurred in many of these patients within the first year so that they never actually achieved a stable CR. Lee and colleagues looked at prognostic factors for disease progression in patients aged under 60 years who had not progressed in the first six months after starting primary chemotherapy, i.e. candidates for a first-remission autograft [48]. The worst prognostic group, accounting for approximately 10% of patients, had a progression-free survival at five years of 57%.

As well as disease status, a number of patient characteristics are important. Most centres have used an upper age limit of 60 or 65 years, although in practice the majority of patients treated in published series are much younger than this, with a median age of 30 years or less. Age has not generally been shown to be an adverse prognostic factor for patients receiving high-dose therapy for Hodgkin's disease, although this probably represents considerable patient selection in the older age group. Most centres have also used selection criteria based upon the physical condition of the patient such as an adequate glomerular filtration rate (typically >60 ml/min), left-ventricular ejection fraction (typically >40–50%) and Karnofsky performance status (e.g. >70%). These criteria are arbitrary and not employed by all.

Overall results with high-dose salvage therapy

Early procedure-related mortality

The early procedural mortality is usually defined as that occuring in the first 90 or 100 days. In some series this includes all deaths within this time frame, whereas in other series deaths due to rapid disease progression are excluded. At UCLH, the proportion of all Hodgkin's disease patients dying within three months of transplantation was 10.4%, with an early procedure-related mortality of 8.3%. In the last 100 patients with at least six months' follow-up, it has fallen to 6.0%. In the EBMT Registry, the early procedure-related mortality following high-dose therapy and autologous blood or marrow transplantation for Hodgkin's disease is 9.9% [49]. There has also been a progressive fall over the years, and although the availability of more recent antimicrobial agents and haemopoietic growth factors and the use of peripheral blood stem cells may have contributed to this improvement, the major factor appears to be the selection of better risk patients. Several investigators have found that patients with resistant disease have an increased procedure-related mortality which is similar to the experience in the non-Hodgkin's lymphomas [50]. Zulian *et al.* reported that 4 of 8 procedure-related deaths occurred in patients who had never achieved CR [51]. In the series described by Yahalom *et al.*, the mortality rate for the group who had never reached remission was 21% which was significantly higher than that for the group who relapsed after CR (11%) [52]. Reece *et al.* [53] also found that patients who had never reached CR had a significantly higher risk of toxic death. Disease bulk at the time of transplantation was also found by Rapoport *et al.* [54] to impact upon the complication rate. Table 9.2 summarizes survivals seen at various centres in poor-risk patients.

Interstitial pneumonitis stands out as the most important cause of procedure-related mortality in most series of patients with Hodgkin's disease. Although such a diagnosis is often made on radiological grounds alone, with no tissue diagnosis available, and will thus include some cases of bacterial, viral or fungal infection or intrapulmonary haemorrhage, drug- or radiation-induced pneumonitis is usually considered to be the major cause. Phillips *et al.* in Vancouver treated 26 patients with cyclophosphamide and total body irradiation (TBI) with optional involved field radiotherapy. Fatal idiopathic interstitial pneumonitis occurred in three patients (11.5%), resulting in this centre switching to CBV (cyclophosphamide, BCNU and etoposide) conditioning [55]. Nine of 56 patients again developed interstitial pneumonitis which was fatal in 7 (12.5%) [53]. Of the initial patients treated with cyclophosphamide and TBI, the 3 deaths from interstitial pneumonitis all occurred in patients who had received prior radiotherapy to the thoracic region [55]. In the CBV recipients, 8 of 42 patients with a history of prior thoracic radiotherapy developed interstitial pneumonitis compared to only 1 of 14 with

Table 9.2 Survival in poor-risk patients

Survival criteria*	Study
DFS at 5 years=40%	Jagannath *et al.* (1989)
OS at 4 years=38%; PFS=27%	Phillips *et al.* (1989) [55]
OS at 2 years=31%	Zulian *et al.* (1989) [51]
OS at 3 years=66%; PFS=51 %	Jones *et al.* (1990) [41]
EFS at 3 years=37%	Kessinger *et al.* (1991) [71]
OS at 5 years=53%; EFS=47%	Reece *et al.* (1991) [53]
OS at 5 years=13%; EFS=14%	Anderson *et al.* (1993) [65]
DFS at 3 years=38.6%	Crump *et al.* (1993) [66]
OS at 5 years=50%	Chopra *et al.* (1993) [58]
EFS at 3 years=49%	Rapoport *et al.* (1993) [54]
DFS at 6.5 years=50%	Yahalom *et al.* (1993) [52]
OS at 5 years=46.9%; PFS=38.6%	Fielding *et al.* (1994) [49]
OS at 2 years=75%; DFS=58%	Nademanee *et al.* (1995) [57]

*DFS=disease-free survival; OS=overall survival; EFS=event-free survival; PFS=progression-free survival.

no such previous treatment. In Seattle, 5 out of 19 TBI recipients who had received previous thoracic radiotherapy died from pulmonary complications following the transplant procedure [56]. Nademanee reported that of 5 patients developing interstitial pneumonitis (2 of which were fatal) following CBV conditioning, all had a prior history of mediastinal irradiation [57]. At the Johns Hopkins Oncology Centre in Baltimore between 1985 and 1988, 50 patients were treated with allogeneic (if a suitable sibling was

available) or autologous bone-marrow transplantation [41]. Twenty-five patients had received extensive prior radiation therapy and so received cyclophosphamide and busulphan conditioning. The other 25 patients received cyclophosphamide and TBI. Fatal idiopathic pneumonitis occurred in 7 patients overall (14%) and did not seem to relate to the conditioning protocol—4 of 7 patients had received busulphan and 3 of the patients had received TBI. Death from pneumonitis occurred in 6 of the 28 patients (21%) who had received prior mediastinal irradiation but in only 1 of 22 (5%) who had not. Prior mediastinal radiation is thus a major risk factor for interstitial pneumonitis particularly if the radiation has been recent. It is our practice to delay high-dose therapy of any type for at least three months after mediastinal radiotherapy, and preferably six months. The other causes of mortality are the expected consequences of myelosuppression, although the toxic colitis associated with BEAM chemotherapy, particularly when doses are escalated, should be noted.

Response rates to high-dose therapy

Assessment of disease response is not straightforward due to the variable criteria used to define a complete response and the protracted period over which resolution of masses can occur following high-dose therapy. For instance, in the UCLH series reported by Chopra *et al.*, of the 72 patients (46%) who were deemed to have had a partial response at three months, 13 were in CR by six months, 8 following consolidation radiotherapy but 5 after no further therapy [58]. Similarly, in the series from the City of Hope National Medical Center [57], at 30 days 61 patients were in a radiological CR and 5 were in partial remission (PR). Further scans were performed at three months without any intervening therapy which demonstrated 68 patients to be in CR and 1 patient in PR. Furthermore, in many patients the lymphoma mass never completely resolves but relapse does not occur, presumably indicating that the residual mass was composed of fibrotic tissue only. The most reliable parameter of response is thus the percentage of patients remaining progression-free, and, of course, the overall survival.

Progression-free and overall survival

In the UCLH series of 336 patients with at least six months follow-up at 10 years, the actuarial progression-free survival was 45% with an overall survival of 51%. (Figure 9.1) Similar data have been reported from other centres (Table 9.3). In the EBMT Registry the actuarial progression-free and overall survivals at five years were 38.6% and 46.9% respectively [49]. The Stanford group have recently compared the results of high-dose therapy for relapsed or recurrent Hodgkin's disease in 60 patients with the results obtained in an historical matched control group treated with conventional salvage regimens at their centre. The actuarial freedom from progression at four years was 62% in the transplant recipients compared to 32% in the conventional salvage recipients (P~0.01), with event-free survivals of 53% and 27% respectively (P~0.01) [59]. The improvement in overall survival was, however, relatively small and not significant.

Approximately ten years ago, the BNLI commenced a randomized trial of BEAM versus up to three courses of mini-BEAM in poor-risk patients as defined in Table 9.1. This trial was prematurely terminated because as experience with high-dose therapy

accumulated, fewer physicians and patients were prepared to participate in the trial. The progression-free survival in the transplant arm and the arm receiving the same drugs at doses that did not require stem-cell support were exactly as predicted from the single-centre experience of high-dose therapy and the BNLI experience of other conventional-dose salvage regimens (Figure 9.2a) [60]. It is interesting that this improvement in progression-free survival has not yet translated into an overall survival benefit (Figure 9.2b). This is at least in part due to the fact that 9 of 20 of the mini-BEAM failures

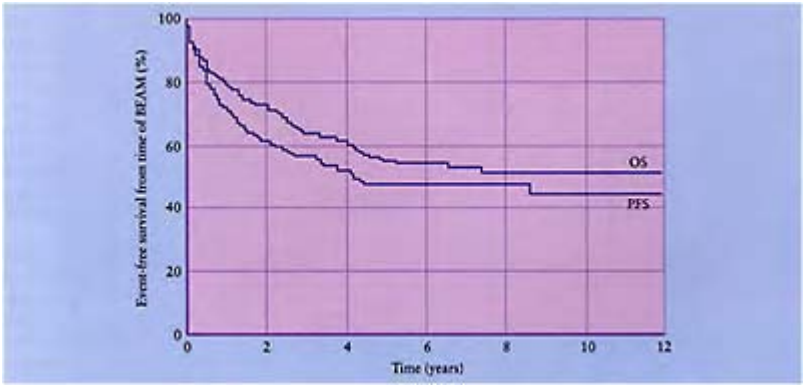

Figure 9.1 Progression-free survival (PFS) and overall survival (OS) after high-dose salvage therapy in the UCLH series.

Table 9.3 Survival in patients with Hodgkin's disease

Centre	Year of study	Patients *(n)*	Complete response (%)	Partial response (%)
Vancouver	1978–1985	26	69	12
EBMT Registry	1979–1994	134	–	–
University College London Hospital	1980–1991	155	28	46
MD Anderson/Nebraska	1982–1986	61	46	31
Memorial Sloan-Kettering	1985–1991	47	62	–
Johns Hopkins Oncology Centre	1985–1988	28	74	23
Vancouver	1985–1987	56	79	4
Royal Marsden	1985–1987	38	46	32

Nebraska	1986–1990	56	52	32
Toronto/London	1986–1991	73	75	–
City of Hope National Medical Center	1988–1993	85	80	1
Rochester, New York	1988–1992	47	–	–

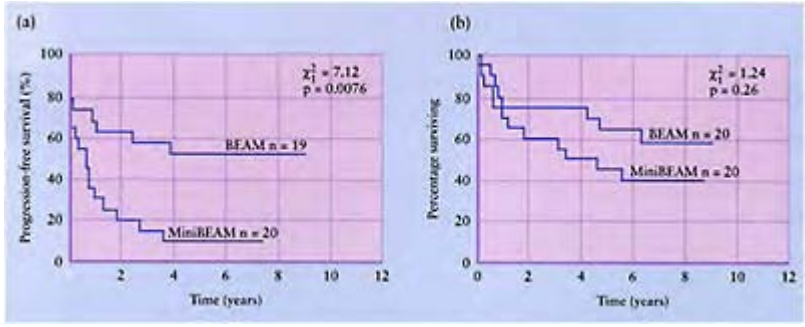

Figure 9.2 (a) Progression-free survival after high-dose therapy and the same drugs at doses not requiring stem-cell support, given as salvage therapy. (b) Improvement in progression-free survival has not yet translated into an overall survival benefit.

have gone on to an autograft procedure and 6 of these are still alive, although only 2 have remained in CR since the autograft.

The German Hodgkin's Disease Study Group are currently carrying out a randomized trial between four courses of DexaBEAM (similar to mini-BEAM) and two courses of DexaBEAM followed by a BEAM transplant. The result of this trial is awaited with great interest but, as previously pointed out, four courses of DexaBEAM is also a very intensive treatment.

Progression most frequently occurs in the first year after high-dose therapy. The median time to relapse is remarkably uniform among different series—9 months [58], 9 months [57], 8 months [63] and 7 months [64]. Relapse beyond two years is less common, although it does occur as can be seen from Figure 9.1. Relapse most commonly occurs at sites of previous disease—33 of 49 relapses [65], 23 of 30 [57], 12 of 13 [62], 10 of 10 [55], 13 of 15 [41]. This pattern of relapse has contributed to the use of adjuvant involved field radiotherapy.

Factors determining outcome after high-dose therapy

In a multivariate analysis of the UCLH patients, the most important prognostic factor was the size of the largest mass at the time of transplantation. Other significant factors were the relapse status at the time of transplant, number of courses of treatment prior to ABMT, and sex (Table 9.4). In this series, the progression-free survival at five years was 61% if the largest mass was <5 cm, 51% if 5–10 cm and 23% if >10 cm. Crump *et al.* reported that patients with no demonstrable disease (i.e. <1.5 cm) at the time of transplantation had an improved disease-free survival (DFS) (68% at four years), compared to patients with non-bulky residual disease defined as <5 cm (26%), which, in turn compared favourably with those transplanted with bulky disease defined as >5 cm (0%) [66]. Rapoport *et al.* similarly showed an improved event-free survival in patients transplanted with masses <2 cm (70% event-free survival (EFS) at two years) compared to >2 cm (15% EFS) [54]. These observations have influenced policies pertaining to pretransplantation conventional-dose salvage therapy.

The second most important factor was relapse status. Patients with primary refractory disease had a progression-free survival of only 33% at five years, but paradoxically, patients transplanted at third or later relapse fared better than those in second relapse who in turn did better than those transplanted at first relapse. This is partly because some patients with Hodgkin's disease have a chronic relapsing form of the disease, and after many relapses can still have a further prolonged remission after high-dose therapy.

Table 9.4 Factors predicting for outcome after BEAM and ABMT salvage therapy in Hodgkin's disease. Adapted from Chopra et al. (1993) [58]

Factor	Univariate analysis	Multivariate analysis
Tumour mass at ABMT	*P*<0.001	*P*<0.001
Relapse status at ABMT	*P*<0.001	*P*=0.007
Number of courses of treatment prior to ABMT	*P*=0.005	*
Sex	*P*=0.001	*P*=0.42
Chemosensitivity status at ABMT	*P*=0.001	*
Length from diagnosis to ABMT	*P*=0.001	*
B symptoms at ABMT	*P*=0.001	*
First remission>12 months	*P*=<0.1	*
*Data not significant.		

Patients transplanted at a late stage of the disease usually have a long period between diagnosis and transplantation, which is a significant factor on univariate analysis only. The poorer results in patients with primary refractory disease have been apparent in other

series of patients [52], although an analysis from Vancouver of patients with induction failure reported better results with an actuarial progression-free survival of 42% at a median follow-up of 3.6 years [67]. Lohri and Connors studied a series of patients with one or more poor prognostic features and suggested that high-dose therapy afforded no benefit in this high-risk group [68]. Andre *et al.,* however, compared the results of 86 patients transplanted for primary refractory disease with matched patients registered on national and international databases who did not receive a transplant [69]. The five-year actuarial survival was 35% in the transplant recipients and 20% in the control group. A recent analysis of the Stanford patients further suggests that it is in the poor-prognosis patients that the maximum relative benefit is achieved [59].

The finding that patients at, or beyond, second remission have a good outcome is not universal and this is in part because in some series this group of patients had a very high procedure-related mortality. The number of courses a patient has received prior to transplantation is obviously closely related to which remission/relapse they are in. Thus, in the UCLH series, the patients who have received the most therapy are those in or beyond second relapse who tend to have an improved outlook. However, on multivariate analysis the receipt of higher numbers of treatment courses then becomes an adverse prognostic factor, suggesting that for any individual patient it is probably better to carry out a high-dose procedure earlier rather than later. Sex was a surprising prognostic factor in the UCLH series, with males having a better outlook than females. This is not apparent in all series but is significant in the collective EBMT series [49]. The reason for this is not known. Chemosensitivity status at the time of transplantation has an important bearing on the outcome [41,63]. Patients with chemosensitive disease (PR to initial therapy or responding relapse) fare better than patients with disease that is resistant to conventional chemotherapy (Figure 9.3), although in the UCLH series this was only significant in univariate analysis [48]. This, in part, relates to disease bulk at the time of transplantation, as patients with resistant disease almost invariably have larger masses at transplantation.

An additional difficulty arises in the comparison of series in that different criteria have been used to define

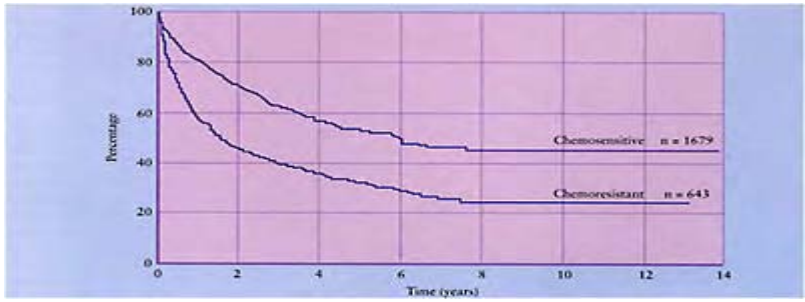

Figure 9.3 Overall survival of patients with Hodgkin's disease following autologous transplantation. EBMT data.

chemosensitivity. Some groups stipulate a reduction of at least 50% in cross-sectional area of disease, whereas other groups accept a lesser response. In the UCLH series, patients with untested relapse had the most favourable outcome, but this does not mean that conventional salvage is deleterious but rather reflects that this subgroup of patients was selected on the basis that they had low bulk disease at the time relapse was detected.

An important point to note is that even in chemoresistant disease, over one-quarter of the patients still have a successful outcome following high-dose therapy, which contrasts markedly with the situation in non-Hodgkin's lymphoma where nearly all such patients die [50,70]. However, it should be pointed out that patients whose disease is progressing on standard-dose therapy or who have B symptoms at the time of transplantation almost invariably do poorly [67 and personal observations].

Several centres have reported the results of transplantation for patients according to the length of first remission, with long first remissions boding well. Nademanee *et al.* reported 17 patients whose initial CR was more than one year [57]. The two-year DFS for this group was 63%, compared to 51% in those with short initial CR, although this difference was not significant. Reece *et al.* reported on 58 patients treated in first relapse and found that patients with an initial CR of more than one year had a lower risk of disease progression than those with an initial CR of under one year [62]. The progression-free survival at four years after transplantation was 85% in patients whose first CR lasted more than one year, and 48% in the group whose CR duration was less than a year. In the series reported by Crump *et al.* the DFS at four years was 53% in those patients with initial CR lasting more than one year and 28% in those with shorter durations, although due to the small patient numbers, this difference was not statistically significant [66]. In the larger UCLH series, however, the length of first remission was not significant [58].

B symptoms at the time of transplantation have been found by some groups to be an adverse prognostic indicator in univariate, although not in multivariate analysis [58]. Disseminated extranodal disease is also potentially a poor prognostic factor. Kessinger *et al.* found that patients with no marrow involvement at the time of peripheral blood stem-cell transplantation had an actuarial EFS of 47% at three years, while those with marrow involvement at the time of stem-cell collection had a significantly worse actuarial EFS of 27% [71]. It should be noted that some of these latter patients received DHAP salvage prior to high-dose therapy and may not all have still had marrow disease at the time of transplantation. At UCLH, it has been our practice not to carry out high-dose procedures in any patient with bone-marrow disease. However, in a study in which bilateral trephines were repeated at the time of the bone-marrow harvest and results not reported to clinician or patient, it was found that 6 patients with marrow disease had been transplanted and 3 were in continuous CR beyond 30 months [72]. Grimwade *et al.* examined the importance of pulmonary involvement at the time of transplant and were unable to demonstrate any adverse effect upon survival [73].

Performance status at the time of transplant is another important factor not evaluated in the UCLH series, as poor performance status was an exclusion criterion. Bierman *et al.* [74] and Reece and Phillips [75] report long-term survival in less than 20% of patients with resistant disease and a poor performance status. Lactate dehydrogenase levels at the time of transplantation have also been shown to be highly predictive of outcome [76].

Most patients who receive high-dose therapy will have received conventional-dose salvage regimens immediately prior to their transplant. As well as allowing the definition of chemosensitivity, this serves the vital process of disease 'debulking' prior to transplantation. Enthusiasm for debulking is derived from the major influence on outcome of the size of the largest mass at transplantation. The potential disadvantage of salvage therapy is that it may encourage development of drug resistance by the tumour, particularly when the same drugs are used for initial salvage therapy and then the high-dose regimen. Sometimes the use of initial salvage treament can delay definitive high-dose therapy if there are significant complications due to salvage therapy, and this is why we proceed directly to high-dose therapy if disease bulk is small and logistics allow. The type of pretransplant salvage varies considerably and there are no comparative studies on their efficacy in this context. Regimens such as MOPP or ABVD can be used to good effect depending on what, with and when, the patient was previously treated. Mini-BEAM and DexaBEAM are more potent salvage regimens but are stem-cell toxic and can mitigate against a later stem-cell collection. We therefore frequently use platinum-based regimens such as DHAP or ESHAP [77,78] for this purpose [79].

Choice of conditioning regimen and further treatment intensification

Hodgkin's disease is a highly radiosensitive tumour and TBI was therefore a logical early choice as a conditioning regimen. Concern about pneumonitis, particularly in those who had received previous mantle field radiotherapy, led many centres to change to a high-dose chemotherapy regimen although, as discussed above, this does not necessarily prevent this problem.

A range of different chemotherapy regimens has been used and the choice of drugs has usually been based on limiting myelosuppression rather than proven efficacy in relapsed Hodgkin's disease. The CBV (cyclophosphamide, carmustine and etoposide) regimen has been the most widely used high-dose chemotherapy in North America, whereas BEAM (BCNU, etoposide, cytarabine and melphalan) has been more commonly used in Europe. An analysis of the EBMT Registry data suggested that BEAM was superior to CBV in Hodgkin's disease (but not non-Hodgkin's lymphoma) [49], although this cannot be proven without a randomized trial. Those centres currently using BEAM would probably not want to enter such a trial. The original CBV regimen [80] used carmustine at a dose of 300 mg/m^2 similar to that used in most BEAM regimens. Subsequently, several centres escalated the dose of BCNU to 600mg/m^2 [81,82] and this was associated with a rise in procedure-related mortality from around 10 to 20%. The major toxicity of higher BCNU doses is interstitial pneumonitis. Zulian and colleagues also found escalated BCNU to be very toxic in the BEM regimen [51], although Carella and colleagues were able to administer 600 mg/m^2 BCNU in the CBV regimen without undue toxicity [61]. Interestingly, the series of patients treated with high-dose BCNU have a similar overall survival to patients treated with standard doses, despite the higher procedure-related mortality, raising the possibility of greater tumour responses at escalated doses. Attempts have also been made to escalate the etoposide dose in high-dose regimens. Wheeler *et al.* carried out a specific dose-finding study using CBV and concluded that the highest tolerated dose of etoposide was 2.0 g/m^2, although this was in combination with escalated

doses of cyclo-phosphamide and BCNU [82]. They found an increase in non-fatal infectious complications with increasing doses of etoposide which probably related to the increased mucositis occurring at the higher doses.

In 1989, Jagannath *et al.* described the effects of increasing the etoposide dosage from 450 to 900 mg/m^2 within the CBV regimen [63]. The 4 toxic deaths in this series were all in the highest dosage group (2 from candidaemia and 2 from interstitial pneumonitis). Similarly, the maximum tolerated dose of etoposide in the BEAM regimen is close to the commonly used dose of 200 mg/m^2×4. Escalation to 400 mg/m2×4 resulted in little additional gastrointestinal toxicity, but at a dose of 600 mg/m^2×4, the incidence of grade III/IV ileitis rose from 7.5% to 77%, and the incidence of gastrointestinal haemorrahge rose from 17.5% to 46%, with 1 death in the high-dose group secondary to the gut toxicity [83].

Various other forms of dose intensification have been attempted. In poor-prognosis patients, we have given one or two courses of mini-BEAM therapy prior to a full-dose BEAM autograft, which, although not requiring stem-cell support, is considerably more intensive than other commonly used salvage regimens. Some success was achieved [58]. A similar approach is being used by the German Hodgkin's Disease Study Group in all patients that receive an autograft. In a study from The Netherlands, an intensive MINE (mitoguazone, ifosphamide, navelbine and etoposide) regimen was given to 100 poor-prognosis patients before high-dose therapy. There were 6 procedure-related deaths, 3 of them occurring during the MINE phase of the treatment [84].

The New York Medical College investigators have taken a particularly difficult group of refractory Hodgkin's patients and considered them for 'double transplants' [85]. They autografted these patients and, if they showed a response, proceeded to a second transplant with a different high-dose chemotherapy regimen. Of the 46 patients reported, there were 5 procedure-related deaths and 28 responses to the first transplant. Twenty-five of these went on to a second transplant and 12 were progression-free at the time of reporting.

Since most relapses occur at sites of previous disease, the addition of adjuvant radiotherapy to the high-dose therapy is appealing, although its role, as in other situations, remains controversial. It has routinely been used in many centres if there have been residual masses after high-dose therapy, precluding controlled trials to prove the efficacy of such therapy. In the Vancouver series reported by Phillips *et al.*, in addition to cyclophosphamide and TBI, selected patients received involved field irradiation to sites of bulk disease [55]. Only 3 of 13 relapses occurred in patients who received such adjuvant local irradiation. The Vancouver group continued to use involved field irradiation once they started to use CBV in place of TBI. Disease progression ocurred in 17 patients, 16 at the site of previous disease, but in only 2 of the 24 patients who received local irradiation pretransplant did the relapse occur in the irradiated site [53].

One of the potential problems of delivering the radiotherapy prior to transplantation is that it may exacerbate the toxicity of the conditioning therapy. Indeed, Reece *et al.* reported that patients receiving radiotherapy in this manner had a higher incidence of procedure-related interstitial pneumonitis than those patients who had either no, or more temporally, remote therapeutic chest irradiation. It has been our practice, therefore, to give the consolidation radiotherapy three months after high-dose chemotherapy. In the UCLH series reported by Chopra *et al.*, 41 patients received post-graft irradiation to

residual masses and 8 achieved a CR, although the benefit of this is difficult to ascertain as 5 patients with a PR subsequently attained CR without further therapy [58]. In the patients reported by Jagannath *et al.* in 1990, 18 patients attained partial remission at three months, and 6 of these reached CR with local radiotherapy [63]. Two of these were sustained complete remissions.

Stem-cell type

Peripheral blood stem-cell (PBSC) transplant versus autologous bone-marrow transplant (ABMT)

PBSC transplant has been shown in randomized trials to result in faster haemopoietic recovery than ABMT with less resource use [86,87]. As a consequence, peripheral blood stem cells have almost replaced autologous bone marrow as the stem-cell source of first choice. An additional potential advantage of PBSC is that they can be collected from patients who have had prior radiotherapy to the pelvis or who have marrow disease, hopefully without tumour contamination. In 1990, Korbling *et al.* described a group of 11 patients with advanced disease and prior radiation to the pelvis in whom it was thought that bone-marrow harvesting would be unrewarding, and yet PBSC transplantation proved possible [88]. Despite this success, there are patients in whom stem-cell mobilization is unsuccessful. In a series of 114 patients with relapsed and resistant Hodgkin's disease in whom stem-cell mobilization was attempted at UCLH with a combination of chemotherapy and granulocyte colony-stimulating factor (G-CSF), 9 patients (8%) failed to achieve the minimum requirement of 1×10^6/kg $CD34^+$ cells or 1×10^5/kg granulocyte-macrophage colony-stimulating factor (GM-CSF). In patients in whom there was a failure to mobilize stem cells, the use of bone marrow did not usually result in prompt engraftment, indicating that poor mobilization is most frequently due to poor bone-marrow function [89]. Prior radiotherapy is a major risk factor for mobilization failure [90,91], other factors being receipt of multiple courses of chemotherapy [92] and receipt of DexaBEAM [93] or mini-BEAM [91]. Kessinger *et al.* reported the results of a group of 56 patients with Hodgkin's disease treated at the University of Nebraska between 1986 and 1990 with PBSC transplant [71]. All patients were selected for this source of stem cells because of prior or current bone-marrow disease, pelvic radiotherapy or marrow hypocellularity at the time of stem-cell harvesting. The EFS at three years of 47% compares favourably with that of ABMT. The results in those patients with bone-marrow disease at the time of stem-cell harvesting were less satisfactory, as discussed above. The transfer to PBSC transplant occurred without consideration of long-term outcome, and a disturbing matched-pairs analysis from the EBMT Registry has suggested that patients receiving PBSC transplant fare less well than those receiving ABMT. Overall survival and progression-free survivals at three years were 73% and 55%, respectively, in the ABMT recipients, and 60% and 44% in the recipients of PBSC transplant [94]. Registry studies are subject to 'centre effects', and a matched-pairs analysis of the UCLH single-centre database shows no such effect, with a progression-free survival at three years in the ABMT and PBSC transplant of 59% and 58%, respectively [95].

Bone-marrow purging

Bone-marrow purging is not widely practised in Hodgkin's disease, although the relatively restricted expression of CD30 by Hodgkin's cells makes immunodepletion a feasibility. Jones *et al.* carried out 28 ABMT purged with 4-hydroperoxycyclophosphamide [41]. The purging resulted in delayed engraftment although there did not seem to be any resulting increase in infective complications. There was no obvious reduction in the relapse rate for these patients compared to other published series, but very large numbers of patients would be required to demonstrate any difference.

Allogeneic versus autologous bone-marrow transplant

There is still limited experience of allogeneic transplantation in Hodgkin's disease, and many of the reports available from single centres are of small size and combine cases of both Hodgkin's disease and non-Hodgkin's lymphoma. It is clear, however, that recipients of allografts suffer many more procedure-related problems than do autograft recipients. In the initial experience of 8 Hodgkin's disease patients treated by allogeneic transplantation in Seattle, 4 died of procedure-related toxicity [96], and in a later report concerning 53 allograft recipients, 28 (53%) suffered non-relapse deaths [65]. In a series of allografts performed in patients with either Hodgkin's disease or non-Hodgkin's lymphomas in Baltimore, the peritransplant mortality was 42% [41]. In the (IBMTR) series of 100 consecutive Hodgkin's disease patients receiving human leukocyte antigen (HLA)-identical sibling allografts, the procedure-related mortality was 54% [97]. The reasons for the high toxicity, when compared to allografts in other situations, include advanced disease stage in some patients, poor performance status of others, prior thoracic irradiation, and possibly the additional deficiencies of cell-mediated immunity due to the disease itself.

With these very high procedure-related death rates, overall survival rates are inevitably poor. In the IBMTR series, the three-year DFS was only 15% with on overall survival of 20%. The key issues for the future are whether there is a graft-versus-tumour effect that can be harnessed and if procedure-related mortality can be reduced. Jones *et al.* reported on a series of 118 consecutive patients with malignant lymphoma treated by autologous (n=80) or allogeneic (n=38) transplantation, of whom 55 had Hodgkin's disease [41]. Although type of graft did not affect overall survival, the relapse rate was 24% higher in the Hodgkin's disease patients who received an autograft than in those who received an allograft. Although the higher relapse rate with autologous transplantation could be due to tumour contamination of the graft, Jones thought it more likely that a graft-versus-lymphoma effect was operative.

In the Seattle series, recipients of HLA genotypically identical sibling allografts also had a significantly lower relapse rate compared to autograft recipients (45% versus 76%, five-year actuarial estimate P= 0.05). Data from the EBMT Registry showed that patients with graft-versus-host disease (GvHD) suffered a lower relapse rate following bone-marrow transplantation for relapsed Hodgkin's disease, adding further support to the notion of a graft-versus-tumour effect in Hodgkin's disease [98]. Once again, there was no overall survival benefit because of the higher procedure-related mortality associated with an allogeneic transplant. In contrast to the EBMT data, information from the

IBMTR on 100 consecutive HLA-identical sibling allografts failed to demonstrate a lower relapse rate in those patients with GvHD. The reasons for this difference are not clear but it should be noted that the number of surviving patients in these series in whom a graft-versus-tumour effect might impact upon relapse rates, is relatively small. Currently, allogeneic transplantation must be considered as a highly experimental procedure, but there is certainly a need for further well-conducted pilot studies in highly selected younger patients.

The role of high-dose therapy in first complete remission

In 1991, Carella *et al.* published the results of high-dose therapy and ABMT in 15 patients in CR, deemed to have a very high risk of relapse. At a median follow-up of 36 months, 13 patients (86%) remained in CR, which compared favourably with the 8 of 24 patients (33%) from a historical control group who attained CR with the same regimen but who did not receive bone-marrow transplantation as adjuvant therapy [99]. In a more recent update, they reported on 22 patients, of whom 18 remain alive in continuous CR [100]. Taylor and colleagues in Newcastle have treated 19 'poor-risk' patients in first remission with high-dose therapy, with little toxicity and no relapses at the time of reporting [101]. In the EBMT experience of 23 patients transplanted in first CR, the results are less encouraging, however, the DFS being only 70% with a median follow-up of 27 months [102].

Late procedure-related mortality and morbidity

Although the majority of patients cured by high-dose therapy and autologous transplantation make a complete physical and psychological recovery, there is occasional long-term (beyond 90 or 100 days) morbidity and even mortality. The deaths can be considered in three categories. First are problems arising early which persist or worsen beyond the arbitrary three-month cut-off. These include the rare engraftment failures and cases of progressive pneumonitis which have accounted for late deaths (albeit usually within six months) in a number of series [55,57]. Second are features of organ damage arising late after an autograft. Most worryingly, Cosset *et al.* reported two sudden cardiac deaths in their series, at 5 and 29 months' post-autograft, although such late cardiac events do, of course, occur after standard therapy for Hodgkin's disease [103]. Third, there are second malignancies, particularly myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML). Several recent reports have highlighted this problem [104–106]. In the Nebraska series [105], the risk of MDS or AML at five years after transplantation was estimated to be 11%, and in the Minneapolis series 15.2% (>18%; 95% confidence interval) [104]. In the UCLH series of 336 patients autografted for Hodgkin's disease, there have been 5 cases of AML with an actuarial risk at six years of 3.9% (1.5–9.4%; 95% confidence interval) [107]. The lower incidence in this series may be due to chance alone but it is possible that BEAM is less leukaemogenic than some other regimens, particularly those containing TBI which was implicated in the Nebraska series [105].

The UCLH patients were also compared with matched patients from the BNLI database who had not received a transplant, and there was no significant difference in

leukaemia incidence, illustrating that a major component of the risk of developing AML is the treatment received before transplantation. It has been previously demonstrated that the risk of leukaemia with conventional chemotherapy rises markedly with each line of therapy attempted, and it may be that high-dose therapy is less leukaemogenic than some other protracted courses of conventional salvage therapy.

An analysis of the EBMT Hodgkin's disease registry found that the actuarial non-relapse mortality at eight years (early and late mortality) was an astonishing 35% [49]. This emphasizes the importance of continued long-term follow-up especially as more patients are transplanted at an earlier stage in the disease process.

The issue of fertility is also important because, although not life-threatening, the psychological consequences are potentially severe. Most males and many females, having received a full course (or more) of MOPP-type therapy prior to transplant, will be infertile, but with more recent regimens, an increasing number of individuals will be potentially fertile at the time they are transplanted. Any male who has spermatozoa in his semen (even low numbers) should have semen cryopreserved before going on to high-dose therapy if this has not been done previously. For females, oocyte preservation is not yet possible and embryo cryopreservation is rarely feasible. Fortunately, a proportion of women who were fertile pretransplant will retain fertility after high-dose chemotherapy regimens such as BEAM [58] although there is still likely to be significant acceleration of the menopause [108]. TBI, by contrast, is nearly always sterilizing. Gonadal failure can also occur many months or even years after transplantation and the sequelae of this are easily overlooked. Ideally, transplant patients should have long-term follow-up at an endocrine clinic.

Financial considerations

The absolute cost of high-dose therapy and autologous stem-cell transplantation will vary from centre to centre and country to country but the advent of PBSC transplant has undoubtedly meant that these costs have fallen in the last few years [87]. The use of cytokines after ABMT [109–111] or PBSC transplant [112,113] is common practice. The beneficial effect on neutrophil recovery has been well established, and this translates into a small reduction in the length of stay in hospital. We therefore believe that the use of post-transplant growth factors is at least cost-neutral. The fact that most Hodgkin's disease patients receiving an autograft are young and that approximately 50% of patients receiving high-dose salvage therapy will have long-term good quality survival and be able to return to work, means that this is a highly cost-effective treatment. Furthermore, it is likely that a single course of high-dose therapy is less expensive than repeated courses of newer intensive salvage regimens that do not require stem-cell support, e.g. four courses of DexaBEAM.

The financial impact of earlier transplantation is difficult to compute. If one considers 100 patients with advanced Hodgkin's disease at diagnosis under the age of 60 years, receiving alternating therapy, approximately 80 will achieve CR. There will be 20 patients with failure of initial therapy, and a further 20 patients will be expected to relapse. Forty patients are therefore potential candidates for salvage therapy, but in fact this is not likely to be feasible in a proportion of patients due to poor physical condition, very extensive disease or bone-marrow infiltration. It is reasonable to assume that 25

patients would therefore come to an autograft, with 13 subsequently being long-term survivors, which would give an overall long-term-survival rate of 73%. The situation in poor-prognosis Hodgkin's disease will crucially depend on how that poor prognosis is defined. Assuming that 10% were defined as high-risk, the following can be calcuated on the basis of some approximate but reasonable assumptions from the literature [47]. Firstly, in the non-poor-prognosis group, the CR rate should be just in excess of 80% so that 66 of the 80 patients in this group will attain CR. There will be approximately 14 primary treatment failures and with a further 15 relapses, this represents 29 possibilities for salvage therapy. Of these, 18 will be expected to proceed to high-dose therapy with 9 long-term survivors. In the poor-prognosis group, so defined, there are likely to be approximately 6 of 20 who do not attain CR, of whom 4 might proceed to a transplant, with 2 expected long-term survivors. If all 14 first CR poor-prognosis patients were transplanted, there would be a total of 36 transplants and to maintain the same overall survival as with a salvage only high-dose strategy, the success rate in the first CR poor-prognosis patients would have to be 80%. A success rate of 90% would be required to save a further 2 lives, this being at the expense of 11 extra transplants. If the criteria for poor-prognosis are loosened, allowing more patients to fall into this category, then the number of transplants performed rises and the cost-benefit situation deteriorates. It is for this reason that many centres have been cautious about undertaking first remission autografts.

Conclusion

The last decade has seen high-dose therapy with autologous stem-cell transplantation established as first-line salvage therapy in the majority of chemotherapy failures, although there is a paucity of data from randomized trials. The optimal form of high-dose therapy has yet to be established, but approximately 40–50% of the recipients of such therapy can expect to be cured. PBSC transplant has largely replaced ABMT, and this has contributed to the cost-effectiveness of high-dose salvage therapy. The role of first-remission transplants, if any, and that of allogeneic transplantation has yet to be determined.

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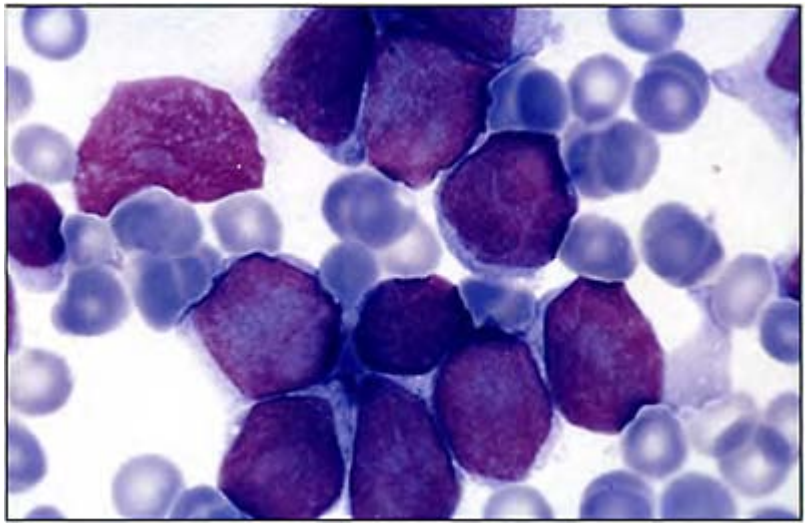

Chapter 10
Non-Hodgkin's lymphomas

Ama Rohatiner and Stephen Kelsey

Low-grade lymphomas

Background

The low-grade B-cell lymphomas are a heterogeneous group in terms of biology, morphology and clinical behaviour. However, they share the following features: the majority of patients present with advanced disease, usually with bone-marrow infiltration, in middle or older age. Conventional therapy is worthwhile in terms of inducing responses, but remissions are rarely complete and hardly ever more than temporary. Immunophenotypic analysis shows clear differences in antigen expression, and this, together with the differences in clinical features between the subgroups, confirms that they represent distinct biological entities.

In 1984, the Kiel Lymphoma Study Group analyzed the clinical course and prognostic relevance of the Kiel classification for more than 700 patients with low-grade lymphoma [1], The results can be summarized as follows: the survival curves of patients with low-

grade lymphoma show an inexorable decline in contrast to the plateau seen for patients with high-grade lymphoma. In terms of histological subtype, in the Kiel study, the survival of patients with follicular lymphoma and small-cell lymphocytic lymphoma/chronic lymphocytic leukaemia was the most favourable, followed by that of patients with lymphoplasmacytoid lymphoma, whereas those with mantle-cell lymphoma had the worst prognosis. These results are paralleled by those reported from St Bartholomew's Hospital [2–5].

This part of the chapter will focus on the use of myeloablative therapy with haemopoietic progenitor cell support in patients with low-grade B-cell lymphoma. However, to put this treatment into some perspective, each histological subtype, and the results of conventional therapy for it, will be summarized at the beginning of each section.

Follicular lymphoma

The disease

Despite responsiveness to both chemotherapy and irradiation, follicular lymphoma remains demonstrably incurable for the majority of patients [6–10]. Thus, death almost always occurs as a consequence of the disease or due to complications of therapy. Various treatment strategies have prolonged duration of remission but none, to date, has convincingly improved survival in a significant proportion of patients. Transformation to high-grade histology occurs, usually after several years, but it can happen early in the course of the disease, or may never happen [7,11–14]. Irrespective of when it occurs, survival afterwards is generally very poor.

The majority of patients present with lymphadenopathy and may otherwise be well. Alternatively, they may have B symptoms, although the latter are seen less frequently than in Hodgkin's disease. Most patients will have advanced disease at presentation, bone-marrow infiltration being most frequently the basis upon which the patient is deemed to have stage IV disease [1]. Involvement of the peripheral blood is therefore not unusual.

Much attention has focused on the molecular biology of follicular lymphoma, the t(14;18) translocation having been described in the majority of cases [15–17]. Consequently, polymerase chain reaction (PCR) analysis [18] is being increasingly used to assess the potential 'curability' of the disease by the new treatments available. The difficulty is knowing what the presence of residual t(14; 18)-containing cells in the blood or bone marrow signifies. Furthermore, equating PCR-negativity with at least the potential for cure assumes that achievement of 'molecular remission', i.e. elimination of t(14; 18)-containing cells, is a prerequisite for cure and should therefore be the aim of treatment. At present, this is only a working hypothesis.

Follicular lymphoma is unusual among haematological malignancies in that treatment may be deferred in some patients, and it has been demonstrated that such deferral of treatment does not result in shorter survival [19]. Conversely, the administration of intensive therapy from the outset does not appear to afford any survival advantage [20].

While 80–90% of patients present with disseminated lymphoma, a small proportion will be found to have limited disease which is potentially curable with radiation therapy [21–24], so treatment of such patients should not be deferred, although this has recently been questioned [25].

In many centres, conventional treatment still comprises chlorambucil in the first instance. With such relatively easy treatment, 'clinical remission' (no palpable disease) will be achieved in 75% of patients, and the median duration of first remission is approximately 18 months [6]. The combination of cyclophosphamide, vincristine and prednisolone (CVP) results in a higher complete response rate than that seen with chlorambucil, but unfortunately, no advantage in terms of survival [26,27]. The CHOP regimen (cyclophosphamide, doxorubicin, vincristine and prednisolone) has also been used extensively in follicular lymphoma [28,29]. Although the conclusion of a retrospective analysis evaluating outcome for patients receiving doxorubicin-containing regimens in open studies was that there is no advantage to adding it [30], many people with follicular lymphoma are treated with CHOP as initial therapy.

Several other strategies have been investigated, including interferon (IFN), fludarabine, and various immunologically mediated approaches. Interferon has been evaluated in two main settings: (i) in combination with chemotherapy; and (ii) as maintenance therapy. A number of phase III studies have been conducted, and the findings can be summarized as follows. Two studies, in both of which a relatively intensive doxorubicin-containing regimen was combined with IFN have shown a 'positive result'. The first, conducted by the Eastern Co-operative Oncology Group (ECOG) showed a higher response rate and better freedom from recurrence for the combination [31,32]. The second, reported by the Groupe d'Etude Lymphome Folliculaire (GELF) for patients with a 'high tumour burden' showed a higher response rate in the interferon-treated group, together with better event-free survival and overall survival [33]. In contrast, three studies in which interferon has been combined with an alkylating agent have not shown any advantage for the combination [34–36]. Interferon given early, i.e. instead of a 'watch-and-wait' policy, has also shown no advantage over prednimustine or, indeed over 'watchful waiting' [37].

Some studies have specifically addressed the question of using or continuing IFN as maintenance therapy. Three show a modest remission duration advantage in favour of the group receiving maintenance IFN but no difference in survival [34,37–40].

The purine analogue fludarabine has generally been used in patients with recurrent or refractory disease. The response rate ranges from 38 to 55% [41–44] and is somewhat higher in newly diagnosed patients [45]. Fludarabine can be useful when other treatments have failed. Fludarabine has also recently been combined with mitoxantrone and dexamethasone, resulting in response rates of over 85% in previously treated patients and high complete response rates [46]. Furthermore, this regimen is reported to result in 'molecular remission' in about half of the patients [47]. Time will tell whether this will translate into a survival benefit.

There is also currently much interest in treatment with the monoclonal antibody anti-CD20 and radiolabelled anti-CD20. The former is a chimaeric antibody, and hence it can be used repeatedly. Clinical trials are in progress. Much of the interest lies in the fact that treatment with anti-CD20 can lead to 'molecular remission' being achieved in some patients [48,49]. Another new approach has been to use anti-CD20 conjugated to iodine-

131. The preliminary results in patients who have been heavily pretreated are encouraging [50].

Myeloablative therapy

It is only relatively recently that myeloablative therapy with autologous haemopoietic progenitor cell support has been considered in patients with follicular lymphoma for a number of reasons. First, there has been understandable reluctance to use treatment which has a potential mortality and significant morbidity in a disease for which alternative treatment is available and which results in relatively long survival. Second, most patients with follicular lymphoma are older and have bone-marrow involvement. Finally, there has perhaps been a degree of negativism about using such treatment for follicular lymphoma, since previous attempts to intensify the treatment have failed to improve survival. Similarly, allogeneic transplantation has not been widely used, largely because of patient age and the potentially high treatment-related mortality in a disease with a relatively long natural history.

Myeloablative therapy with autologous bone-marrow transplantation (ABMT) following recurrence (Table 10.1)

In the light of the promising results for high-dose treatment in patients with recurrent high-grade

Table 10.1 High-dose treatment+ABMT in second or subsequent remission of follicular lymphoma

Centre	Treatment	Outcome (percentage free of disease)	Reference
St Bartholomew's Hospital	CY+TBI	61 at 5 years	[51]
Dana Farber Cancer Institute	CY+TBI	60 at 3 years	[60]
EBMT Registry	TBI cont./CT	77 at 2.5 years	[56]
Minnesota and Barcelona	TBI cont./CT	18 at 2 years	[58]
'France Autogreffe' study	TBI cont./CT	58 at 3.5 years	[57]
CY=cyclophosphamide; TBI=total body irradiation; TBI cont.=total body irradiation-containing regimen; CT=chemotherapy-only regimen.			

lymphoma (see below), the same principles have been applied in younger patients with follicular lymphoma. The total number of patients treated is still quite small and the follow-up time relatively short. Most patients have been treated with cyclophosphamide+total body irradiation (TBI) although 'drug only' regimens such as

BCNU, etoposide, ara-C and melphalan (BEAM) have also been used. Because of the propensity of follicular lymphoma to involve the bone marrow, at some centres the marrow has been purged *in vitro* prior to re-infusion.

The results from St Bartholomew's Hospital can be summarized as follows: at present, with a median follow-up of 4.5 years, one-third of patients have developed recurrent lymphoma. Freedom from recurrence does not correlate with the length of time from diagnosis, the presence or absence of residual disease at any site at the time of treatment, or the specific remission in which the treatment was given (the second as opposed to later). However, comparison with a historical control group treated in second remission at St Bartholomew's Hospital prior to the introduction of this treatment, shows a significant advantage in favour of myeloablative therapy (P= 0.001) [51] (Figure 10.1). Currently, there is no difference in survival, either because there will not be such a difference, or because the follow-up is still too short. These results are essentially similar to those reported from the Dana Farber Cancer Institute where the marrow was also treated *in vitro* with antibodies and complement [52–54], and to data reported from other centres [55–61] (Table 10.1).

A recent European Bone Marrow Transplant (EBMT) Registry analysis has reported outcome for 374 patients with low-grade lymphoma [62], the majority of whom had follicular lymphoma. At five years, the predicted progression-free survival was 38%, and survival 60%, irrespective of whether a TBI-containing or drug only regimen (BEAM) was used. On multivariate regression analysis, remission status at the time of the high-dose treatment (first remission versus chemosensitive recurrence versus refractory disease), younger age and *in vitro* treatment of the marrow were favourable prognostic factors for both progression-free and overall survival.

The question of whether re-infusion of lymphoma cells contributes significantly to recurrence remains contentious. Data from the Dana Farber Cancer Institute suggest that PCR negativity for the t(14;18) is the most important prognostic factor for freedom from recurrence [63]. In contrast, at St Bartholomew's Hospital, using one or four monoclonal antibodies, such PCR negativity is almost never achieved [64,65] and yet the clinical results are identical [66]. Furthermore, the results in patients in whom the marrow has not been treated *in vitro* are also similar (Table 10.1).

Myeloablative therapy with peripheral blood progenitor cells (PBPC) (Table 10.2)

With the general move away from autologous bone-marrow transplant (ABMT) to peripheral blood progenitor-cell (PBPC) support, the latter is also now being evaluated in follicular lymphoma. The greatest experience is from Omaha, Nebraska; Bierman *et al.* have recently reported an actuarial failure-free survival of 44% for 100 patients with follicular lymphoma receiving myeloablative therapy supported by (in the majority) unmanipulated PBPC [67]. High-dose therapy supported by PBPC has also been used in Heidelberg. Although the follow-up time for the latter study was relatively short, two statements can be made: in the majority of patients, PBPC collections contained PCR-detectable lymphoma cells. However, in 6 of 22 patients, bone-marrow samples

subsequently became PCR-negative between 3 and 16 months after re-infusion of PBPC [68].

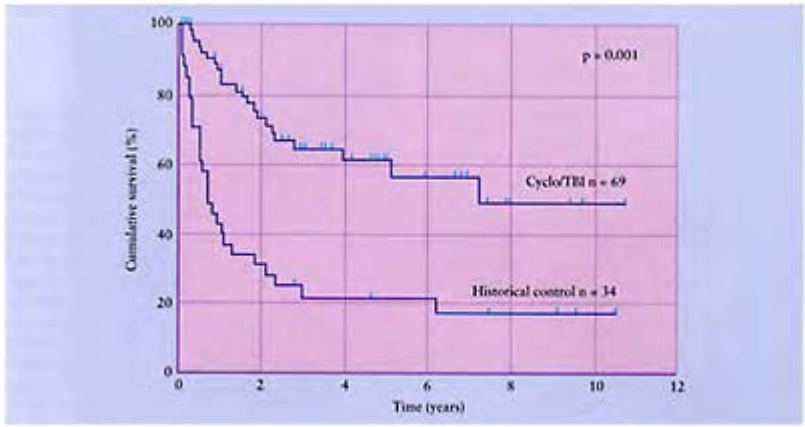

Figure 10.1 Follicular lymphoma. Duration of second remission; cyclophosphamide/TBI versus historical control group.

Table 10.2 High-dose treatment+PBPC support for follicular lymphoma

Centre	Treatment	Outcome (percentage free of disease)	Reference
Heidelberg	TBI cont./CT	Not stated	[68]
Omaha	TBI cont./CT	46 at 4 years	[67]
France (three centres)	TBI cont./CT	53 at 2 years	[69]
TBI cont.=total body irradiation-containing regimen; CT=chemotherapy-only regimen.			

In a French study, the feasibility and therapeutic efficacy of high-dose therapy with autologous PBPC support were evaluated in 60 patients with follicular lymphoma deemed to have a poor prognosis [69]. At the time of treatment, 65% of the patients had persistent bone-marrow involvement, and 82% were in partial remission. The majority of patients received a TBI-containing regimen. At a median follow-up of 21 months, the estimated overall survival and failure-free survival at two years were 86% and 53%, respectively, without a plateau. The results were significantly better for patients treated ‘earlier’, i.e. in first or second remission as compared to those treated subsequently.

Thus, PBPC appears to be a better and safer alternative to ABMT, with blood count recovery times being approximately half of those seen with marrow. However, at St

Bartholomew's Hospital there has been difficulty in collecting sufficient cells, particularly with the intention of performing CD34-positive selection. Whether this reflects previous treatment such as chlorambucil or fludarabine, or whether it represents an inherent property of follicular lymphoma is unknown. Similar problems have been reported from University College Hospital London [70] where the time from the last treatment significantly affected the feasibility of obtaining sufficient cells, six months being considered a minimum.

Myeloablative therapy+ABMT in first remission

Not surprisingly, myeloablative therapy is now also being tested as consolidation of first remission. Eight patients originally reported by Fouillard *et al.* received the BEAM regimen followed by ABMT with marrow treated *in vitro* by mafosfamide at individually adjusted levels. At the time of publication, all 8 were in unmaintained complete remission (CR) 15–43 months later [71]. A larger number of patients has been treated in Boston [72]; patients presenting with advanced stage disease received CHOP followed by myeloablative therapy with ABMT, the marrow being treated *in vitro* with four anti-B cell monoclonal antibodies. Eighty-three patients entered the study; CR was achieved in 36% and 77 proceeded to the high-dose therapy (cyclophosphamide+TBI). Seventy patients had a known t(14;18); all were found to be PCR-positive in the bone marrow before *in vitro* treatment. Disease-free survival and overall survival were estimated to be 63% and 89% at three years respectively, with a median follow-up of 45 months. Patients whose bone marrow converted to PCR negativity after *in vitro* treatment had a significantly longer freedom from recurrence compared with those whose bone marrow remained PCR-positive. Continuing PCR negativity in

Table 10.3 High-dose treatment in first remission of follicular lymphoma

Centre	Treatment	Source of cells	Outcome (EFS)	Reference
Paris	BEAM	BM*	8 of 8 at 15–43 months	[71]
Boston	CY+TBI	BM*	63% at 3 years	[72]
Paris and Lille	Beam	BM*	75% at 4 years	[73]
'France Autogreffe'	TBI cont./drugs	BM	58% at 3.5 years	[74]
France (three centres)	TBI cont./drugs	PBPC	55% at 3 years	[69]

BEAM=BCNU, etoposide, ara-C, malphalan; CY=cyclophosphamide; TBI cont./=total body irradiation-containing regimen.
BM=bone marrow; PBPC=peripheral blood progenitor cells.
*With *in vitro* treatment.
EFS=event-free survival.

follow-up bone marrow samples was also strongly predictive of continued complete remission.

Morel *et al.* have also evaluated early high-dose therapy with ABMT using marrow treated with mafosfamide in patients with follicular lymphoma considered to be at high risk for recurrence [73]. Thirty-four patients entered the programme, 21 of whom had bone-marrow involvement. On reaching a 'minimal disease' state with an doxorubicin-containing regimen (ACVBP), 26 patients received BEAM. At four years, the estimated time to treatment failure was 55% and the probability of continuing remission was 75%. Comparison by 'intention to treat' of the results with 14 historical, 'high-risk' patients who received ACVBP at the same hospital but without high-dose therapy, showed an advantage for high-dose treatment (P= 0.04) in terms of probability of continuing remission.

A retrospective France Autogreffe study of 42 patients receiving myeloablative therapy in first partial remission (or chemosensitive relapse) shows, with a median follow-up of 43 months, relapse-free survival to be 60%, event-free survival 58% and overall survival 83% [74]. In the recent EBMT analysis, patients treated in first complete or partial remission had a significantly better outcome in terms of both progression-free and overall survival [62] compared to those treated following a 'chemosensitive recurrence'. However, there was no difference in outcome between those treated in first CR as compared with partial remission (Table 10.3).

Thus, there are no conclusive data, but a suggestion from the EBMT Registry data (which has to be interpreted with caution because of all the problems with any registry data) that using high-dose treatment in first remission may be advantageous. However, this needs to be balanced against the potential mortality and very real morbidity of the treatment. None of the curves show a plateau. At St Bartholomew's Hospital, myeloablative therapy is therefore only used in first remission in patients in whom initial therapy results in a less than complete, or good partial response. Under these circumstances, it is considered to be justified, in view of the significantly worse prognosis in such patients [6]. A problem with deferring treatment is that at each recurrence, the proportion of patients responding will be approximately 75% [6], and the denominator will therefore correspondingly decrease.

Myeloablative therapy+ABMT following transformation

The prognosis following transformation to high-grade histology is extremely poor [6,7,11–14]. Several centres have therefore used high-dose treatment in this situation. A report from the Dana Farber Cancer Institute described 18 such patients; the event-free survival at four years was only 23%, as compared to 53% for patients in whom there was no evidence of transformation [60]. Similarly, Schouten *et al.* have reported the results of treatment after transformation had occurred which were considerably inferior to those achieved when the treatment was used earlier [61]. Using PBPC, the results are similar. In the series reported by Bastion *et al.* [69], 16 patients were treated following transformation. Their 'failure-free survival' was worse than that of the patients with *follicular* lymphoma but there was no difference in overall survival.

This poor prognosis may reflect the natural history of the disease following transformation, but is also influenced by patient selection. For 28 patients treated at St

Bartholomew's Hospital who were only considered for cyclophosphamide+TBI+ABMT if chemotherapy given at the time of transformation resulted in a complete or very good partial remission, the results are more encouraging, with 55% of patients remaining free of disease at 3.5 years [75]. The median survival was 5.6 years without an evident plateau. The EBMT Registry results for 48 patients show overall and progression-free survival to be 50% and 33%, respectively, at five years; 88% of patients were treated following a 'chemosensitive' recurrence [76]. Thus, in younger patients treated in remission, high-dose therapy with autologous bone marrow or PBPC support is appropriate, in view of the very poor prognosis with conventional therapy. It should be remembered that recurrence following high-dose therapy can be with *follicular* lymphoma (2 of 9 in the St Bartholomew's Hospital series) so that re-biopsy is mandatory.

Myelodysplasia and secondary AML

A problem which has only relatively recently come to light is that, although the early treatment-related mortality of high-dose therapy is <5%, the overall figure is probably twice that, since about 5% of patients [62,77,78] have developed myelodysplasia and rarely, acute myelogenous leukaemia. It is difficult to know whether this represents the cumulative effect of all the treatment for follicular lymphoma that the individual had received prior to the myeloablative therapy, or a function of the latter *per se*. It is described both with TBI-containing regimens and 'drug only' ones and is clearly a cause for major concern.

Detection of lymphoma at the molecular level following myeloablative therapy

There has been much interest in attempting to establish whether myeloablative therapy, i.e. the treatment as a whole, as well as the *in vitro* component can eradicate 'minimal residual disease'. As mentioned above, when bone-marrow samples were analysed before and after *in vitro* treatment at the Dana Farber Cancer Institute, 'PCR negativity' of the re-infused marrow was the single most important prognostic factor for disease-free survival [63,72]. Sequential bone-marrow samples from patients deemed to be in complete remission after the myeloablative therapy were also examined. Again, disease-free survival was longer in patients with no PCR-detectable lymphoma cells in the marrow [72,79]. An alternative to tumour-cell depletion is to use an Avidin/Biotin system for CD34-positive cell selection. When used with autologous bone marrow, this has been reported to result in PCR negativity in a proportion of patients [80].

The use of peripheral blood progenitor cells as opposed to autologous marrow raises the same questions. Several studies have now shown that progenitor cells collected from peripheral blood from patients in whom the marrow was morphologically uninvolved at the time of collection are positive by PCR analysis [68,81]. The clinical significance and prognostic implications of such residual cells detected by PCR and evaluation of the risk of further recurrence will require prospective long-term studies, but as a basic principle, if 'molecular remission' is a worthwhile aim, it would seem reasonable at least to try and use marrow or peripheral blood contaminated with as few lymphoma cells as possible.

Myeloablative therapy with allogeneic bone-marrow transplantation (BMT)

There are very few patients worldwide—for obvious reasons—who have received high-dose treatment supported by allogeneic bone-marrow transplantation. The results for 10 patients with chemotherapy-refractory and recurrent low-grade lymphoma have, however, recently been reported. All had poor prognostic features and had been extensively pretreated. Despite this, complete remission was achieved in 8 of 10 and none have relapsed at a median follow-up of over two years [82]. Two patients died of early complications. For surviving patients, the duration of remission exceeded that of any previous remission. Allogeneic grafting is therefore worthy of further investigation in younger patients who have a matched sibling donor.

Small lymphocytic lymphoma

The disease

Small lymphocytic lymphoma accounts for only 4% of adult non-Hodgkin's lymphoma and is predominantly a disease of older people. The relationship between it and chronic lymphocytic leukaemia (CLL) has long been the subject of debate and controversy but it is often considered to be the nodal counterpart of CLL. The majority of patients present with peripheral lymphadenopathy, and hepatosplenomegaly is often also present. The bone marrow is usually involved, as may be the peripheral blood. Up to 15% of patients develop high-grade lymphoma eventually [1,83,84].

Not all patients require treatment at presentation. Those with few symptoms other than lymphadenopathy may be managed expectantly in the first instance, with careful follow-up and intervention when symptoms supervene. Conventional treatment comprises an alkylating agent such as chlorambucil, although doxorubicin-containing regimens, and more recently fludarabine, have also been used. The median survival is approximately ten years [85–88]. However, the advent of the purine analogues has to some extent altered the philosophy of treatment in younger patients with this diagnosis. The interest in fludarabine lies in the fact that *complete* remissions can be achieved which is very unusual with any other treatment for CLL or small lymphocytic lymphoma. The possibility of achieving complete remission, if albeit transiently, has led to small numbers of younger patients with CLL being treated with high-dose therapy.

Myeloablative therapy

Data for small lymphocytic lymphoma *per se* are extremely sparse but it is helpful to look at the results for patients with CLL who have received high-dose treatment with allogeneic or autologous bone-marrow transplantation.

With regard to autologous marrow, overall, approximately one-third of patients appear to do well in the long term [89,90] but the treatment is associated with a particularly high incidence of opportunistic infections. This may relate to the low immunoglobulin levels

associated with CLL or to prior treatment with fludarabine and its effect on T-helper cells.

The largest series of allogeneic transplants is represented by registry data. The median age of a series of 54 patients was 41; treatment comprised cyclophosphamide+TBI. The probability of three-year survival was 46%, but 25 patients (46%) died of treatment-related complications [91]. This very high incidence of treatment-related deaths may, as in the autologous setting, be specific to CLL and means that very careful consideration must be given to making the decision to proceed. The fact that a person has a human leukocyte antigen (HLA)-identical sibling donor is not in itself a reason to carry out an allogeneic transplant.

Lymphoplasmacytoid lymphoma

The disease

The majority of patients present with lymphadenopathy, with or without splenomegaly. The bone marrow is very often involved and some patients have a serum paraprotein. Since most patients are older, the aim of treatment is generally to keep the person as well as possible for as long as possible. Conventional therapy comprises an alkylating agent such as chlorambucil, or doxorubicin-containing regimens but these have not been found to be superior. Fludarabine can be useful, with response rates of approximately 30% in both newly diagnosed patients [92] and those treated at recurrence [93]. Altogether, this is an unsatisfactory illness to treat, the median survival of patients treated at St Bartholomew's Hosptial being only five years [4].

Myeloablative therapy

There is a dearth of data for myeloablative therapy in this disease, mainly because, as with small lymphocytic lymphoma, patients tend to be older and because complete remission is rarely achieved. At St Bartholomew's Hosptial, 12 patients have received cyclophosphamide+TBI+ABMT, the marrow mononuclear cell fraction being treated *in vitro* with anti-CD20 and complement. Four patients continue in remission between 'two and eight years. Given the incurability of this disease with conventional treatment, in younger patients it would seem reasonable to attempt to achieve first CR with a view to proceeding to high-dose treatment.

Mantle-cell lymphoma

The disease

Previously called 'centrocytic lymphoma' and included amongst the low-grade lymphomas in the updated Kiel classification [94], mantle-cell lymphoma has been found, in the majority of cases, to be associated with the chromosomal translocation t(11;14)(q13;q32) [95,96] which has confirmed it to be a discrete entity. Like the other

low-grade B-cell lymphomas, this is typically an illness of older adults. Again, the present-ation is usually one of generalized lymphadenopathy, with or without hepatosplenomegaly. Bone-marrow infiltration is almost invariably seen [97–100] with involvement of the peripheral blood in more than one-third of patients [89,94–100]. Extranodal involvement is relatively common, particularly of the gastrointestinal tract [1,97–100].

The clinical course of the disease represents an inexorable pattern of progression in the majority of cases, irrespective of which treatment is used and despite responsiveness to treatment. With conventional therapy, the median survival is only between 2.5 and 3.5 years [1,97–100].

Myeloablative therapy

Myeloablative therapy supported by autologous bone-marrow transplantation has been used in very small numbers of patients, mainly because mantle-cell lymphoma is a disease of older people, and again, because complete remissions are rare. However, a report from Omaha for patients treated in first remission suggests that this treatment may be useful [101]. The preliminary results with peripheral blood support from Heidelberg are also encouraging: 13 patients received cyclophosphamide+hyper-fractionated TBI in first or second remission. One patient died of interstitial pneumonia, but although the median follow-up is still short, 10 of 13 remain free of disease between ten months and four years [102]. A recent report from MD Anderson Hospital is also of interest: a very intensive alternating chemotherapy regimen was used as initial therapy in 44 patients. Nineteen subsequently proceeded to high-dose treatment comprising cyclophosphamide+TBI, 4 with allogeneic and 15 with autologous haemopoietic cell support. One patient died at three months of interstitial pneumonia; with very short follow-up, all of the remaining patients are well and without recurrence [103]. For younger patients, this approach is therefore certainly worth investigating.

Conclusions

Although the number of patients with low-grade lymphoma who have received high-dose treatment is still relatively small, there is now a sufficient body of data at least in follicular lymphoma to allow some conclusions to be drawn. The treatment is feasible and can result in prolonged remissions. Prolongation of survival has not been demonstrated, and there is currently no plateau in the survival curves for patients treated in first or subsequent remissions. The risk of myelodysplasia and AML is small but real.

Nonetheless, this treatment with PBPC support is probably the best option for younger patients with follicular lymphoma and small lymphocytic lymphoma in whom a second remission has been achieved. For younger patients with lymphoplasmacytoid and mantle-cell lymphoma, it should be considered as consolidation of first remission. However, as is the case with every treatment modality, it requires consideration in the context of both the conventional and the other experimental options available for each individual patient. Above all, it needs to be refined.

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Intermediate and high-grade non-Hodgkin's lymphoma

Conventional chemotherapy for intermediate and high grade non-Hodgkin's lymphoma (NHL) results in long-term disease-free survival in around 30–40% of patients. Variants of the conventional CHOP regimen, often including increases in the dose of at least one of the drugs involved, have failed to improve significantly on these results. For nearly twenty years it has been suspected that escalation of chemotherapy to doses requiring haemopoietic progenitor cell support may be curative. However, the precise role of high-dose chemotherapy for patients with histologically aggressive high grade NHL remains unclear.

Factors determining the outcome of autologous transplantation as salvage therapy for high-grade non-Hodgkin's lymphoma

High-dose chemotherapy with autologous bone-marrow rescue was first reported by Appelbaum in 1978 [1]. Since then, a number of studies have suggested that autologous transplantation can induce long-term disease-free survival in patients who have a poor initial response to, or relapse following, conventional chemotherapy [2–4]. These observations represented a major advance in the treatment of high-grade NHL as patients who are not cured with conventional chemotherapy have an extremely poor prognosis despite experimental salvage regimens [5–8]. Autologous transplantation for relapsed or refractory high-grade NHL became adopted as standard therapy in many centres worldwide, making randomized trials difficult to conduct. Despite this, it has now been conclusively demonstrated that high-dose chemotherapy with autologous bone-marrow support is superior to a conventional cisplatin-based salvage regimen for patients with chemotherapy-sensitive relapse after CHOP-like chemotherapy, with event-free survival increasing from 12% to 46%, and overall survival at five years increasing from 32% to 53% [9] (Figure 10.2).

The outcome for autologous transplantation in relapsed high-grade NHL is dictated by a number of factors, each of which will determine the desirability of this approach in an individual patient. The major determinant of outcome is the status of the disease at the time of high-dose therapy (Table 10.4). Patients with primary refractory disease or refractory relapse do worse than those with responsive disease at any stage [2,10,11]. In fact, the outcome for patients with chemotherapy-resistant disease is so dismal that alternative approaches are badly required, and conventional autografting is difficult to justify as palliative therapy. It is not clear whether the extent of response to salvage chemotherapy is an important predictor of outcome. One study suggested that subjects achieving complete remission with salvage chemotherapy performed better than those in partial remission at the time of transplant [12], but those subjects in partial remission had a lower median survival (16 months) and four-year survival rate (26%)

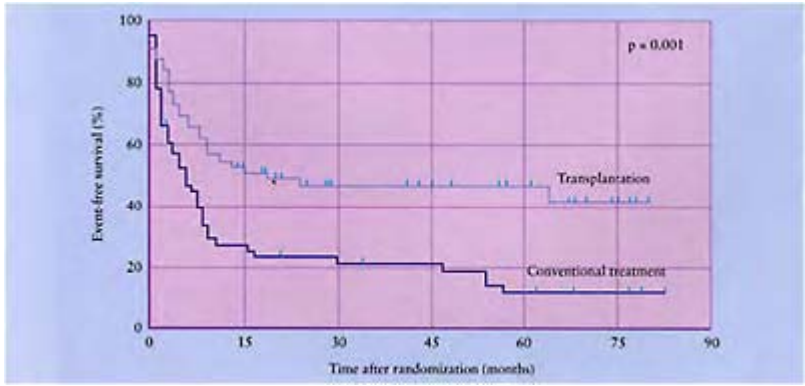

Figure 10.2 Progression-free survival for patients with chemotherapy-sensitive relapse of aggressive non-Hodgkin's lymphoma. From Philip et al. *[9].*

Table 10.4 Factors adversely affecting outcome from autologous transplantation in high-grade NHL

• Primary refractory disease
• Chemotherapy-resistant relapse
• Persistent CNS disease
Possibly:
• Age >65 years
• Transformation of low-grade NHL
• Extensive previous therapy
• Poor performance status

than might have been expected [13–15]. In addition, it is not clear whether intensive salvage actually confers a benefit directly, or whether this approach merely selects patients with disease that is more likely to respond favourably to high-dose therapy. The question is not academic, as both the nature and intensity of salvage chemotherapy are in themselves associated with adverse effects which may compromise long-term survival or quality of life following high-dose therapy.

Other factors have been variably proposed as predictors of outcome following autologous transplantation in high-grade NHL. These include poor performance status, transformation of low-grade

Table 10.5 High-dose chemotherapy regimens used for high-grade NHL

BEAM	BCNU	300 mg/m^2	d1
	Ara-C	400 mg/m^2	d2–5
	Etoposide	200 mg/m^2	d2–5
	Melphalan	140 mg/m^2	d6
BEAC	BCNU	300 mg/m^2	d1
	Ara-C	200 mg/m^2	d2–5
	Etoposide	200 mg/m^2	d2–5
	Cyclophosphamide	35 mg/kg	d2–5
CBV	Cyclophosphosphamide	1.5 g/m^2	d1–4
	Etoposide	300 mg/m^2	d1–4
	BCNU	300 mg/m^2	d4

NHL, and extent of therapy prior to transplant [16,17]. Age is a major determinant of outcome with many chemotherapy approaches, and in allogeneic BMT. However, patients aged between 55 and 66 years with high-grade NHL had similar progression-free and overall survival following autologous BMT as those under 55 years, providing that total body irradiation (TBI) was avoided [18].

There is little evidence that conditioning regimens differ substantially in their efficacy. Most established regimens use a combination of agents which includes an alkylating agent, either cyclophosphamide or melphalan, given at high doses (Table 10.5). Drug doses in the current BEAM regimen are close to dose-limiting, as further escalation of the etoposide dose in an attempt to improve efficacy results in a substantial increase in mucosal toxicity. Total body irradiation, in combination with high-dose chemotherapy, is as efficacious as chemotherapy-only regimens but probably not more so. In addition, TBI is associated with greater toxicity and procedure-related mortality, mainly due to opportunistic pulmonary infection [19,20].

Growth factors and peripheral blood-derived stem cells

The procedure-related mortality from high-dose chemotherapy with autologous bone-marrow support for patients with high-grade NHL is in the region of 10% [21]. Thus, any manipulation which may improve the safety of the procedure may increase desirability of the approach, may increase the number of patients who may benefit from it, and, ultimately, may improve outcome. Haemopoietic growth factors (granulocyte colony-stimulating factor, G-CSF; granulocyte-macrophage colony-stimulating factor, GM-CSF) administered following autologous marrow transplantation significantly increase the rate of neutrophil regeneration but have failed to reduce infection-related mortality or improve overall survival in randomized trials [22–24] (Table 10.6). Transplantation of

peripheral blood-derived progenitor cells (PBPC) has dramatically reduced the duration of neutropenia and thrombocytopenia following high-dose chemotherapy [25]. A recent study has demonstrated that a further reduction in the duration of neutropenia may be obtained by administration of G-CSF following PBPC rescue [26]. However, although transplant costs may be reduced by this approach, overall survival is not significantly altered. Nevertheless, across Europe the trend towards use of PBPC, and general improvements in supportive care, have reduced transplant-related mortality to a few percent, suggesting that small improvements not detected by small randomized studies are having a cumulatively beneficial effect.

High-dose chemotherapy has been administered to patients without haemopoietic stem-cell support. Laporte *et al.* [27] administered BEAM chemotherapy plus GM-CSF to 5 patients with extensive end-stage lymphoma and bone-marrow involvement, 3 of whom survived with engraftment. However, while of interest, this approach cannot be routinely recommended.

Table 10.6 Colony-stimulating factors in high-grade NHL

G-CSF and GM-CSF will:
• Permit mobilization of peripheral blood-stem cells
• Reduce the duration of severe neutropenia after PBSC transplant and ABMT
• Permit high-dose therapy without stem-cell rescue in some patients.

Role of transplantation as initial therapy for high-grade non-Hodgkin's lymphoma with poor prognostic features

Despite the relative success of salvage therapy for relapsed lymphoma with autologous transplantation, the relapse rate is still high and long-term survival is only around 40%. This has led to the search for therapeutic approaches which reduce the proportion of subjects failing to respond to initial therapy. Using the International Prognostic Index, those subjects with bulky disease, poor performance score and a high serum lactate dehydrogenase can be predicted to do poorly with conventional chemotherapy [28]. The primary reason for the reduced survival of patients with poor prognostic indicators is failure to attain complete remission [29].

A number of pilot studies have reported the efficacy of consolidating first remission in poor-risk high-grade NHL with high-dose chemotherapy and autologous transplantation. Using locally developed risk criteria, Jackson *et al.* [30] reported the results of autologous transplantation following high-dose melphalan and TBI in 30 subjects with poor-risk high-grade NHL who had attained complete remission after CHOP-like chemotherapy. Event-free survival at median follow-up of 44 months was 83%. These results compare favourably with similar data from patients not receiving transplants. Similarly encouraging results have been reported by other authors [31–33].

Randomized studies have been conducted to address this issue with conflicting and controversial results. In one study, patients in partial remission after three courses of CHOP were randomized to receive further CHOP or an autologous transplant after high-

dose cyclophosphamide and TBI. Patients with refractory disease were excluded from the analysis. There was no significant difference in event-free or overall survival between the two groups, with those receiving autografts tending to do worse than those receiving conventional chemotherapy [34]. Early intervention with BEAM chemotherapy and autologous bone-marrow transplant (ABMT) similarly failed to improve complete response rates or prevent relapse in poor-risk patients as defined by the International Prognostic Index [35]. Similar

Table 10.7 Indications for high dose chemotherapy in high-grade NHL

Proven:
• Chemotherapy sensitive relapse
Possible:
• Poor-risk NHL in first complete remission
Not substantiated or under evaluation:
• Poor-risk disease in partial remission
• Chemotherapy-resistant relapse
• Primary refractory disease.

randomized trials based on poor prognostic indicators are in progress. However, if these results are confirmed, the place of high-dose chemotherapy as initial therapy for high-grade NHL becomes unclear and the criteria for selection of patients who are subjected to autologous transplantation as first-line therapy will have to be revised. Interestingly, those patients with high risk disease in the GELA study [36] who achieved first complete remission prior to undergoing autologous transplantation did appear to have improved overall survival. However, it is not clear whether this self-selecting subgroup represents those poor-prognostic patients who do surprisingly well with conventional chemotherapy, or whether they are precisely the group for whom high-dose therapy in first remission should be reserved. Table 10.7 summarizes the current situation regarding high-dose chemotherapy in high-grade NHL.

Sequential high-dose therapy with progenitor-cell support

The concept of dose escalation in high-grade NHL has been borne out by improved results from high-dose therapy in selected groups of patients. The natural extension of these results was to establish whether two or more courses of high-dose therapy could improve the results achieved from one autograft. Such an approach proved problematic because of the technical difficulties of harvesting sufficient numbers of progenitor cells after high-dose therapy containing alkylating agents or nitrosoureas. However, peripheral blood stem-cell mobilization now permits the collection of enough stem cells to allow two, if not several, transplant procedures. Preliminary data from Marseille, France,

suggest that six sequential cycles of chemotherapy with progenitor-cell rescue are feasible, although each course is less myeloablative than the BEAM regimen [37]. In the first series of 22 patients with poor-risk high-grade NHL, overall and failure-free survival at three years are 75% and 55% respectively. The regimen is clearly intensive, with one toxic death (5%); however, the whole course may cost no more than a single autograft and the approach clearly warrants further evaluation.

Purging of autologous marrow or stem cells in high-grade non-Hodgkin's lymphoma

Highly sensitive polymerase chain reaction (PCR) assays for lymphoma cells suggest that harvested bone marrow or peripheral blood-derived stem cells may contain lymphoma cells even if the marrow is morphologically uninvolved by disease. As gene-marking experiments in other tumours have shown that infused stem cells may contribute to relapse, it is reasonable to attempt to purge the graft of any contaminating tumour prior to re-infusion. The clinical benefits of doing so, however, are unproven. First, it is impossible to ensure that the purging technique is completely successful. Second, many purging techniques result in depletion or damage to the stem cells and delayed engraftment. Third, it is not clear whether any component of post-transplant relapse in high-grade NHL comes from infused stem cells, as appropriate gene-marking studies have not yet taken place. No randomized studies of purging in high-grade NHL have been performed. However, a case-matching analysis from the EBMT Registry suggests that purging in high-grade NHL, while not detrimental, is of no benefit [38].

Allogeneic transplantation in high-grade non-Hodgkin's lymphoma

Allogeneic transplantation has the potential advantages of a graft-versus-lymphoma effect and freedom from the risk of infusing malignant cells with the autologous graft which may precipitate subsequent relapse. However, in NHL as in other diseases, these advantages are offset by a considerable increase in morbidity and mortality compared with autologous transplantation. Shepherd *et al.* [39] reported on 15 patients with advanced intermediate or high-grade NHL undergoing matched sibling allogeneic transplantation. Four died of transplant-related complications, 3 died of progressive NHL and 8 remained alive and well at 187–1732 days' post-BMT. Those patients undergoing BMT in complete or partial remission performed better, suggesting that any potential graft-versus-lymphoma effect was not sufficient to overcome the adverse biology of chemotherapy-resistant disease.

Similar results, with outcome not dramatically different from those achieved with autologous transplantation, were reported by Mendoza *et al.* [40]. By contrast, the MD Anderson Cancer Center reported disease-free survivals of 10–17% for patients with high-grade NHL undergoing matched sibling BMT [41], and the Seattle Group reported a 62% transplant-related mortality with a 17% two-year event-free survival in a group of patients who received allogeneic BMT following previous dose-limiting radiotherapy [42].

Data from the EBMT Registry suggest that a graft-versus-lymphoma effect does exist, particularly in lymphoblastic lymphoma, although the advantages of such an effect are outweighed by transplant-related mortality. As a result, overall survival is not significantly different between autologous and allogeneic BMT [43]. A comparative trial, where 66 patients with relapsed or refractory NHL received an allogeneic BMT if they had a matched sibling donor, or an autologous transplant otherwise, has recently been reported [44]. Over 50% of patients in each group died, although as expected the majority of allograft recipients died of transplant-related causes, whereas autograft recipients died of relapsed lymphoma. Nevertheless, relapse after allogeneic transplant was uncommon. These data do not necessarily favour the use of allogeneic transplantation in relapsed or refractory high-grade NHL. However, they suggest the existence of a graft-versus-lymphoma effect, and the potential for immunotherapy after autologous transplantation to eradicate minimal residual disease. Table 10.8 summarizes the current situation regarding allogeneic transplantation in high-grade NHL.

Table 10.8 Allogeneic BMT in high-grade NHL

Associated with:
• A low relapse rate
• High procedure-related mortality
The results of allogeneic BMT in high-grade NHL are not convincingly different from autologous transplantation except possibly in lymphoblastic lymphomas.

Lymphoblastic lymphoma

Lymphoblastic lymphoma is characterized by extensive extranodal disease at presentation, and in many instances it is difficult to distinguish from acute lymphoblastic leukaemia (ALL). As a result, many of the therapeutic regimens for this disease are based on ALL-type regimens. Therefore, the role of autologous or allogeneic transplantation for this disease, particularly in adults, is only as clear as for ALL. Allogeneic transplantation from an HLA-identical sibling can be recommended in first complete remission. For those subjects in whom no donor is available, the decision rests between intensive consolidation and maintenance, or autologous transplantation. The EBMT and UK Lymphoma Group are currently running a randomized trial to address this issue.

Data from the EBMT Registry suggest that overall survival at three years for adults with Burkitt's and Burkitt-like lymphoma undergoing autologous BMT is 53%, increasing to 72% for patients transplanted in first complete remission [45]. These results compare favourably with the results of allogeneic BMT and conventional chemotherapy regimens, with the exception of the IVAC-CODOX-M regimen [46] which is, as yet, unvalidated.

Transplantation of patients with CNS disease

Patients with high-grade NHL of the CNS tend to have limited survival and respond poorly to conventional chemoradiotherapy. Recent strategies for the treatment of CNS

infiltration include the use of escalating doses of drugs which penetrate the CNS such as idarubicin, cytarabine and methotrexate, in an attempt to induce CNS remission. Although these remissions may be short-lived, they may permit successful consolidation with high-dose therapy and autologous transplantation. Patients with active CNS disease at the time of autologous transplant do badly, with only 9% progression-free survival at six years. By contrast, those patients undergoing an autograft in CNS remission may do as well as patients with no pre-existing CNS disease [47]. CNS disease appears to behave like nodal disease in that chemotherapy-responsive disease is potentially curable, whereas disease that does not respond to conventional CNS salvage is unlikely to respond to autologous transplantation.

The role of radiotherapy after high-dose chemotherapy

Attempts to address the issue of whether radiotherapy is of value following high-dose chemotherapy have been confounded by a number of problems. These include a lack of consensus among clinicians on the criteria for randomized trials, differences in interpretation of follow-up radiological studies, and refusal by patients to enter such studies after aggressive chemotherapy. Some patients with apparently residual disease following high-dose chemotherapy remain alive and well some time after involved-field radiotherapy, but no formal assessments of active disease have been made, and patients with apparently extensive nodal enlargement immediately following autologous transplantation may undergo radiological regression over the subsequent few months without further therapy [48]. There is some evidence that results may be improved by involved-field radiotherapy to residual masses. However, this must be offset against the direct risks of irradiation to sensitive structures such as the heart and lungs, and the increased risk of secondary cancers [49].

Complications and considerations of high-dose chemotherapy for high-grade non-Hodgkin's lymphoma

The early and late complications of allogeneic transplantation are well established. However, the late effects of autologous transplantation may be more subtle. Nevertheless, these disadvantages must be taken into account when considering appropriate therapy for any individual.

The immediate transplant-related mortality from peripheral blood stem-cell transplantation in high-grade NHL is less than 5%. However, opportunistic infections may still occur, and idiopathic pneumonitis is a problem if conditioning regimens include TBI. Reactivation of varicella zoster is a cause of major morbidity in up to 25% of subjects, and Epstein-Barr virus (EBV)-related lymphoma has been reported in a patient who underwent a B-cell purged autologous transplant following TBI [50].

Most high-dose alkylating agent-based regimens are associated with infertility, although pregnancies have been reported in younger subjects. Gonadal failure, particularly in females, may be associated with premature osteopenia and a tendency to osteoporosis. Other long-term effects, such as secondary cancers, may not necessarily be attributable to high-dose chemotherapy *per se*, but to antilymphoma therapy in general.

Although the actuarial risk of myelodysplasia or therapy-related acute myeloid leukaemia is reported as being as high as 18% six years following autologous BMT, evidence suggests that it is damage to haemopoietic cells sustained prior to the transplant that is primarily responsible [51]. Ultimately, the financial consequences of dose-escalated therapy with progenitor cell rescue will need to be addressed. At present, this is difficult to evaluate as the true clinical value of high-dose therapy in most circumstances is unclear. No intervention can be cost-effective unless it is clinically effective, and thus far no economic evaluations have been performed in the one indication where high-dose therapy has been shown to work for high grade NHL; that is, in chemotherapy-responsive relapse. Further clinical trials are required before the true financial burden of high-dose therapy for NHL is likely to be known.

Future advances

High-dose therapy for NHL has dramatically altered the survival of a few selected individuals with high-grade NHL, but has been disappointingly ineffective for many. Nevertheless, the concept of dose escalation has been established and requires refinement. Clearly, the applicability of high-dose therapy needs to be increased. Firstly, the procedure needs to be applicable to older subjects. Secondly, the biology of the disease that renders patients resistant to chemotherapy needs to be understood. In this respect, novel agent combinations may be beneficial, and sequential high-dose therapy looks encouraging. Clinical trials of multidrug resistance modifiers are underway. Third, immunotherapy may be an effective way of eradicating minimal residual disease, although effective agents have been elusive and alternative approaches thus far experimental.

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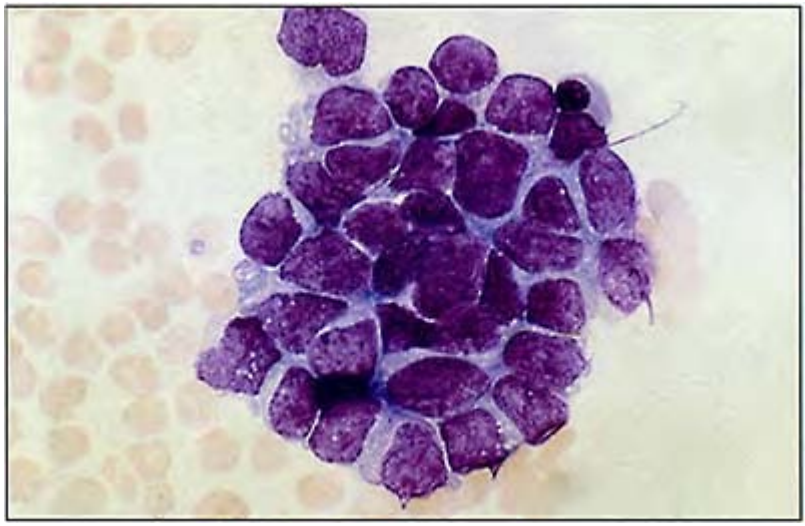

Chapter 11
Solid tumours in children

Simon Meller and Ross Pinkerton

Introduction

Autologous bone-marrow transplantation (ABMT) following high-dose nitrogen mustard in children was first reported more than 35 years ago [1]. The feasibility of treating neuroblastoma with high-dose melphalan and ABMT rescue was first described by Tim McElwain (Figure 11.1) at the Royal Marsden Hospital [2,3]. Worldwide experience of high-dose therapy (HDT) employing alkylating agents is now extensive and the role of HDT in neuroblastoma well established [4–6]. The European Bone Marrow Transplant (EBMT) Solid Tumour Registry holds data on more than 1600 paediatric transplants (Table 11.1) and is a valuable source of information [7]; nonetheless the place of HDT in other childhood solid tumours remains less clear [8] and there are several reasons for this:

- childhood cancers are rare and approximately one-third of cases are leukaemias;
- approximately 60% of children survive cancer and most of these cures are achieved with conventional-dose chemotherapy;

- although children are more tolerant to the short-term effects of chemotherapy than adults, developing organs are particularly vulnerable to the late effects of high-dose chemotherapy and radiotherapy;
- HDT is mainly reserved for salvage therapy after relapse and the success of salvage therapy is determined at least in part by the nature of the preceding primary treatment. Furthermore, in certain childhood cancers, for instance Wilms' tumour [9] and germ-cell tumours [10], successful salvage can be achieved with conventional-dose second-line chemotherapy;
- primary HDT is still largely experimental and is reserved for very poor risk tumours with an expected survival rate of less than 20–30% at three years with conventional therapy;
- with the exception of neuroblastoma, there have been no randomized trials of primary HDT compared with conventional-dose chemotherapy alone in advanced childhood solid tumours;
- single institution studies are usually one-arm studies with few patients and are usually compared to historical controls. Historical controls are notoriously unreliable in childhood cancer because of continuing improvements in survival with conventional-dose chemotherapy;
- national cooperative clinical trials also tend to be single arm with inadequate numbers and may be subject to selection bias;
- international collaborative studies of HDT in advanced childhood cancer are very few;
- although a dose-response relationship has been demonstrated for several childhood cancers using cell lines *in vitro*, it has been much more difficult to demonstrate such a close relationship *in vivo* with the possible exceptions of neuroblastoma, rhabdomyosarcoma and Ewing's sarcoma.

The place of HDT with stem-cell rescue will be reviewed in six childhood solid tumour groups (Table 11.2). HDT for childhood leukaemia and lymphoma is reviewed in other chapters in this book. A large number of high-dose chemotherapy and radiotherapy regimens have been employed using both single-agent chemotherapy, single agent with total body irradiation (TBI) and multi-agent HDT with or without TBI. The main limitation of TBI in solid tumours is that total doses above 12 Gy are rarely used, and while this may be adequate for haematological malignancies, it is of less benefit in solid tumours where curative doses generally exceed 40 Gy. Furthermore, the use of TBI in young children is associated with increased short-term procedure-related mortality and major long-term endocrine morbidity. It has yet to be shown that the addition of TBI to single- or multiple-agent high-dose chemotherapy in solid tumours produces improved survival. Allogeneic stem-cell rescue following high-dose chemotherapy and radiotherapy in non haematological childhood cancer produces increased morbidity from graft-versus-host disease and usually no benefit owing to a lack of graft-versus-tumour effect. There is limited experience of allogeneic bone-marrow transplantation (BMT) in childhood solid tumours other than neuroblastoma where the results are no better than with autologous BMT [11].

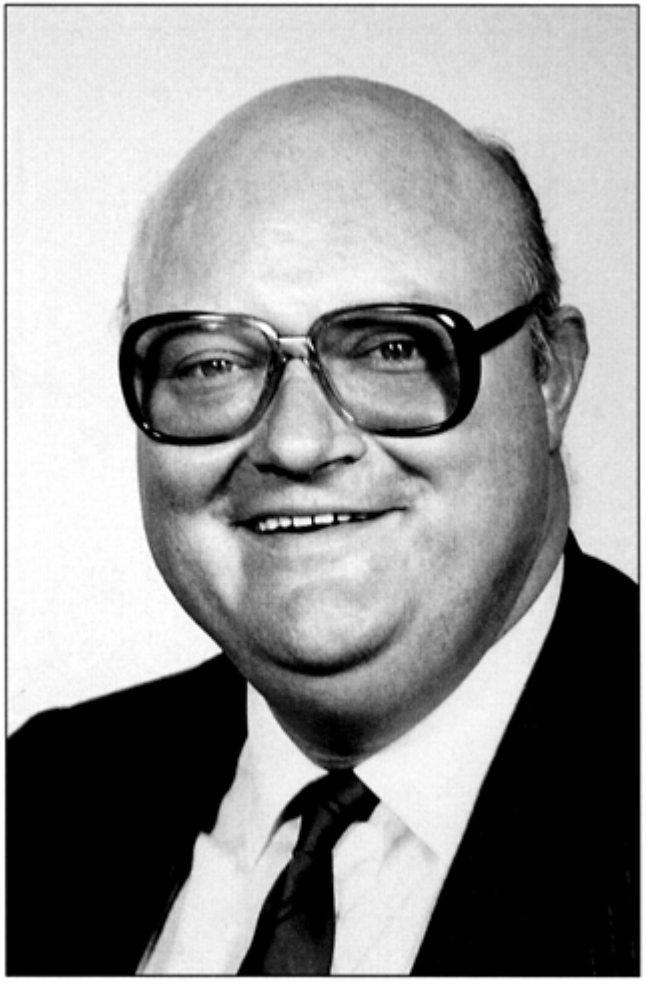

Figure 11.1 The late Professor Tim McElwain.

Table 11.1 Distribution of solid tumours treated by HDT and BMT in the paediatric age group. EBMT Solid Tumour Registry 1984–1995.

Paediatric (≤16 years) n=1658		
	Number of patients	
Neuroblastoma	925	(55.8%)
Soft tissue sarcoma	204	(12.3%)
Ewing sarcoma	182	(11.0%)
Glioma	67	(4.0%)
Wilms' tumour	56	(3.4%)
Germ-cell tumours	52	(3.1%)
PNET	36	(2.2%)
Other tumours	135	(8.2%)

Table 11.2 High-dose chemotherapy for childhood solid tumours

- Neuroblastoma
- Rhabdomyosarcoma
- Ewing's sarcoma
- Wilms' tumour
- Germ cell tumour
- Brain tumours

Advances in supportive care, central venous catheters and stem-cell technology have improved the procedure-related morbidity and may in turn lead to improved overall survival and it is therefore important to consider both disease-free survival and overall survival as in all other forms of cancer treatment. More emphasis is placed on disease-free survival in the evaluation of success in the treatment of childhood cancer and this is not always appreciated by medical oncologists dealing mainly with adults: a small improvement in disease-free interval and overall survival, although it may indicate that a new treatment is promising, is of little solace to the parents of children with cancer and the child himself may have to pay a high price in terms of procedure-related morbidity in order to achieve a modest prolongation of life. However, it is encouraging to know that the quality of later life for the survivors is good [12], even if in the short term they have to go through a difficult period of adjustment [13]. Paediatric oncology has now moved into an era of 'cure at least cost' rather than 'cure at any cost' [14].

Neuroblastoma

Neuroblastoma has lagged behind other childhood solid tumours with very little improvement in survival for those with advanced disease over the past three decades (Figure 11.2). Low-stage neuroblastoma is relatively uncommon and the majority of cases present with stage IV disease and, despite relatively high chemosensitivity and complete response rates to conventional chemotherapy exceeding 60%, fewer than 20% of patients with stage IV neuroblastoma are long-term survivors despite intensive treatment strategies. A single institution historical series of 42 consecutive cases of stage IV neuroblastoma treated at Great Ormond Street Hospital with multi-agent chemotherapy in the 1970s prior to the introduction of HDT resulted in a stable survival curve, which plateaued at 18% five years from diagnosis (Figure 11.3).

Single-arm studies from around the world reported since 1991 have employed various regimens with or without irradiation and claim survival figures of between 20 and 40% [15–19]. A recent review of more than 700 high-dose chemotherapy treatments with ABMT in advanced neuroblastoma reported to the EBMT Registry gives a good overview of current results [20]. Patients have been categorized into those who received treatment in first complete remission (CR) or partial remission (PR) and those in relapse or with progressive disease: 80% received a single ABMT procedure and 20% a double graft; 80% of autografts were purged and 20% were unpurged. The five-year progression-free survival was 20% irrespective of the type of HDT or purging. For patients receiving primary HDT the most important clinical prognostic factors were age (Figure 11.4) and continuing evidence of bone metastases at the time of HDT (Figure 11.5): improved survival occurred in infants under 18 months of age at diagnosis and in those with clearing of skeletal metastases. Patients receiving HDT and ABMT after relapse were divided into those in whom the initial treatment had included HDT, where there was only

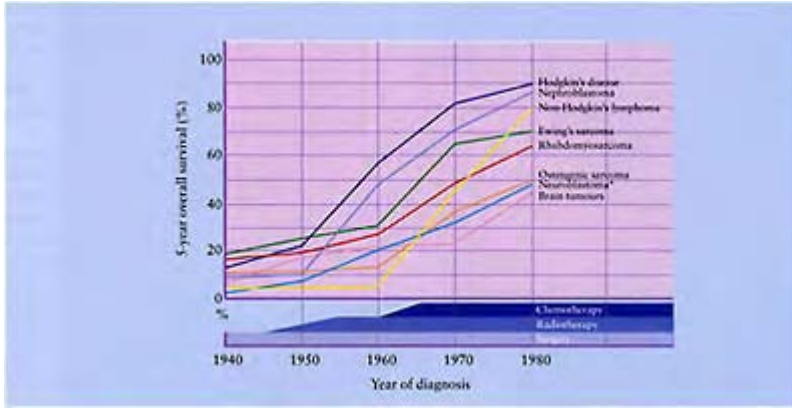

Figure 11.2 Improved survival of childhood solid tumours prior to the introduction of HDT.

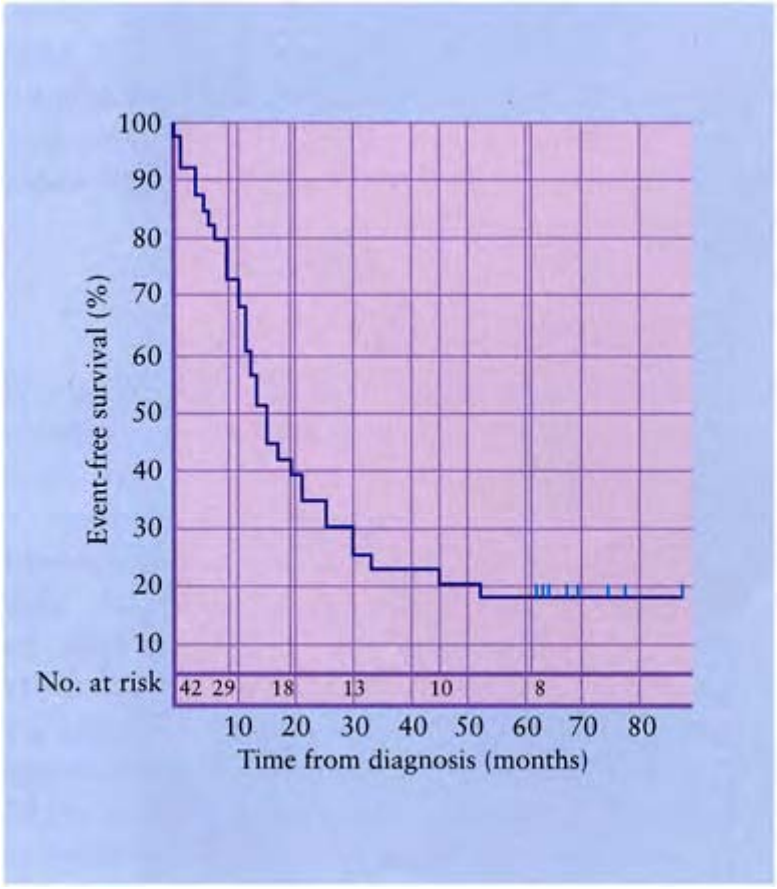

Figure 11.3 Survival of 42 consecutive patients with Stage IV neuroblastoma treated at the Hospital for Sick Children, Great Ormond Street, London prior to the introduction of high-dose melphalan in the late 1970s. (Courtesy of Dr Jon Pritchard.)

1 patient out of 18 alive but with disease beyond one year from HDT, and those who were treated after conventional-dose primary treatment (30 patients), where there were 12% survivors at two years (Figure 11.6).

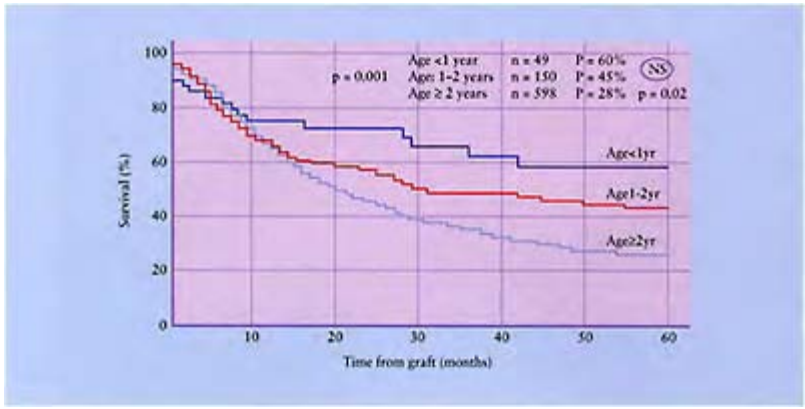

Figure 11.4 EBMT 1995—Neuroblastoma. Age at diagnosis.

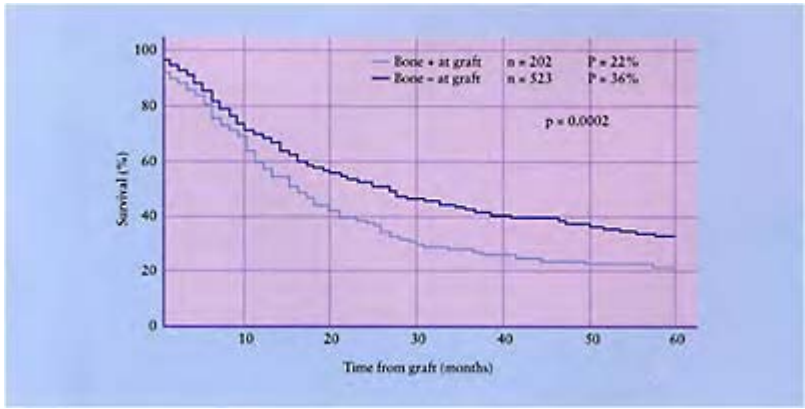

Figure 11.5 EBMT 1995—Neuroblastoma. Bone metastases versus no bone metastases at time of HDT.

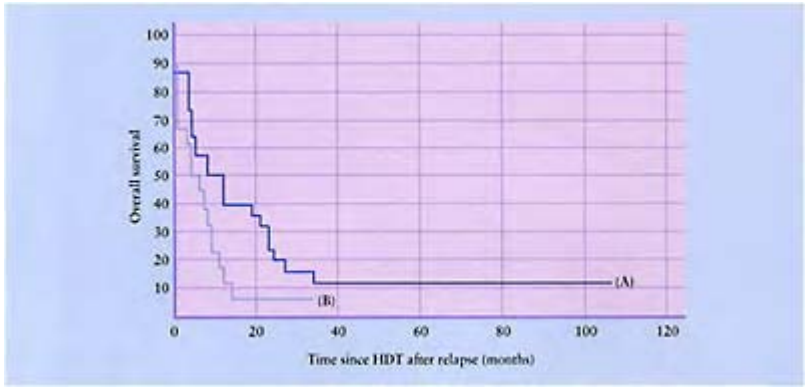

Figure 11.6 Neuroblastoma. Overall survival after HDT following relapse. Initial treatment not including HDT (A) versus initial treatment including HDT (B).

The only randomized study of HDT in neuroblastoma was carried out in the early 1980s by the European Neuroblastoma Study Group (ENSG1) [21]. In this study, 167 patients with stage III or IV disease received standard initial treatment with OPEC (vincristine, cisplatin, teniposide and cyclosphosphamide). Following between six and ten courses, 65 patients who achieved at least a good partial response (PR primary site, clearance of distant metastases and no progression on bone scan) were randomized to either stop therapy or receive a single dose of high-dose melphalan (140 mg/m^2) followed by unpurged non-cryopreserved ABMT (Figure 11.7). This study demonstrated that consolidation with high-dose melphalan significantly prolonged progression-free survival, although because of late relapses, the long-term survival was only marginally, although significantly, improved [22]. For patients over 12 months of age at diagnosis with stage IV disease, the median progression-free survival was 23 months for those receiving melphalan, versus six months for those receiving OPEC alone (Figure 11.8). A further randomized Children's Cancer Group study (CCG-3891) is comparing a high-dose chemotherapy and radiotherapy regimen with continued intensive pulsed chemotherapy and the result of this study is awaited with interest.

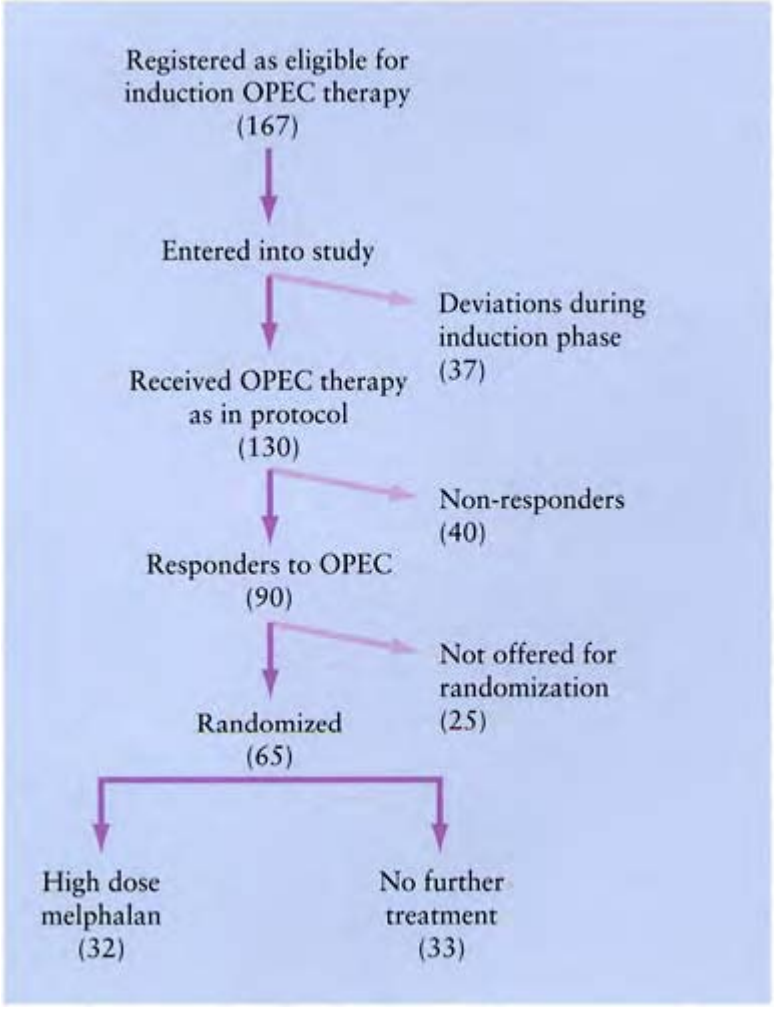

Figure 11.7 ENSG1—Neuroblastoma. Patient entry for randomization.

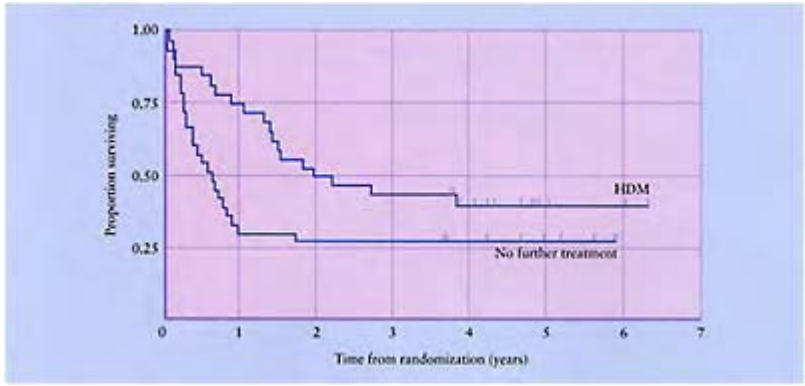

Figure 11.8 ENSG1—Neuroblastoma. Survival: High-dose melphalan (HDM) versus no further treatment.

The use of double autograft procedures with different high-dose therapy regimens prior to each graft has also been studied [23]. This inevitably entails considerable morbidity following the second procedure and, to date, has not been shown to have any significant advantage (Figure 11.9).

A review of patients registered with the EBMT has failed to show any survival advantage for those who received TBI as opposed to high-dose chemotherapy alone (Figure 11.10). Combining high-dose chemotherapy with radiolabelled meta-iodobenzyl-guanidine (mIBG) targeted irradiation is under

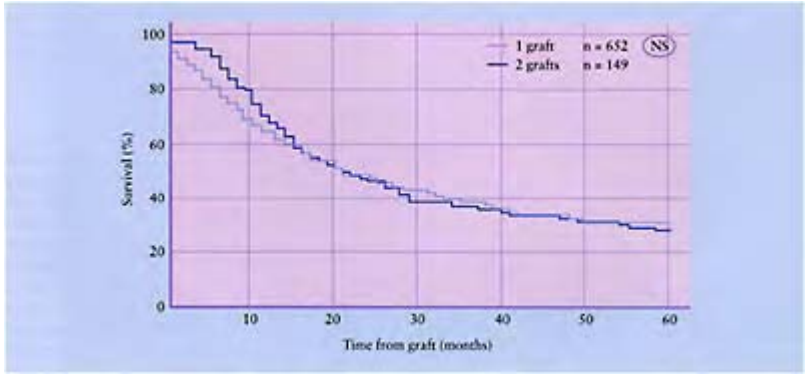

Figure 11.9 EBMT 1995—Neuroblastoma: one graft versus two grafts.

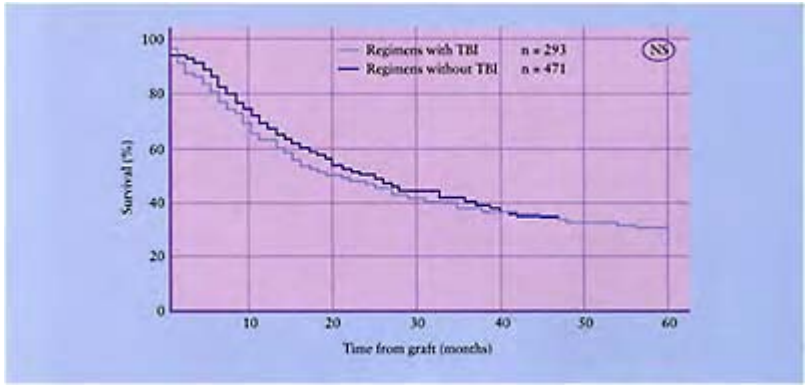

Figure 11.10 EBMT 1995—Neuroblastoma. TBI versus no TBI.

evaluation and although responses have been encouraging, studies remain preliminary [24].

It is now generally felt by paediatric oncologists that this improvement in long-term survival can be achieved with acceptable short-term and long-term morbidity and HDT now has an established place for patients over one year of age at diagnosis who achieve either CR or very good partial response at metastatic sites to initial chemotherapy. It remains controversial whether multi-agent regimens are better than melphalan alone and combination regimens such as vincristine, melphalan, etoposide and carboplatin (OMEC) or the same combination without vincristine (MEC) may have unacceptable toxicity [25,26].

Several different methods of purging neuroblasts from marrow harvests have been employed [27–29], but it is not clear whether purging produces better results than the use of unpurged marrow: in an attempt to address this question, the EBMT studied results from similar patient populations at two large participating centres and could find no difference in outcome [30]. There are case reports of unusual relapse sites, such as malignant pneumonitis following unpurged marrow reinfusion which may reflect reintroduction of disease [31], but the main problem in these patients is recurrence of disease which has remained in the body despite HDT rather than the reintroduction of a small number of tumour cells. Recent studies employing reinfused stem cells contaminated by genetically-marked neuroblasts indicate that re-infusion of tumour cells does contribute to later relapse [32].

There has been an exponential growth in knowledge about prognostic factors in advanced stage neuroblastoma as a result of more precise definition of clinical and diagnostic criteria [33] and mIBG scanning has proved a highly sensitive staging procedure, which has altered the definition of complete response [34] and also may result in upstaging at the initial assessment of neuroblastoma. Furthermore, advances in molecular biology of neuroblastoma now define poor prognostic subgroups of stage 4 disease: for instance, a neuroblastoma with amplification of the N-*myc* oncogene and deletion on chromosome 1p carries an extremely poor prognosis regardless of the therapy employed. Future therapeutic trials in advanced neuroblastoma must take account of

these important prognostic variables and will require large numbers of cases to be included in any meaningful clinical trials.

No attempt has yet been made to set up a randomized study of purged versus non-purged marrow and one reason for this is the current trend to replace autologous bone marrow by peripheral blood stem-cell (PBSC) rescue because of more rapid engraftment and patient recovery. It will require international collaboration to mount a new randomized study of HDT in advanced neuroblastoma and it remains to be seen whether such a study should have a common HDT regimen and examine the question of purging, or whether all stem-cell transplants should be purged thus allowing the opportunity to compare two different HDT regimens. It is unclear whether PBSC harvests carry a reduced risk of tumour cell re-infusion and studies of gene-tagged peripheral blood stem-cell harvests are awaited with interest. Within Europe, the national neuroblastoma study groups are now working in close collaboration with the intention of finding common ground and devising a treatment protocol which would be both internationally acceptable and answer one of these important questions in the next decade.

Rhabdomyosarcoma

High-dose chemotherapy has been employed for primary treatment of advanced stage rhabdomyosarcoma as well for salvage therapy. Low-stage rhabdomyosarcoma has a long-term survival of 60% with conventional multi-agent chemotherapy and it is only a small group of poor prognosis rhabdomyosarcomas which should be considered for primary HDT—these include those presenting with distant metastases and those with parameningeal disease or alveolar histology. The majority of high-dose therapies in rhabdomyosarcoma have been and will be performed in second CR rather than as primary treatment.

A single institution series of 43 children with stage III and stage IV rhabdomyosarcoma treated with rapid VAC and high-dose melphalan and ABMT showed a three-year disease-free survival of 55% and 25%, respectively [35]. A review of HDT in rhabdomyosarcoma including 113 cases registered with the EBMT showed a three-year disease-free survival of 28% in those treated in first CR and 12% in those treated in second CR or PR. The cases registered with EBMT are heterogeneous (Figure 11.11) and it is therefore difficult to draw firm conclusions as to whether this form of treatment is superior to conventional-dose chemotherapy in these high-risk cases (Figure 11.12). The current SIOP MMT 1991 study evaluates the role of high-dose melphalan as consolidation of CR in stage IV patients: the outcome will be compared with that using the same six-drug intensive pulsed chemotherapy regimen used in MMT 1989, where no consolidation was employed.

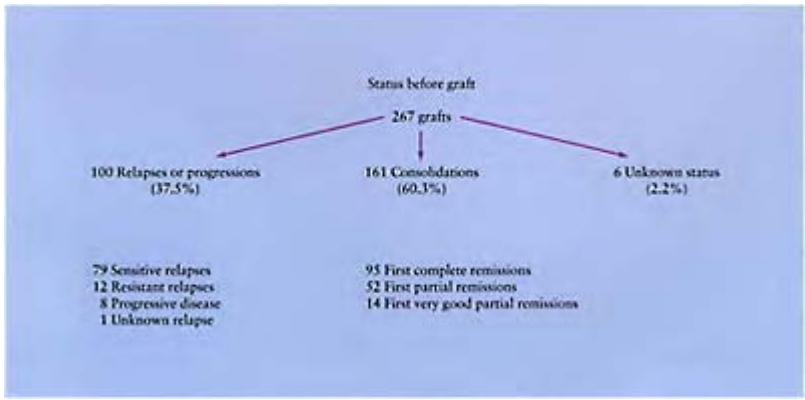

Figure 11.11 EBMT soft-tissue sarcoma 1982–1995. Status before HDT.

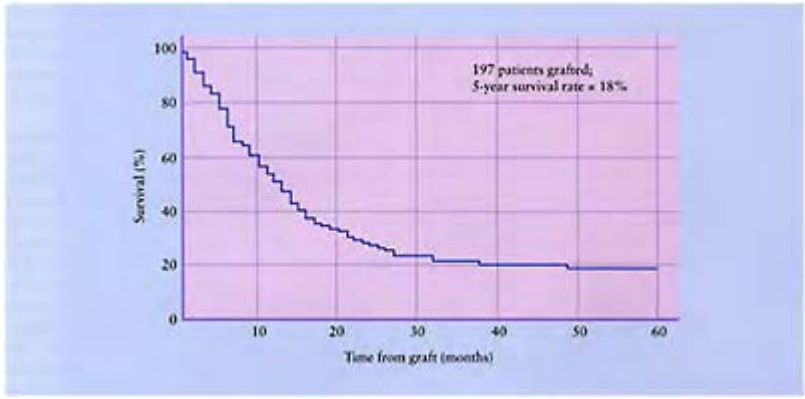

Figure 11.12 EBMT 1995—Rhabdomyosarcoma. Survival (all cases).

Ewing's sarcoma

Ewing's sarcoma is also a cancer in which high-dose chemotherapy has been employed because there is good evidence that dose escalation of alkylating agents used in primary chemotherapy has improved disease-free survival [36,37]. It is possible to define poor prognosis subgroups of Ewing's sarcomas as being those with distant metastases at diagnosis and very bulky axial primary tumours. As with neuroblastoma, HDT regimens with or without TBI have been used [38,39]. Experience at the Royal Marsden Hospital

with high-dose busulphan and melphalan with ABMT included 13 high-risk CR1 patients. There was 1 treatment-related death, 2 relapses and 10 patients continued in first CR with a median follow-up of 24 months [40], An analysis of patients registered with EBMT include 63 cases treated in CR (32 CR1, 31 CR2) with a wide range of HDT regimens and 9 patients received double autografts. The five-year actuarial disease free survival was 61% for CR1 patients and 32% (with no stable plateau) for CR2 patients (Figure 11.13). Again, the patients are heterogeneous and it is difficult to draw conclusions other than regimens including busulphan and melphalan seem to be superior and TBI-based regimens appear to be inferior [41] (Figure 11.14).

Wilms' tumour

Wilms' tumour is probably the most chemosensitive of childhood solid tumours: 85% of Wilms' tumours with favourable histology are cured by multimodality conventional therapy [42]. Although dose escalation is an effective strategy in Wilms' tumour, HDT would only be considered for relapsed or refractory cases and possibly those with unfavourable histology. Furthermore, as the salvage rate for Wilms' tumour is approximately 50% without employing HDT, case selection is important and there is no randomized trial to indicate whether survival can be improved by HDT. High-dose melphalan has been successfully employed in refractory Wilms' tumour [43]. The results of HDT in Wilms' tumour reported from the EBMT Registry [44] should be interpreted with caution because of the heterogeneity of the sample (Figures 11.15 and 11.16).

Germ-cell tumours

The place of HDT in germ-cell tumours of childhood is extremely limited as current cure rates with multimodality conventional chemotherapy exceed 80% at five years. Salvage rates in relapsed cases

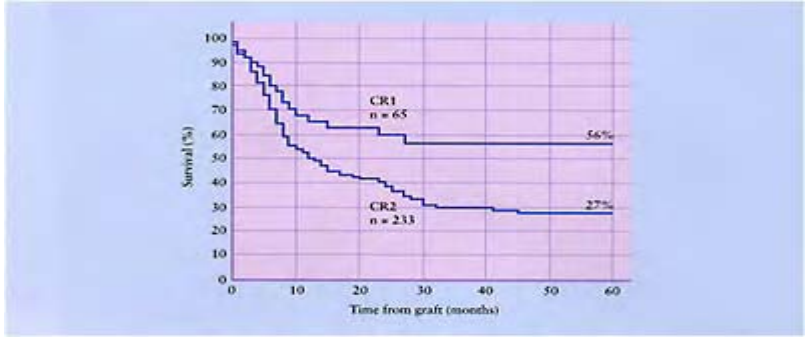

Figure 11.13 EBMT 1995—Ewing's sarcoma. Survival CR1 versus CR2.

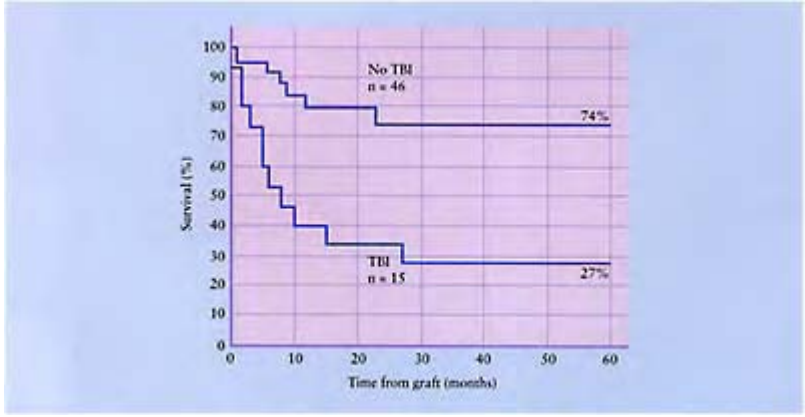

Figure 11.14 EBMT 1995—Ewing's sarcoma. Survival HDT with TBI versus no TBI.

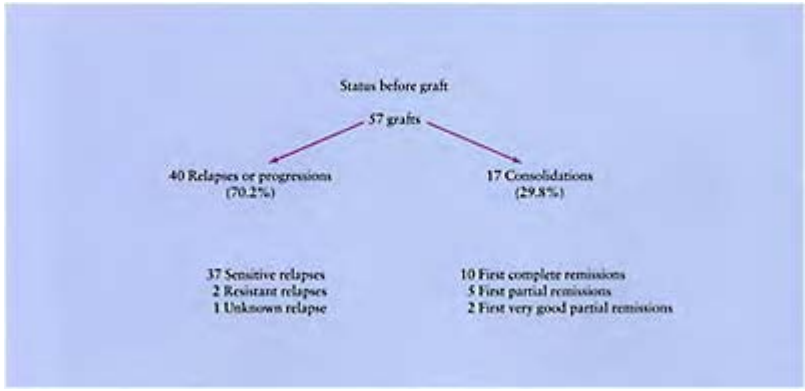

Figure 11.15 EBMT Wilms' tumours 1984–1995. Status before HDT.

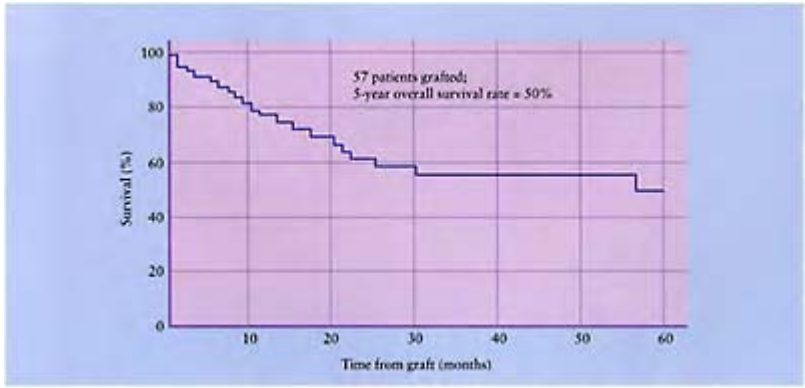

Figure 11.16 EBMT 1995—Wilms' tumour. Survival.

employing conventional-dose chemotherapy are also good and so the literature only contains anecdotal reports of high-dose chemotherapy in childhood germ-cell tumours. The German Testicular Cancer Cooperative Study Group have reported results in 74 adults treated with high-dose cisplatinum/etoposide/ ifosfamide with PBSC transplant and have shown a 35% event-free survival at two years [45]. The French experience of HDT in germ-cell tumours of adults and children has also been reported [46].

Malignant brain tumours

There is still only limited experience with conventional-dose multi-agent chemotherapy in the malignant brain tumours of childhood. Useful responses have been demonstrated in high-grade gliomas treated with BCNU, procarbazine, thiotepa, cyclophosphamide, platinum derivatives and epipodophyllotoxins. A dose-response relationship has not been shown *in vivo* and the high-dose chemotherapy employed has significant toxicity and so far no survival advantage has been demonstrated. Recent pilot studies of single-agent high-dose cyclophosphamide 4.5 g/m^2 with PBSC transplant in medulloblastoma have produced promising results [47,48]. The EBMT Registry has data on 67 children and 285 adults with gliomas receiving various forms of high-dose chemotherapy with extremely poor long-term survival and, although it is difficult to draw any firm conclusions from this heterogeneous group, it is generally felt that at present HDT has a very limited place in the treatment of childhood brain tumours (Figure 11.17).

Is bone marrow purging necessary in childhood solid tumours?

As mentioned above, many of the tumours for which HDT is considered either have a tendency to metastasize to bone marrow or have initially presented with bone-marrow involvement. Significant residual bone-marrow disease following appropriate initial chemotherapy indicates a high level of chemoresistance and in general is a contraindication to HDT. Under these circumstances, dose escalation is not curative and the short-term benefits of a partial response rarely justify the inevitable morbidity and prolonged hospitalization. The issue of purging arises for patients in whom minimal involvement or no residual marrow involvement is evident.

In the case of neuroblastoma, there is no question that a number of techniques have proved effective in the laboratory for removing tumour cells from involved marrow or from marrow that has been artificially seeded with tumour cells. Techniques include cytolytic antibodies [27], monoclonal antibodies bound to immunomagnetic beads [28], *in*

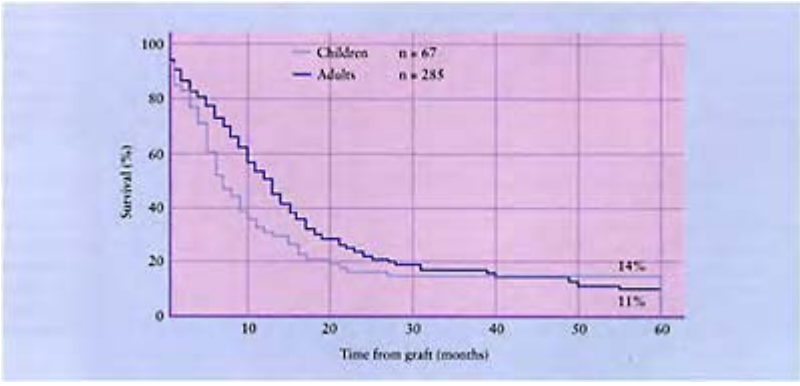

Figure 11.17 EBMT 1995—Glioma. Survival in children versus adults.

vitro chemotherapy and photosensitive cytotoxic agents [29]. Although a several log reduction in tumour cell number can be demonstrated using clonogenic assays [49] and only a small minority of the tumour cells reinfused will be clonogenic and capable of producing recurrent disease [50], the value of these techniques in practice has never been proven and there has been no randomized study to date evaluating purging in any childhood solid tumour.

Future trends

Stem-cell rescue employing PBSC rather than marrow holds considerable promise in paediatric oncology because of the reduced engraftment time and more rapid platelet recovery. Until recently, there were technical difficulties associated with PBSC collection in small children, but several institutions now have experience in harvesting PBSC from children down to a body weight of around 15 kg [51]. Combined autologous marrow and PBSC rescue results in faster engraftment [52] but most institutions are now employing PBSC rescue alone after HDT [53,54]. Several different stem-cell mobilizing schedules have been employed [55–57], but priming with both chemotherapy and cytokines seems to result in a better stem cell yield than harvesting after a single mobilizing stimulus [58]. It will be of great interest to see whether tumour contamination is related to the method of stem-cell mobilization either using chemotherapy, cytokines or a combination.

There is little doubt that dose escalation in certain paediatric solid tumours produces significantly increased cytoreduction. The problem is that, unlike leukaemia or lymphoma, the cell kill achieved by one single treatment even at high dose is not likely to be sufficient for cure. Unless complete responses are demonstrable in phase II studies indicating a several log tumour cell kill, one course of HDT is unlikely to be of significant benefit when given as consolidation of first remission. The addition of TBI to melphalan requires a reduction of the melphalan dose by around 30% in order to avoid unacceptable procedure-related morbidity and mortality. Furthermore, it is unlikely that complex multi-agent HDT regimens will be shown to have any significant advantage over use of single high doses of alkylating agents and the additional toxicity associated with other agents may limit the amount of alkylating agent that it is feasible to deliver [25,26].

The use of biological response modifiers following autografting is a novel area that has stimulated interest. It has been noted that cell populations with natural killer cell function predominate during the period of bone-marrow recovery. The potential cytotoxic effect of this cell population may be enhanced by administration of interleukin-2 after HDT [59] or by immunizing patients with autologous neuroblastoma cells transduced with a retroviral vector encoding interleukin-2 [60].

The future may lie in the use of repeated courses of HDT followed either by PBSC rescue or given in conjunction with bone-marrow growth factors. With this approach, it may be possible to administer three or more courses and incorporate a significant dose escalation. One such pilot study is in progress at collaborating centres in the UK: the CECC regimen employs cyclophosphamide 6 g/m^2 on two occasions followed by mobilization of stem cells on one or two occasions in combination with granulocyte colony-stimulating factor (G-CSF). Etoposide in a dose of 2.4 g/m^2 often does not require PBSC rescue and generally the harvested stem cells only need to be re-infused after the final component (carboplatin at an area under the curve (AUC) of 24), so four sequential high-dose therapies can be given usually needing only one PBSC harvest and return (Figure 11.18). This regimen has been well tolerated to date and it is hoped that sequential HDT, as well as being less toxic, may become a more effective approach to the poor prognosis sarcomas of childhood.

Despite such manoeuvres, it is likely that HDT will continue to have a role only in the consolidation of remission achieved by conventional or moderately high-dose

chemotherapy. The main thrust of research must therefore revolve around improving the quality of the initial remission by exploiting new drugs, employing new methods of scheduling old drugs and considering methods of attenuating drug resistance.

Acknowledgement

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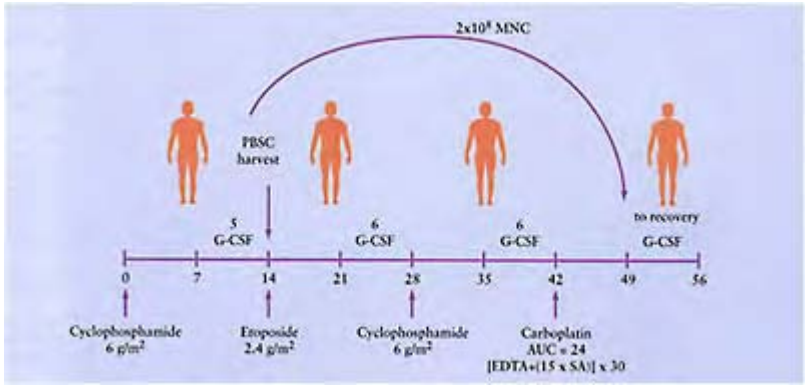

Figure 11.18 CECC protocol.

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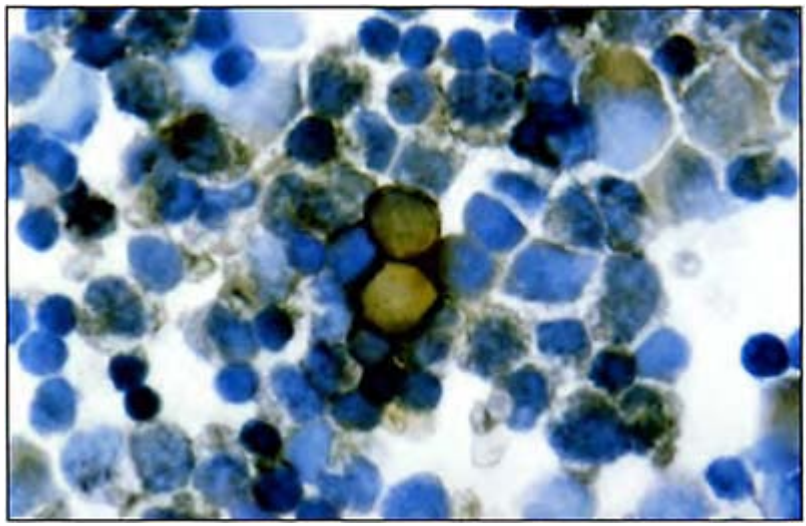

Chapter 12
Breast cancer

Pablo J.Cagnoni and Elizabeth J.Shpall

Introduction

Breast cancer is the second leading cause of cancer death among American women. A National Institute of Health consensus development conference reported that from the year 1990 to 2000, more than 1.5 million women in the United States will be diagnosed with breast cancer and 30% of them will ultimately die of their disease [1]. Following standard-dose adjuvant therapy, 40–50% of stage II–III patients with four to nine positive axillary lymph nodes and 70–80% of those with ten or more axillary lymph nodes containing tumour will relapse [2,3]. These high recurrence rates for patients treated in the adjuvant setting are disconcerting in light of the fact that stage IV breast cancer is generally incurable with standard therapy [4]. The median progression-free and overall survival duration rates for patients with oestrogen-receptor-negative stage IV breast cancer who receive Adriamycin-containing regimens for metastatic disease are 8.6 and 22 months [4]. Newer agents such as paclitaxel or vinorelbine, respectively, produce complete responses in 12% and 21% of patients with untreated metastatic breast cancer,

with few long-term disease-free survivors [5,6]. It is clear that new therapeutic strategies need to be developed for the treatment of breast cancer, both in the high-risk adjuvant and metastatic settings.

Rationale for high-dose chemotherapy in the treatment of breast cancer

Several *in vitro* [7] and *in vivo* [8] studies support the use of high-dose chemotherapy for the treatment of several malignancies, including breast cancer. The dose-response curve with alkylating agents for the treatment of murine leukaemia [9] and solid tumours [10] has been shown to be steep. In quantitative *in vitro* studies with a human melanoma cell line, Tsuruo and Fiedler [11] have demonstrated that resistant subpopulations differ from the sensitive subpopulations by a factor of less than 10-fold. Frei *et al.* [12] induced resistance to alkylating agents *in vitro* in several human cell lines by selection pressure. They found that the maximal resistance to alkylators varied between 3-and 15-fold. Dose increases between 3- and 30-fold can be achieved with haematopoietic cell transplantation, thus supporting the rationale for dose-escalating alkylating agents for the treatment of high-risk disease [13]. This *in vitro* data has been confirmed in animal studies [14].

A well-established principle of curative chemotherapy is the use of a combination of agents to reduce the chances for the development of resistance. Studies in breast cancer cell lines have shown that alkylating agents exert a synergistic antitumour activity when combined [15]. Also, several alkylating agents have been shown to lack cross resistance in *in vitro* and *in vivo* studies [15].

A retrospective analysis by Hryniuk and Bush provides support for a dose-response effect in the treatment of metastatic breast cancer [16]. A recent study conducted by the CALGB, compared three dose levels of CAF (cyclophosphamide, adriamycin and 5-fluorouracil). The study concluded that patients treated in the high- or intermediate-intensity arms had a longer disease-free survival than those treated in the low-intensity arm [17].

Results with high-dose chemotherapy in patients with metastatic breast cancer

Single-cycle chemotherapy

Peters *et al.* developed a high-dose chemotherapy regimen with a combination of three alkylating agents: cyclophosphamide (5.6 g/m^2 over three days), cisplatin (165 mg/m^2 i.v. over three days) and BCNU (600mg/m^2) (CPA/cDDP/BCNU) [18]. They showed that a significant dose escalation of the drugs was possible with acceptable toxicity. When this regimen was tested as initial treatment in oestrogen-receptor-negative untreated metastatic breast cancer patients, 54% of the patients achieved a complete response with

an overall response rate of 73% [19]. These preliminary results appeared to be superior to those achieved with conventional chemotherapy, which produces a complete response rate of 10–20% [20]. Moreover, with a minimum follow-up of eight years, 14% of the patients in that study remain disease-free [21].

Several groups have subsequently administered high-dose chemotherapy programmes to metastatic breast cancer patients as shown in Table 12.1.

Jones *et al.* [22] treated 45 patients with breast cancer, oestrogen-receptor-negative or hormone

Table 12.1 Selected single-cycle high-dose chemotherapy trials

Induction	High dose	CR (%)	DFS (%)	Reference
AFM	CCB	60	20 at 2 years	Jones *et al.* [22]
LOMAC	CT	44	13 at 3 years	Williams *et al.* [24]
CAF/AFM	CTC	45	27 at 2 years	Ayash [28]

AFM: Adriamycin, 5-fluorouracil, methotrexate, LOMAC: leucovorin, vincristine, methotrexate, Adriamycin, cyclophosphamide, CAF: cyclophosphamide, Adriamycin, 5-fluorouracil, CCB: cyclophosphamide, cisplatin, BCNU, CT: cyclophosphamide and thiotepa, CTC: cyclophosphamide, thiotepa and carboplatin, DFS: disease-free survival, CR: complete remission.

refractory disease, and no prior treatment for metastases with three or four cycles of induction chemotherapy (AFM: Adriamycin, 5-fluorouracil and methotrexate) [23] followed by high-dose CPA/cDDP/BCNU. The complete response rate was 68%, and 20% of the patients remained disease-free at a median follow-up of two years. The mortality rate in this study, due to regimen-related toxicity, was 22%. In subsequent studies described below, the regimen-related mortality rate is ≤5%.

The group from the University of Chicago also used an Adriamycin-containing regimen (LOMAC: leucovorin, vincristine, methotrexate, Adriamycin and cyclophosphamide) as induction, prior to high-dose chemotherapy with cyclophosphamide (7.5 g/m^2×1) and thiotepa (225 mg/m^2×3) followed by autologous stem-cell rescue in 102 patients with metastatic breast cancer with responsive or stable disease after induction therapy [24,25]. They obtained a 44% complete rate response, with 13% of the patients alive and disease-free at a median follow-up of three years. Response to induction therapy was a very important prognostic factor in their study. Of the patients that received high-dose chemotherapy in complete response, 40% are alive and disease-free at three years [26]. Ayash *et al.* administered induction chemotherapy with CAF/AFM followed by high-dose chemotherapy with cyclophosphamide, carboplatin and thiotepa, all given as 96-hour continuous infusions to 62 patients with responsive metastatic breast cancer [27]. The complete response rate was 45%, with 27% of the patients alive and disease-free at a median two years. Their mortality rate with this regimen was only 3%. In this study, of the patients that received high-dose therapy in

complete remission (CR), 36% remained in CR with a median follow-up of 43 months [28].

It appears from these preliminary studies that patients who receive high-dose therapy with minimal disease have better clinical outcomes than those with bulky or refractory disease. The University of Colorado designed and conducted a protocol of high-dose chemotherapy using the CPA/cDDP/BCNU regimen in patients with untreated breast cancer and no evidence of disease (NED) or minimal disease at study entry, defined as either a resected metastatic lesion or a single metastatic site which could be encompassed within a single radiation field [29]. To date, 23 patients have been enrolled on study. With a lead follow-up of >2.5 years, 53% of the patients are alive and disease-free [30].

In all of the stage IV studies described above, there is a proportion of patients (10–40%) who remain in untreated complete remission beyond 2.5 years. Further follow-up is needed to confirm these early promising results, together with the results of an ongoing intergroup randomized trial comparing high-dose chemotherapy with conventional chemotherapy in patients with responsive metastatic disease (Figure 12.1).

Multi-cycle chemotherapy

A number of investigators have explored the possibility of giving more than one high-dose chemotherapy cycle using autologous haematopoietic progenitor cell

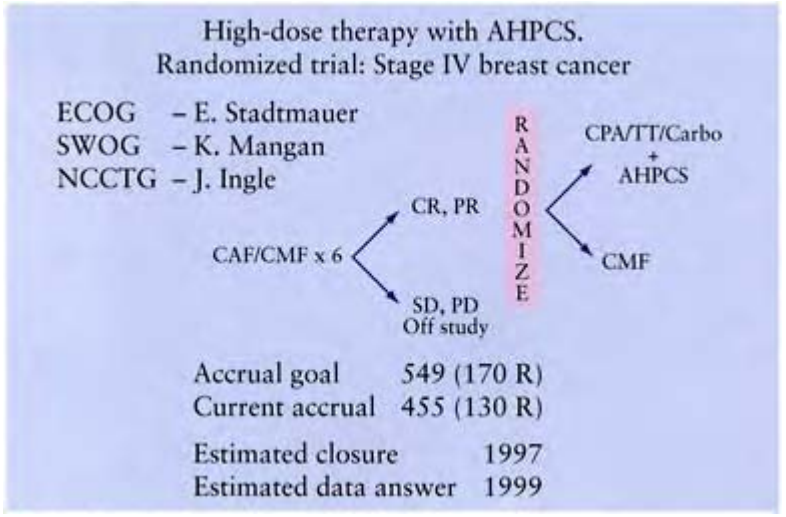

Figure 12.1 Treatment scheme and current accrual for the Intergroup Randomized Trial of high-dose versus conventional-dose chemotherapy in patients with chemotherapy-responsive metastatic disease. CAF:

cyclophosphamide, Adriamycin and 5-fluorouracil, CMF: cyclophosphamide, methotrexate and 5-fluorouracil, ECOG: Eastern Cooperative Oncology Group, SWOG: Southwest Oncology Group, NCCTG: North Central Group, CR: complere response, PR: partial response, SD: stable disease, PD: progressive disease, CPA: cyclophosphamide, TT: thiotepa, Carbo: carboplatin.

support (AHPCS). Some of these studies have reduced the dose intensity of each cycle in an attempt to give more than one sequential treatment (Table 12.2). Dunphy *et al.* administered two courses of high-dose cyclophosphamide, etoposide, and cisplatin followed by autologous haematopoietic progenitor cell support (AHPCS) to patients with metastatic breast cancer [31,32]. At the time of the last report, with a median follow-up of 83 weeks, 18% of the patients were progression-free from the start of induction chemotherapy. The mortality in this study was 6%.

Ayash *et al.* administered two sequential high-dose chemotherapy treatments to 21 patients with responsive metastatic breast cancer [27]. The first treatment was single-agent melphalan at a dose of 140–180 mg/m^2, and the second regimen included the high-dose CPA/carboplatin/thiotepa regimen previously reported [28]. The study showed that patients received the second cycle at a median of 25 days following the melphalan, with acceptable toxicity. Bitran *et al.* treated 27 patients with high-dose thiotepa and cyclophosphamide, followed within 180 days by single-agent melphalan [33]. Eighteen of 22 eligible patients received the second cycle of high-dose chemotherapy. With a median follow-up of 24 months, the actuarial freedom from relapse was 56%.

Ghalie *et al.* treated 39 patients with high-dose cyclophosphamide, thiotepa and carboplatin, followed later by high-dose busulfan and etoposide [34]. They reported a two-year event-free survival of 25.4% for

Table 12.2 Selected multi-cycle high-dose chemotherapy trials

Induction	High-dose	CR (%)	DFS (%)	Reference
AC	CCV×2	55	18 at 3 years	Dunphy and Spitzer [32]
AC	Mel-CTC	55	–	Ayash [28]
NA	CT-Mel	56	56 at 2 years	Bitran *et al.* [33]
NA	CTC-BuVP	31	25 at 2 years	Ghalie *et al.* [34]
C-C	T-T	42	21 at 28 months	Vahdat *et al.* [36]

Not given	CMV	51	80 weeks (median)	Bezwoda *et al.* [37]
Variable	ICE×2 or 3	–	–	Rodenhuis *et al.* [35]

AC: Adriamycin and cyclophosphamide, C: cyclophosphamide, CCV: cyclophsphamide, cisplatin, etoposide, Mel: melphalan, CTC: cyclophosphamide, thiotepa, carboplatin, BuVP: busulfan and etoposide, CT: cyclophosphamide and thiotepa, CMV: cyclophosphamide, mitoxantrone, etoposide, ICE: ifosfamide, carboplatin, etoposide, DFS: disease-free survival, CR: complete response.

complete responders, which was comparable to previous experience using high-dose thiotepa and cyclophosphamide alone.

Rodenhuis and collaborators have recently demonstrated that it is feasible to administer multiple cycles of high-dose cyclophosphamide, thiotepa and carboplatin with AHPCS [35]. They treated 48 patients with metastatic breast cancer or germ-cell tumours, with two or three cycles of cyclophosphamide (6000 mg/m^2), thiotepa (480 mg/m^2) and carboplatin (1600 mg/m^2), followed by AHPCS. The dose of carboplatin used is significantly higher than the one reported by Antman *et al.* Their main finding was that 80% of the second courses were given on time and without the need for dose reduction; only one toxic death was reported.

Vahdat *et al.* from Memorial Sloan-Kettering have explored the possibility of giving multiple cycles of intermediate-dose chemotherapy supported by AHPC [36]. Breast cancer patients received two courses of cyclophosphamide followed by two cycles of thiotepa. All cycles were supported by granulocyte colony-stimulating factor (G-CSF) and the two thiotepa courses were also supported by AHPC. Forty-two patients were enrolled; 10% did not receive the second cycle of thiotepa. Of the patients with measurable disease at study entry, 42% achieved a complete response, and 21% of the 42 patients are progression-free at a median follow-up of 28 months. More recently, Bezwoda *et al.* reported the results of a randomized trial in patients with metastatic breast cancer [37]. Ninety patients without prior chemotherapy in the metastatic setting were enrolled. Patients were treated with either two cycles of high-dose cyclophosphamide (2.4 g/m^2), mitoxantrone (35–45 mg/m^2) and etoposide (2.5 g/m^2) followed by AHPCS, or six cycles of standard-dose cyclophosphamide, mitoxantrone and vincristine. Fifty-one percent of the patients in the high-dose arm achieved a complete response in contrast to only 5% in the conventional-dose arm. Both the median duration of response (34 versus 80 weeks) and survival (45 versus 90 weeks) were significantly longer for patients enrolled on the high-dose arm. Further follow-up and larger randomized studies are needed to determine if multi-cycle treatment is comparable to single-course high-dose chemotherapy.

Results with high-dose chemotherapy in patients with primary breast cancer

Based on the high relapse rate following standard adjuvant therapy in patients with ten or more positive axillary lymph nodes, Peters *et al.* initiated a study of high-dose

chemotherapy and autologous bone-marrow support in patients with high-risk primary breast cancer [38]. Patients were entered in the study if they had stage II or III breast cancer with ten or more involved axillary lymph nodes. These patients were initially treated with conventional chemotherapy (cyclophosphamide, Adriamycin and 5-fluorouracil) for four cycles, followed by high-dose chemotherapy with the CPA/cDDP/BCNU regimen and bone marrow alone or in addition to peripheral blood progenitor cells. Post-transplant, 85 patients were evaluated, and with median follow-up of five years, the actuarial event-free survival is 71% [39]. Radiation therapy was administered to the chest wall and axillary areas in 90% of patients. The treatment-related mortality was 12%. Pulmonary toxicity of variable severity occurred in 31% of the patients and was reversible in the majority of cases. These results appear to be superior to the historical results with conventional chemotherapy and are currently being tested prospectively in two randomized intergroup trials (Figures 12.2 and 12.3).

The University of Colorado in collaboration with the Cleveland Clinic and the University of Arizona Cancer Center have completed a pilot high-dose study in patients with stage II and III breast cancer involving four to nine axillary lymph nodes [40]. Fifty-four patients were treated with four cycles of Adriamycin and cyclophosphamide followed by high-dose CPA/cDDP/BCNU with AHPCS. The only modification of the high-dose regimen developed by Peters *et al.* was a reduction in the dose of BCNU from 600 mg/m^2 to 450 mg/m^2 in an attempt to reduce the incidence of pulmonary toxicity associated with the regimen. Patients enrolled on this protocol were discharged after the high-dose chemotherapy administration was completed and followed as outpatients during the pancytopenic period. With a median follow-up of 16 months, 47 patients (87%) are alive and disease-free. Post-transplant, all patients received radiation therapy to the chest wall and axilla.

To confirm these promising preliminary results, a randomized intergroup trial has been initiated in this patient population (Figures 12.3 and 12.4). Patients will receive either the treatment regimen described above or an intensive sequential chemotherapy regimen, including three cycles each of doxorubicin, cyclophosphamide and paclitaxel, supported by G-CSF [41].

Graft processing

Rationale for purging

A potential obstacle to the use of high-dose chemotherapy followed by autologous haematopoietic progenitor cells is the possibility of reinfusing viable tumour cells with the graft. Patients with breast cancer have a high incidence of bone-marrow involvement, even at the time of initial diagnosis. Mansi *et al.* studied the bone marrow of 307 patients with primary breast cancer using immunocytochemistry and found micrometastasis in 26.4% of the cases [42]. The presence of micrometastasis was a poor prognostic factor in these patients.

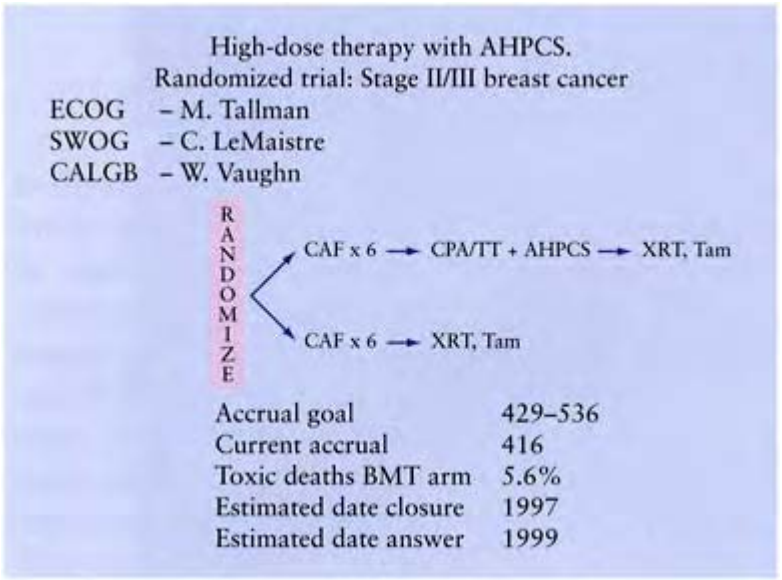

Figure 12.2 Treatment scheme and current accrual for the Intergroup Randomized Trial of high-dose versus conventional-dose chemotherapy in patients with stage II–III breast cancer and ≥10 positive axillary lymph nodes. CAF: cyclophosphamide, doxorubicin and 5-fluorouracil, ECOG: Eastern Cooperative Oncology Group, SWOG: Southwest Oncology Group, CALGB: Cancer and Leukaemia Group B, CPA: cyclophosphamide, TT: thiotepa; XRT: radiation therapy, Tam: tamoxifen.

A similar study was conducted by Porro *et al.* using a monoclonal antibody technique with a level of detection of 1 breast cancer cell in 200000 bone-marrow cells [43]. They studied 159 patients with negative axillary lymph nodes, no evidence of metastatic disease, and negative bone marrow by conventional histology. Seventeen percent of the patients had immunohistochemical evidence of breast cancer cells in their bone marrow. These results have been confirmed by Cote *et al* [44]. This group showed that the presence of occult metastatic disease detected by immunohistochemistry in patients

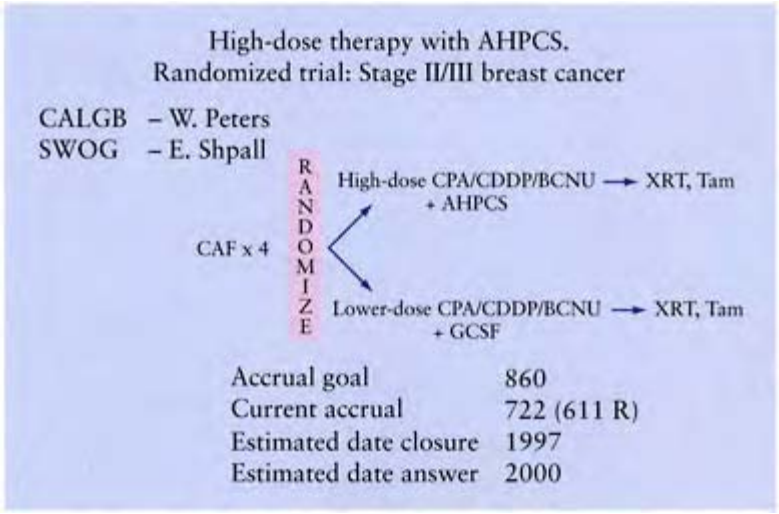

Figure 12.3 Treatment scheme and current accrual for the Intergroup Randomized Trial of high-dose versus conventional-dose chemotherapy in patients with stage II–III breast cancer and ten or more positive axillary lymph nodes. CAF: cyclophosphamide, doxorubicin and 5-fluorouracil, SWOG: Southwest Oncology Group, CALGB: Cancer and Leukaemia Group B, CPA: cyclophosphamide, CDDP: cisplatin, XRT: radiation therapy, Tam: tamoxifen, AHPCS: autologous haematopoietic progenitor cell support.

with stage I–II breast cancer was predictive of a higher relapse rate. In patients with metastatic disease, the incidence of bone-marrow involvement when evaluated by conventional histology is as high as 40–60% [45]. Immunohistochemical studies of haematopoietic cell grafts at the University of Colorado have shown comparable results [46].

Over the last ten years, it has been shown that peripheral blood progenitor cells (PBPCs) can provide durable multilineage engraftment after high-dose chemotherapy [47]. Many considered the use of PBPCs because of the lower likelihood of reinfusing tumour cells when compared to bone marrow. Several studies have reported the detection of breast cancer cells in the PBPC collections ranging from 10 to 24% [48–50] and

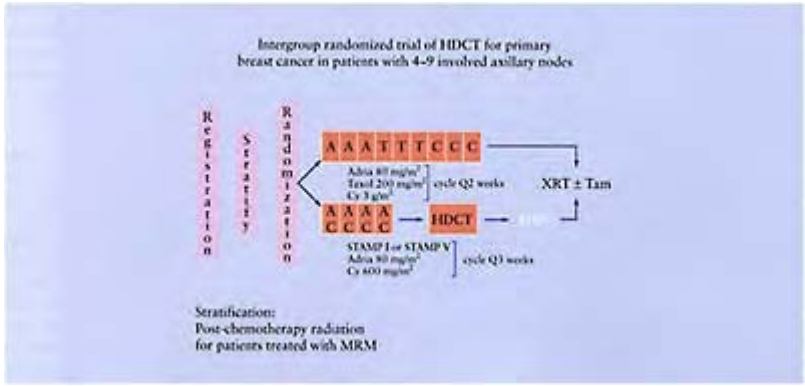

Figure 12.4 Treatment scheme for the the Intergroup Randomized Trial of high-dose chemotherapy in patients with stage II–III breast cancer and four to nine involved axillary lymph nodes. A: adriamycin, T: paclitaxel, C: cyclophosphamide, HDCT: high-dose chemotherapy, AHPC: autologous haematopoietic progenitor cell, XRT: radiation therapy, Tam: tamoxifen, MRM: modified radical mastectomy.

in some cases as high as 78% if mobilization regimens are used [51]. One study also showed that these cells appear to have clonogenic potential *in vitro* [52]. At the present time, there are no definitive data demonstrating that re-infused tumour cells contribute to relapse in breast cancer patients, but indirect evidence suggests that re-infused tumour does contribute to relapse in acute myeloblastic leukaemia, non-Hodgkin's lymphoma and neuroblastoma [53].

Particularly important are the studies conducted by Brenner and collaborators [54]. They performed gene marking of bone-marrow grafts of eight neuroblastoma patients using a marker gene (neomycin resistance). At the time of report, three of the eight patients had relapsed. All three had phenotypic and genotypic evidence of the marker gene in the relapsed tumour cells. The authors estimated that at least 200 malignant cells with clonogenic potential were introduced with the transplant and contributed to relapse. Two reports have shown that the presence of bone marrow micrometastases detected either by immunocytochemistry [55] or polymerase chain reaction [56] predict for a lower disease-free survival after high-dose chemotherapy.

Several conclusions can be extracted from the above data:

1. In breast cancer patients, bone-marrow involvement is a common event in all stages of the disease.

2. The presence of occult bone-marrow metastases is associated with a poor prognosis (increased incidence of relapse and shorter survival).
3. The use of PBSC may reduce, but certainly will not eliminate, the chances of re-infusing tumour cells into the patient.
4. The re-infused tumour cells may contribute to relapse after transplant in minimal disease settings.

Thus, procedures were developed to eliminate the presence of tumour cells in the graft. These techniques can be divided into two categories:

- Negative purging methods which attempt to kill tumour cells or remove them from the bone marrow.
- Positive selection, which involves the specific isolation of normal haematopoietic progenitor cells for transplantation, discarding more mature marrow elements, and hopefully tumour cells.

Purging methods and results

Negative purging

Several methods have been used to purge the bone marrow of breast cancer patients. In 1986, Coombes *et al.* reported on the use of a monoclonal antibody-toxin conjugate to purge the bone marrow in three patients with metastatic breast cancer prior to high-dose chemotherapy [57]. They showed that this processing of the bone marrow did not significantly impair engraftment. Anderson *et al.*, using an immunomagnetic separation technique, were able to eliminate 2–4 logs of clonogenic breast cancer cells from cell lines that had been mixed with a ten-fold excess of irradiated human bone marrow from donors [58]. Similar treatment of non-irradiated bone-marrow cells did not produce significant effect on Colony Forming Unit Granulocyte-Macrophage (CFU-GM).

One of the more widely used bone marrow purging reagents is 4-hydroperoxycyclophosphamide (4-HC). 4-HC is a synthetic analogue of 4-hydroxycyclophosphamide, the active metabolite of cyclophosphamide. *In vitro*, 4-HC hydrolyses spontaneously to produce 4-hydroxycyclophosphamide. Large numbers of patients with acute leukaemia have received 4-HC-purged autologous bone marrow. Retrospective analysis suggests an advantage in disease-free survival compared to unpurged marrow with the use of 4-HC [59]. Shpall *et al.* demonstrated that a significant delay in the time to engraftment was seen when bone marrow was incubated with 4-HC [60]. In a subsequent study, the same group randomized patients to have the bone marrow treated in addition to 4-HC, with or without the bone-marrow protectant WR-2721 [61]. The group of patients that had their bone marrow purged with 4-HC+WR-2721 had significantly shorter time to engraftment (mean days to white-blood cells >1000/mm^3 26 versus 36 days), received fewer platelet transfusions and days of antibiotic therapy. There was no significant difference in the rate or pattern of relapse between the two groups. Similar data was obtained in patients with non-Hodgkin's lymphoma [62]. More recently, Passos-Coelho *et al.* showed that bone-marrow specimens from five patients with

responsive breast cancer can be rendered disease-free by incubation with 4-HC as evaluated by immunocytochemistry and a tumour-cell clonogenic assay [63].

In summary, purging the bone marrow of breast cancer cells with the use of 4-HC appears possible and reasonably safe. The true significance of this approach in terms of tumour relapse or even treatment-associated morbidity and mortality remains to be determined.

Positive selection

The CD34 antigen that is expressed on early haematopietic progenitors is not present on most tumour-cell types [64]. This led to the development of methods for positively selecting CD34-positive normal progenitors, with the objective of reducing the tumour contamination of the graft, without the potential damage to the haematopoietic progenitors produced by negative purging. Several positive-selection methods are currently being investigated. The majority of these involve the separation of the target cells on relatively large macroscopic, immunospecific surfaces including plastic plates, columns or magnetic beads. A detailed description of these methods can be found elsewhere [65].

Two of the methods use the Dynal superparamagnetic microsphere as the solid-phase matrix for cell separation. The indirect immunomagnetic positive-selection method consists of three steps: sensitization with antibody, rosetting between the target cells and beads, and finally releasing the selected cells from the microspheres. The average enrichments of CD34 cells achieved using this system were 54±5-fold for bone marrow and 169±17-fold for cord blood. Civin *et al.* used this system to select bone marrow from 13 patients with refractory solid tumours that underwent high-dose chemotherapy [66]. Patients were disqualified from the study if their CD34 purities were low. In the ten patients eventually transplanted, the mean CD34 purity was 91% and the patients received a mean of 1.4×10^6 $CD34^+$ cells/kg. All the patients engrafted. An absolute neutrophil count of at least 500 was reached in a mean of 41 days. The direct immunomagnetic positive-selection method uses microspheres precoated with anti-CD34 monoclonal antibodies (Dynabeads CD34). The cells are first incubated with washed Dynabeads CD34, then the rosetted cells are collected by exposure to a magnet and the non-target cells are aspirated. An anti-mouse Fab polyclonal antibody is added (Detachabead®) to release the target cells. Clinical application of this method has not yet been reported.

Miltenyi Biotech has developed an immuno-magnetic cell separation system based on the use of 60 nm diameter biocompatible iron-dextran particles that behave as colloid (Figure 12.5). The particles are coated with a chemically modified anti-immuno-globulin which reacts with the anti-CD34 monoclonal antibody used to sensitize the target haematopoietic progenitor cells. The microbead-cell complexes are then passed through a coarse mesh to remove any aggregates and then over a column containing a metallic matrix in which a high-gradient magnetic field has been induced by externally placed permanent magnets. The bead-coated cells are retained on the matrix, and the non-rosetted cells are washed through. The target cells are simply eluted by removal of the column from the magnetic field and flushing with buffer. The eluted cells still have the microbeads which do not interfere with flow cytometric analysis or culture. These beads

are biodegradable by the manufacturer, but it is not presently known whether their presence may interfere with the functional activity of the cells following the re-infusion. Using this method, a 200–500-fold enrichment can be obtained using one column, which can be increased to 5000–20000-fold using a second column. Less than 10% of the target-cell population is lost in the negative fraction during each separation. This method has not yet been clinically tested.

We have conducted a clinical trial using the CEPRATE stem-cell concentrator. This method uses immunoadsorption of target cells, coated with biotinylated anti-CD34 monoclonal antibodies to avidin-coated polyacrylamide beads in a column configuration (Figure 12.6). In a summary of nine experiments performed using the laboratory scale separator, the mean purity achieved was 83±7.0% with a yield of 32±6.4%, representing a 61.2-fold enrichment. In preclinical studies, the purity achieved with this method is consistently >75% (range 50–91%). We have evaluated the capacity of this $CD34^+$-enriched product to engraft patients with breast cancer after high-dose chemotherapy [67]. Immunhistochemical staining for breast cancer was performed on all haematopoietic cell products before and after the positive-selection procedure. A breast cancer cell depletion of a mean of 2 logs (range 1 to >4) was documented, which was sufficient to select to negativity 20–30% of the grafts [68]. The engraftment

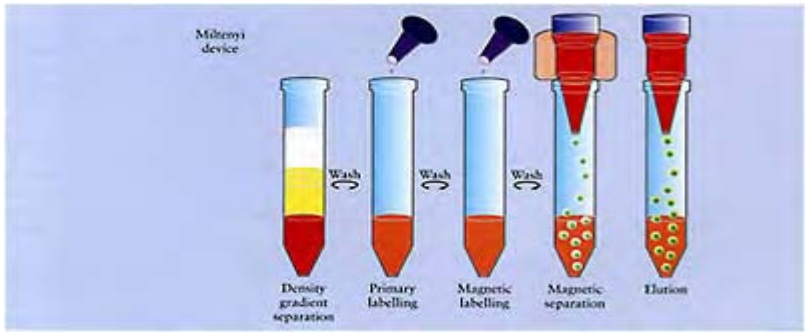

Figure 12.5 Positive selection with the Miltenyi Biotech immunomagnetic device. Bone marrow, peripheral blood or cord blood mononuclear cells are incubated with QBend10 for 15 minutes at 4°C, then incubated with 200 l microbeads/18 cells for 15 minutes at 6°C followed by washing. The microbead-labelled cells are applied to a column, retained by a magnet and then eluted for further processing and/or evaluation.

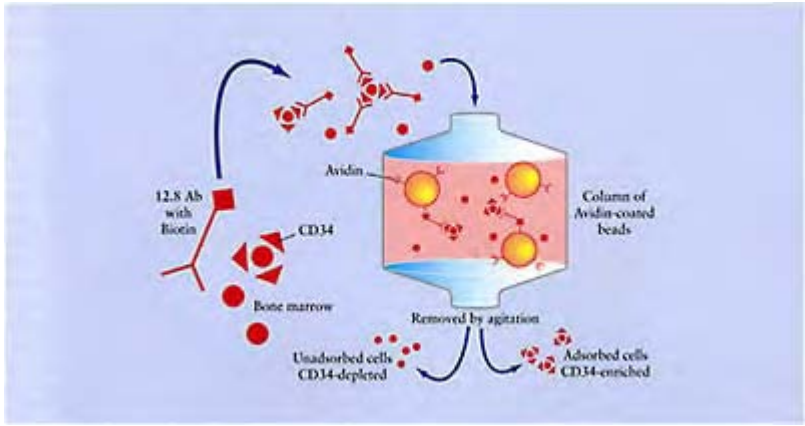

Figure 12.6 CD34 selection procedure with the CellPro column. The buffy-coat marrow fraction is incubated with 20 μg/ml of the biotinylated-12.8 anti-CD34 monoclonal antibody. The washed cells, in a volume of 300 ml of PBS, are then applied to a sterile column of avidin-coated polyacrylamide beads (CellPro Inc., Bothell, WA). After washing with PBS, the $CD34^+$ cells were removed from the beads by gentle mechanical agitation and eluted with 90 ml of peripheral blood serum containing 10 units/ml heparin and 4 ml of human serum albumin.

rates have been comparable to those achieved using non-selected marrow or PBPCs. We have extended this experience to a total of 155 patients and the same conclusions apply.

New high-dose chemotherapy regimens

As discussed above, only 15–20% of the patients with chemotherapy-responsive metastatic breast cancer will be rendered disease-free at two or more years from transplant with the current high-dose chemotherapy strategies. Thus, several investigators are exploring new chemotherapy combinations to improve these results.

Fields *et al.* attempted to further increase the intensity of the high-dose chemotherapy regimen. They performed a phase I study of ifosfamide, carboplatin and etoposide in patients with poor-prognosis malignancies (99 of 154 patients had breast cancer) [69]. The maximally tolerated dose (MTD) for this combination was ifosfamide 20100 mg/m^2, carboplatin 1800 mg/m^2 and etoposide 3000 mg/m^2, all given in divided doses over six days. The dose-limiting toxicity for the combination included central nervous system toxicity and acute renal failure. In a subsequent analysis of this study, the authors suggested that patients with anthracycline-responsive breast cancer treated at the higher dose levels had a higher response rate than those treated at the lower dose cohorts [70].

At least two groups have explored the possibility of using busulfan-cyclophosphamide, a commonly used regimen for haematological malignancies, in patients with breast cancer [71,72]. The results did not appear better than those obtained with other regimens.

Somlo *et al.* conducted a phase I study of etoposide (60 mg/kg) and cyclophosphamide (100 mg/kg) with escalating doses of Adriamycin [73]. The MTD for Adriamycin in this combination given as a continuous infusion of 96 hours was determined to be 165 mg/m2.

One potential way to improve the current results obtained in patients with metastatic breast cancer is by the incorporation of drugs with novel mechanisms of action. In a recently published phase I study, investigators from the University of Colorado Bone Marrow Transplant Program demonstrated the feasibility of incorporating paclitaxel into a high-dose chemotherapy regimen, including CPA and cDDP [74]. The rationale for this study was based on paclitaxel's promising antitumour activity in breast cancer, the lack of life-threatening extra-haematological toxicity with a conventional dose of paclitaxel, the preclinical evidence for a dose-response effect in solid tumours, and the *in vitro* synergism exhibited when paclitaxel and cDDP are combined [75–78]. The paclitaxel MTD for this regimen was 775 mg/m^2 over 24 hours, cyclophosphamide 5625 mg/m^2 in three consecutive daily doses and cisplatin 165 mg/m^2 given by continuous i.v. infusion over 72 hours followed by AHPCS. We are currently conducting a follow-up study testing the possibility of incorporating BCNU into this high-dose paclitaxel CPA/cDDP-based regimen with a reduced dose of paclitaxel [79].

Summary

Over the last ten years, significant advances have been made in the treatment of breast cancer with high-dose chemotherapy and autologous haematopoietic progenitor cell support. The mortality from the procedure has been reduced from 20–30% to less than 5% with the use of growth factors, mobilized peripheral blood progenitor cells, and increased expertise in the management of bone-marrow transplant patients. High-dose chemotherapy studies have shown for the first time that it is possible for 13–40% of breast cancer patients to be alive and free of disease for two years to more than ten years. The superiority of this approach over traditional chemotherapy in patients with metastatic disease has been demonstrated in prospective trials. Randomized trials in the stage IV, as well as the adjuvant setting, are currently ongoing in the Cooperative Breast Cancer

Intergroups, so additional data will be forthcoming. These studies will clearly define the role of high-dose chemotherapy in the treatment of this disease.

Numerous questions remain, however. Which is the most effective high-dose chemotherapy regimen? Is induction chemotherapy necessary? Which patients are more likely to benefit from high-dose chemotherapy? Is single-cycle therapy superior to multi-cycle approaches? These and many other questions are likely to remain unanswered without well-designed multicentre trials. Thus, it is imperative that breast cancer patients be enrolled into clinical studies if we expect to continue to make progress in this field.

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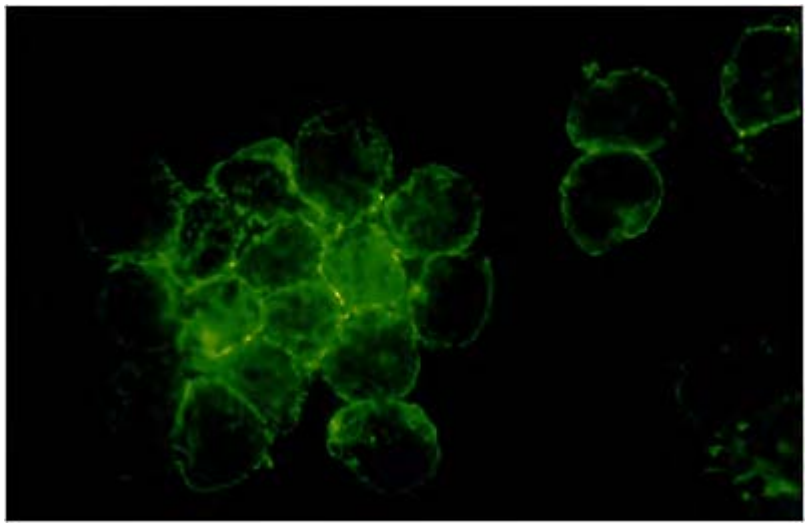

Chapter 13
Solid tumours in adults (excluding breast and germ cell)

Mark Hill and Martin Gore

Introduction

Over the last three decades, there have been a number of advances made in the treatment of haematological malignancies including an increasingly defined role in curative therapy programmes for high-dose chemotherapy (HDC) with stem cell support. This has provided an impetus for similar approaches to be tested in the more common solid tumours of adults. Drug resistance is one of the most important reasons for treatment failure in these diseases, and therefore attempting to overcome it with HDC is an obvious strategy to investigate. This rationale is supported by laboratory data demonstrating that dose correlates with number of cells killed, and that increasing drug doses by 5–10-fold can overcome resistance [1].

Clear evidence of a dose-response effect in patients is provided by numerous clinical trials of chemotherapy in solid tumours [2–4]. A large number of studies have

investigated HDC in solid tumours, particularly in those malignancies which frequently demonstrate initial chemosensitivity, but later relapse. Examples include epithelial ovarian cancer, soft-tissue sarcoma, small-cell lung cancer and gastric cancer. Myelosuppression is often the dose-limiting toxicity of HDC and therefore haematopoietic support is required. Until recently, this has been almost exclusively in the form of autologous bone-marrow transplantation (ABMT) but increasingly, peripheral stem cells (PSC) are used. This technique is technically much simpler than ABMT and also reduces the duration of myelosuppression with consequent shortening of inpatient time. The emphasis of this review, however, is on treatment results and future prospects.

For solid tumours there are no randomized trials defining the role of HDC (except in breast cancer) and the studies outlined below are largely phase I/II trials. Many of the trials are small pilot studies in heavily pretreated patients with large volume disease and therefore the true role of this mode of therapy earlier in the course of these cancers can not yet be fully assessed. Any conclusions must be guarded.

Epithelial ovarian cancer

Epithelial ovarian cancer is a relatively chemosensitive solid tumour, but frequently relapses, often remaining within the peritoneum until very late in the natural history of the disease. It can remain responsive to second-, third- and even fourth-line conventional-dose chemotherapy, but inevitably drug resistance supervenes. It is thus an ideal tumour in which to investigate HDC, both in refractory disease and as a consolidation of remission.

There have been a number of trials reported involving small numbers of patients with recurrent or refractory ovarian cancer [5–11]. In general, the procedure-related mortality is low and the response rate high, but duration of response tends to be short (Table 13.1). The results reported by Stiff and coworkers are representative [10,11]. This group combined mitoxantrone with carboplatin and cyclophosphamide in a dose-escalation study involving 25 patients with various refractory malignancies. Maximum tolerated doses were established (75 mg/m^2, 1.5 mg/m^2 and 120 mg/kg, respectively) and 6 of 7 patients with platinum-resistant ovarian cancer responded, 5 achieving complete remission (CR). In their phase II study, these doses were given to 30 patients with relapsed/refractory disease with a response rate of 89% but a disappointing three-year progression-free survival (PFS) of 23%. In an attempt to lengthen the remission duration, another group [9] has used a two-step sequence of therapy, with moderately high-dose melphalan and PSC transplants before combination high-dose chemotherapy and a second transplant. It is too early to assess the results, but the approach has attracted considerable interest, and is also being tested in the adjuvant setting (see below).

There have been a comparable number of studies in patients with tumours which have responded to platinum-based therapy, with the HDC usually administered with the intention of consolidating remissions [12–17]. Once again, the procedures have been generally well tolerated, but with only a moderate disease-free survival of around 40% at three years (Table 13.2). Among the best results reported are those from Benedetti-Panici *et al.* [17] using high-dose cisplatin, etoposide and carboplatin followed by PSCT (16 patients) or ABMT (4 patients) in 20 stage III/IV patients after induction therapy.

Progression-free survival was 57% at four years with an overall survival of 62%. Engraftment was significantly quicker in the PSC transplant group. Lotz *et al.* [15] used two sequential courses of a three-drug combination

Table 13.1 Studies of high-dose chemotherapy in patients with recurrent ovarian cancer

Study	Patients *(n)*	Conditioning	Response rate (%)	Median relapse-free survival
Mulder *et al.* (1989) [5]	11	Cyclophosphamide/etoposide	55	15 months
Shpall *et al.* (1990) [6]	12 (8 evaluable)	Cyclophosphamide/ thiotepa/cisplatin (i.p.)	75	6 months
Viens *et al.* (1992) [7]	17	Melphalan	53	4 months
Broun *et al.* (1994) [8]	9 (8 evaluable)	Ifosfamide/carboplatin	87	6 months
Shea *et al.* (1995) [9]	10 (9 evaluable)	Mitoxantrone/etoposide/ thiotepa/carboplatin (i.p.)	78	8 months
Stiff *et al.* (1995) [10]	30	Mitoxantrone/carboplatin/ thiotepa	89	7 months

Table 13.2 Trials of HDC in platinum-sensitive ovarian cancer

Study	Patients treated *(n)*	Conditioning	Disease-free survival (%)
Dauplat *et al.* (1989) [12]	9	Melphalan	36 at 2 years
Dufour *et al.* (1991) [13]	11	Melphalan±flash abdominal radiotherapy	45 at 3 years
Legros *et al.* (1992) [14]	31	Melphalan (n=18); carboplatin. cyclophophamide (n=13)	35 at 3 years
Lotz *et al.* (1993) [15]	14	Ifosfamide/carboplatin/ tenoposide (×2)	29 at 1 year (calculated)
Viens *et al.* (1995) [16]	28	Melphalan±cyclophosphamide ± carboplatin (n=24) Thiotepa/cyclophosphamide/ carboplatin (n=4)	52 at 3 years (projected)
Benedetti-Panici *et al*	20	Cisplatin/carbophosphamide/ etoposide	57 at 4 years

(1995) [17]

(ifosfamide, carboplatin and tenoposide) to produce a response rate of 56% in 16 evaluable patients, but with a treatment-related death rate of 9%. An ongoing South-Western Oncology Group study is testing the feasibility of HDC in a cooperative group setting, using one of two randomly assigned conditioning regimens in patients with both refractory and responding but persistent residual disease [18]. The least toxic regimen will be taken forward into a phase III study of HDC versus standard therapy.

An alternative approach is to consolidate remissions obtained surgically with HDC. Murakami and colleagues treated 42 chemo-naive patients with tandem courses of high-dose cisplatin, doxorubicin and cyclophosphamide followed by ABMT [19]. There were no toxic deaths and the five-year survival rate of the 22 patients with no residual disease after surgery was 78%, but only 26% for the 20 with residual disease. Thus, this strategy may be worth pursuing in those patients in CR after surgery.

Newer drugs, such as the taxanes, can also be dose-escalated and are non-cross-resistant to platinum in a proportion of patients [20]. In the light of this and evidence that multiple applications of high-dose treatment rather than a single course may be beneficial [21], several groups have investigated this approach using taxane-containing regimens. In a dose-escalation study reported in 1995, 16 chemo-naive patients were treated with two 14 day courses of escalating doses of paclitaxel (150–300 mg/m^2) and 3 g/m^2 cyclophosphamide [22]. Granulocyte colony-stimulating factor (G-CSF) was administered after course one to facilitate PSC harvest, and 12 patients went on to receive four cycles of rapidly cycled carboplatin (1 g/m^2) and cyclophosphamide (1.5 g/m^2) with PSC support. All assessable patients responded, but there was considerable toxicity including one episode of fatal sepsis. The authors concluded that the high response rate was encouraging, but that further developmental work was required before this strategy could be fully tested.

In summary, therefore, there is evidence that HDC in advanced epithelial ovarian cancer can be delivered safely and with the expectation of high response rates. Improvements in survival are more difficult to evaluate and likely only to be detected in large randomized trials but none have been published to date. It is reasonable to continue to investigate the accelerated multiple high-dose cycle approach involving newer agents, especially in patients in first remission or with minimal residual disease, but only within the context of a clinical trial.

Sarcoma

Tumours of the Ewing sarcoma family (ESF), rhabdomyosarcoma (RMS) and soft-tissue sarcomas are frequently chemosensitive to first-line conventional-dose chemotherapy, but are often chemotherapy resistant at relapse. These tumours thus present a good model for investigating the high-dose strategy as a means to overcome drug resistance. Several groups have performed trials of HDC followed by ABMT in patients with sarcoma, often with young adults and children being reported together. One of the larger such series included 91 patients treated on three consecutive American National Cancer Institute

protocols between 1981 and 1986 [23]. Patients in CR after chemotherapy and radiotherapy for high risk ESF and RMS were given 8 Gy total body irradiation (TBI) followed by ABMT. Nineteen patients failed to achieve CR, all of whom died, and a further 7 declined TBI. Of these patients, 3 of 7 (43%) are alive at six years. A total of 65 patients received the consolidation therapy, 20 (31%) of whom are long-term survivors. Not surprisingly, patients with metastatic disease fared substantially worse than those with localized disease (Table 13.3). The authors concluded that the prognosis was not improved by this therapy.

The European Bone Marrow Transplantation Registry for Solid Tumours has recently reported the collective results from many centres across Europe [24]. Of the 104 patients with ESF, the two-year overall survival was 31% in the 14 patients with multifocal disease in first CR and was 37% for 15 patients in second CR. Of the 67 patients with measurable disease, the response rate was 72%. These outcomes are rather better than might be expected with conventional-dose chemotherapy, and the authors advocated continued research into the optimum conditioning procedure and better delineation of high-risk groups prior to embarking on randomized studies.

Table 13.3 Survival in 65 patient with sarcoma undergoing TBI+ABMT as consolidation of CR

Patient group	Event-free survival (%)	Overall survival (%)
All	25	30
Metastatic	14	20
Non-metastatic	38	40

Small-cell lung cancer

This is one of the most chemosensitive solid tumours, with a high response rate to conventional doses of cytotoxic agents. However, relapse within two years is almost inevitable, and a theoretical role for HDC with haematopoietic support is in the consolidation of remissions obtained with conventional-dose chemotherapy [25]. A number of groups have investigated this possibility, and initially trials involved patients with relapsed disease. Activity was demonstrated with agents such as etoposide, cyclophosphamide and carmustine when used at high doses [26–28]. Trials of HDC for patients with extensive disease (ED) in first remission have not, however, demonstrated a survival advantage over conventional treatment [29] and for those with partial responses there is similarly no increase in the proportion of two-year survivors [30].

In contrast, a phase II study in patients with limited disease (LD) has suggested that the proportion of complete responders can be increased with a higher intensity regimen, and that there is a suggestion of a prolongation of survival (39% at four years) [31]. In the only randomized study comparing HDC after induction therapy with a single further conventional dose of chemotherapy, HDC had no benefit for patients with ED, but was associated with a statistically significant improvement in overall survival for LD patients

from 10 to 35 weeks [32]. However, only 41% of patients who commenced induction therapy were randomized, and the two-year survival for all patients entered was only 7% which is similar to that expected without HDC in this patient group.

It was concluded from these trials that any benefit from this approach was limited to those in at least partial remission (PR), and that since few patients with ED obtain good remissions, future trials should be restricted to patients with LD [33]. Furthermore, high local relapse rates suggested that additional chest irradiation may improve outcome, as was subsequently demonstrated in other trials where conventional doses of chemotherapy were used [34]. A recent study has taken these two factors into account, and treated stage III patients in PR or CR after conventional therapy with high-dose cyclophosphamide, cisplatin and carmustine plus ABMT, followed by chest irradiation [35]. The two-year survival in the 19 patients treated was 57%, but in the absence of results from the ongoing randomized trial by the same group, no firm conclusions can as yet be drawn since the apparently favourable results may be due to case selection.

Malignant melanoma

The low response rate to chemotherapy in advanced melanoma has led a large number of groups to investigate the possibility that higher dose treatment could improve both response rate and duration [36–48]. Many drugs have been used including melphalan, cisplatin, cyclophosphamide, BCNU and combinations thereof, as well as thiotepa and DTIC plus ifosfamide or melphalan. Response rates have generally been acceptable for this tumour type, in the range 40–60% with up to one-third of patients achieving CR (Table 13.4). Median response duration, however, has been universally disappointing, at less than six months. Whilst the improved response rate with HDC is encouraging, the short duration of response leads most authors to conclude that metastatic melanoma is unsuited to this approach.

There has been one phase III trial of adjuvant HDC in patients at high risk of relapse [49]. Participants were enrolled within eight weeks of surgery and received either HDC (BCNU, cisplatin, cyclophosphamide) and ABMT, or no further therapy. Those who were randomized to observation only but subsequently relapsed received HDC without induction chemotherapy. The conditioning regimen was identical to that used in the treatment arm. A total of 39 patients was randomized. The median time to progression was 16 weeks in the observation arm and 35 weeks in the immediate HDC arm, a difference which was not statistically significant, possibly as a consequence of the small trial size. Overall survival in all patients receiving HDC was superior to historical controls. Although this was a trial investigating the *timing* of HDC rather than its overall merits, the evidence was felt adequate to conclude that HDC did not offer sufficient benefits to warrant its use in this setting.

Table 13.4 Selected trials of high-dose chemotherapy in malignant melanoma

Agent	Patients *(n)*	Response (%)	CR (%)	Median response duration	Reference
Melphalan	48	58	19	3–6 months	[36–38]
Thiotepa	51	57	8	3 months	[39]
BCNU	29	38	13	6 months	[40]
BCNU*	38	50	8	2–4 months	[41–44,47]
DTIC**	37	49	14	4 months	[48]

*With melphalan, cisplatin or cyclophosphamide. **With melphalan or ifosfamide.

Gastrointestinal cancer

Colon

The poor outlook of advanced colonic cancer and the steep dose-response effect seen *in vitro* has prompted a small number of trials of HDC. An early study investigated high-dose melphalan in 20 patients with metastatic disease, reporting a response rate of 45% and median survival of seven months [50]. A subsequent phase II trial with escalating doses of melphalan and ABMT, however, reported 15 patients, none of whom responded [51]. Other studies with thiotepa and carmustine plus Mitomycin C have been equally disappointing [52,53]. There is scant evidence to justify pursuing this approach in patients with advanced colorectal cancer.

Stomach

Advanced gastric cancer also carries a poor prognosis but is rather more chemosensitive than colon cancer. Unfortunately, there are even fewer trials of HDC in this disease, the largest being from a Japanese group who treated 10 patients with high-dose etoposide, adriamycin and cisplatin followed by ABMT [54]. There were no toxic deaths and 8 of 9 (89%) assessable patients responded (all PR), but median survival was only nine months. The lack of complete responses and short survival may, however, be improved by cytoreduction with conventional-dose chemotherapy prior to HDC. This approach is currently being investigated in an ongoing randomized trial at the Royal Marsden Hospital.

Summary

Despite extensive investigation over many years, HDC does not yet have an established role in the management of the common solid tumours. It has, however, been determined which patients will not benefit from this toxic and expensive treatment. Both side-effects and cost have been reduced by use of PSC transplant and growth factors, prompting continued investigation of this approach. In general, future trials must be randomized and should involve selected patients with good performance status, minimal tumour burden and chemosensitive disease, with a view to increasing cure rates.

Acknowledgement

Table 13.4 is reproduced with permission from *Cancer Principles and Practice of Oncology,* 4th edition (1993). VT DeVita, S Hellman and SA Rosenberg (eds) (New York: JB Lippincott).

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Chapter 14
Germ-cell tumours

Alan Horwich

Introduction

Germ-cell tumours occur predominantly between the ages of 18 and 45 years. The incidence is increasing in most Western countries, such that in young men it has risen from 6 per 100000 during the 1960s to just over 12 in the early 1990s. Fortunately, the success of combination chemotherapy has prevented this being translated into a problem of increasing mortality. The introduction of cisplatin in combination with vinblastine and bleomycin was a seminal advance in the treatment of patients with metastatic disease [1]. Further improvement in treatment results has come from the replacement of vinblastine by etoposide in this regimen; the combination of bleomycin, etoposide and cisplatin (BEP) currently represents a standard approach internationally [2–4]. Furthermore, there has been publication of sufficient long-term results to judge that chemotherapy is curative. For example, in 229 patients treated in Indiana between 1974 and 1980, recurrence more than three years after chemotherapy was rare [5]. This is supported by a decade of Royal Marsden Hospital results in 320 patients treated between 1976 and 1985 [6].

Of patients presenting with metastatic germ-cell cancer, some 85% should be cured [7]. The prognosis depends upon the extent of disease at presentation, and a recent overview analysis based on just over 5000 patients with metastatic germ-cell cancer, treated in a variety of centres in Europe, North America and Australasia has found that the adverse key prognostic factors are the presence of a mediastinal primary, the presence of non-pulmonary visceral metastases, and the degree of elevation of the serum tumour markers α-fetoprotein (AFP), human chorionic gonadotrophin (HCG) and lactate dehydrogenase (LDH) [8]. These define a poor prognosis subset, as shown in Table 14.1. A second group of patients who may benefit from improved chemotherapy efficacy are those who have relapsed after first-line chemotherapy. This occurs in some 20–30% of patients and confers a poor prognosis [9,10]. High-dose chemotherapy of germ-cell tumours has been evaluated very predominantly in this context of salvage after failure of first-line chemotherapy.

A difficulty in evaluating the results of new approaches to the salvage treatment of germ-cell tumours is the heterogeneity of patients. There is considerable variability of not only the extent of the disease at presentation, but also the pattern of response to primary chemotherapy, the details of chemotherapy employed in first-line treatment together with residual organ tolerance (especially renal function in view of the toxicity of cisplatin), the disease-free interval before progression and the extent of the disease at relapse [11,12]. In a series of 105 patients treated for relapse with conventional-dose chemotherapy at the Royal Marsden Hospital between 1980 and 1988 [13], 73 patients were in stage IV (extranodal metastases) at the time of relapse, and the disease-free interval was between 0 and 8 months in 30 patients, between 8 and 15 months in 48 patients and more than 15 months in 27 patients. Three-year survival probability from the time of beginning salvage chemotherapy was 30%. A multivariate analysis of survival demonstrated a higher risk of mortality in those with a disease-free interval of less than 8 months and in those with extensive disease at relapse as shown in Table 14.2. The analysis allowed subgroups to be identified whose prognosis on standard-dose salvage chemotherapy was extremely poor with a three-year survival probability of less than 10%, in contrast to those with a long disease-free interval and limited extent of disease with a probability of conventional-dose salvage of more than 70%. Therefore, the results of high-dose chemotherapy in the salvage of germ-cell tumours must be evaluated on the background of there being a group of patients who can still be cured with conventional-dose salvage chemotherapy, often associated with salvage surgery [14].

High-dose salvage chemotherapy for germ-cell tumours

The rationale for considering high-dose chemotherapy in salvage of patients who have failed first-line chemotherapy for germ-cell tumours is based on chemosensitivity of this tumour type, the evidence for dose-response of cisplatin at lower doses and the feasibility of significant dose escalation for a number of key drugs including carboplatin, etoposide and either cyclophosphamide or ifosfamide. When supported by haematopoietic precursor support, dose-limiting side-effects are mucositis for etoposide, renal toxicity for

ifosfamide, especially in cisplatin-pretreated patients, and bowel toxicity for combination of these drugs with cisplatin analogue.

Table 14.1 Prognosis at presentation with metastatic non-seminoma germ-cell tumours (IGCCG, 1995) (from Mead et al. 1995 [8])

Prognostic group	Definition	Three-year progression-free survival (%)	Three-year survival (%)
Good	All of: No EPVM No mediastinal/primary AFP<1×10^3 HCG<5×10^3 LDH<1.5×N	89	92
Intermediate	All of: No EPVM No mediastinal/primary AFP 1–5×10^3 HCQ 5−50×10^3 LDH 15–10×N	78	81
Poor	Any of: EPVM Mediastinal/primary AFP>5×10^3 HCG>50×10^3 LDH>10×N	45	49

IGCCG=International Germ Cell Collaborative Group.
EPVM=extrapulmonary visceral metastases, such as bone, liver, brain, etc.
AFP=α-fetoprotein. (International units)
HCG=human chorionic gonadotrophin. (Intenational units)
LDH=lactate dehydrogenase, upper limit of normal (N) range.

High-dose chemotherapy was first evaluated using support by autologous bone-marrow transplantation. Trials of this approach began in Indiana University in 1986 using high-dose carboplatin and etoposide. In 32 patients registered for this study in the first two years, the chemotherapy comprised etoposide 1200 mg/m^2, together with carboplatin in an escalating-dose schedule from 900 to 2000 mg/m^2. Seven patients died of treatment-related problems; however, there were 8 patients with complete remissions within a 42% overall response rate [15]. Further follow-up after the first 40 patients had been treated in the same study found that 6 patients (15%) were alive and continuously disease-free at a minimum of 36 months follow-up, but a further patient died of acute myelogenous leukaemia while in remission 28 months after autologous bone-marrow transplantation (ABMT) [16].

At the Royal Marsden Hospital, our treatment approach for patients who have failed the standard first-line schedules of cisplatin-based chemotherapy is to undertake a four-week course of intensive weekly induction based on bleomycin, vincristine and cisplatin [13]. Patients with disease which stabilized or responded to this approach went on to receive high-dose carboplatin and etoposide and, if the response was continued, a second cycle of high-dose carboplatin and etoposide was administered two to three months later. In this early experience, the high-dose chemotherapy was supported by autologous bone-

*Table 14.2 Prognosis after conventional-dose salvage chemotherapy**

	Probability of salvage in relation to volume of disease at relapse (%)**		
Disease-free Interval	Small	Large	Very
<8 months	20	5	5
8–15 months	20	20	5
>15 months	70	20	5

* Horwich *et al.* (1993) [11], **Volumes-defined by MRC Prognostic Analysis (1985) [32].

marrow stem-cell transplantation. Thirty-three patients were eligible for this programme between 1991 and 1993, but 3 patients declined the high-dose approach and, of the remaining 30 patients, 7 patients progressed during conventional dose-induction chemotherapy [17]. High-dose etoposide was administered at a fixed dose of 1200 mg/m^2 in each course. However, the carboplatin dose was based on renal function to achieve a desired [serum concentration×time] [18]. The carboplatin dose was escalated to achieve a [serum concentration×time] ranging from 15 to 40 mg/ml×mins, and based on a range of toxicities (especially gastrointestinal toxicity), it was concluded that further studies should be pursued at a [serum concentration×time] of 30 mg/ml×mins.

An example of the pattern of myelosuppression is shown in Figure 14.1, which illustrates a patient treated with two cycles of high-dose chemotherapy based on carboplatin at a [serum concentration×time] of 30 mg/ml×mins with an interval of two months. It can be seen that there was no evidence for cumulative myelosuppression, and this conclusion was supported by results from all 12 patients who had a second cycle of high-dose therapy, as shown in Table 14.3. The bone-marrow harvest yield before the first high-dose treatment comprised a median of 2.5×10^8 nucleated cells/kg, and prior to the second cycle a median of 2.7×10^8 nucleated cells/kg.

In many patients, non-haematological toxicity was only mild to moderate. Thirteen patients (57%) experience only grade I toxicity. With pretreatment anti-emetics no patients had more than grade IB nausea. Three patients (13%) had maximal grade II

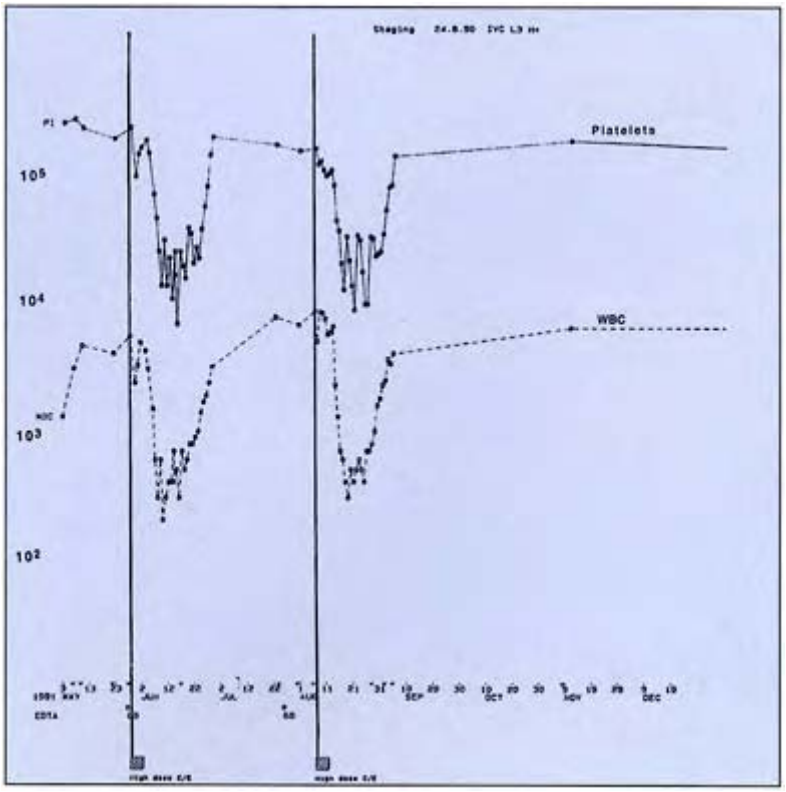

Figure 14.1 Leukocyte and platelet count during two cycles of high-dose chemotherapy with an AUC of 30 mg/ml× mins. AUC=area under the serum concentration-time curve.

toxicity comprising cardiac arrhythmias (grade IID) in 1 patient, and symptomatic reversible heart failure (grade IIA) in 2 patients. Four patients (17%) suffered grade III toxicity, including 2 with grand mal seizures (grade IIIB), 1 of whom had grade IIA mucositis and grade IIB diarrhoea. These patients had received carboplatin doses to give [serum concentration×time] of 30 and 35 mg/ml/min. One patient developed grade IIIB congestive cardiac failure (at 30 mg/ml×mins),

Table 14.3 Myelosuppression during two cycle of high-dose chemotherapy (Royal Marsden Hospital 1991–1993)

Cycle	WBC		Platelets	
	Nadir×10^9/litre	Recovery to 1×10^9/litre (days post-chemotherapy)	Nadir×10^9/litre	Recovery to 50×10^9/litre (days post-chemotherapy)
Cycle 1 (n=23)	0.3	19 (range 14–25)	7	27 (range 23–43)
Cycle 2 (n=12)	0.3	23 (range 16–27)	8	29 (range 23–52)

and the fourth patient had grade IIIB diarrhoea (25 mg/ml×mins). Three patients (13%) died as a result of treatment, 2 following their first cycle and 1 after his second. Two patients died with sepsis, renal failure and necrotizing enterocolitis at post-mortem. Post-mortem of the third patient showed severe toxic lung changes and necrotizing enterocolitis. The 3 patients who died with necrotizing enterocolitis had received carboplatin doses to give [serum concentration×time] of 30 mg/ml×mins in one cycle, 35 mg/ml×mins in two cycles and 40 mg/ml×mins in one cycle. Two patients developed renal failure in connection with sepsis, and they had received carboplatin doses to give [serum concentration×time] of 35 mg/ml×mins in two cycles and 40 mg/ml×mins in one cycle.

The results achieved by high-dose chemotherapy salvage are summarized in Table 14.4. Eight of the 23 patients are alive and in remission, 12–36 months from the start of salvage chemotherapy. Although these results overall do not provide conclusive evidence that high-dose chemotherapy is superior to standard approaches to salvage, there is clear evidence from individual cases that those who are refractory to standard-dose drugs may, nevertheless, be cured by high-dose treatment. Since the concentration of serum tumour markers provides a sensitive monitor of disease bulk, a good example of the efficacy of high-dose treatment is illustrated by the marker patterns shown in Figure 14.2, indicating progressive disease after conventional-dose chemo-therapy, with normalization of markers after two cycles of high-dose therapy, in a patient who has now remained in remission for two years.

Table 14.4 High-dose salvage in germ-cell tumours (CGT) (Royal Marsden Hospital 1991–1993)

Parameter	Patients (*n*)
Treated	23
Evaluable for response	19.
Early death	4

Alive in CR	6	(34%)
Alive in marker CR	2	
Alive with disease	7	
Died of germ-cell tumours	5	
Toxic death	3 (13%)	

A review of a number of series describing results of high-dose chemotherapy salvage included 272 patients reported between 1984 and 1992 [19]. There were 80 complete responses (31%), but only 44 of these (17%) were durable. In the same series, there were 29 (11%) treatment-related deaths. The review suggested that inclusion of either cyclophosphamide or ifosfamide in the high-dose regimen may increase the proportion of patients with durable complete remissions. However, good results have also been reported using only carboplatin and etoposide [20].

A more recent review from published reports included a total of 388 patients treated with high-dose chemotherapy with ABMT [21]. This review concluded that higher doses of etoposide and an oxazophosphorine derivative increase the cure rate.

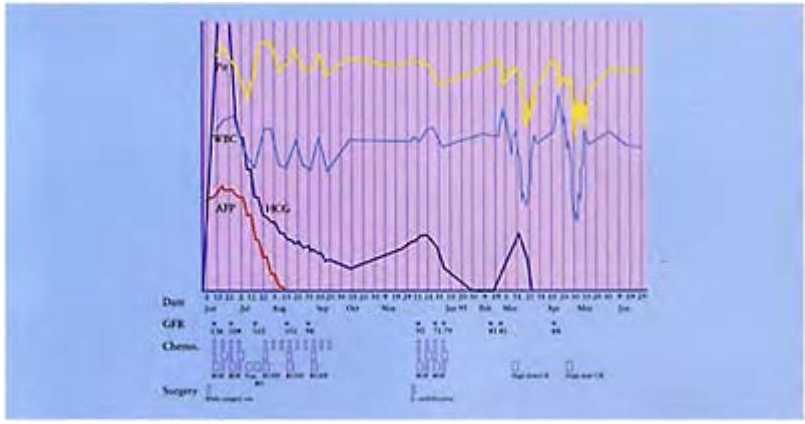

Figure 14.2 Royal Marsden Hospital Testicular Tumour Monitoring System for blood counts and tumour markers during chemotherapy. In this patient with widespread choriocarcinoma, the serum HCG was 8×10^5 U/litre at start of chemotherapy and surged to 2.4×10^6 U/litre. On relapse, there was only temporary HCG response to conventional-dose salvage, but

complete remission after two cycles of high-dose carboplatin/etoposide which has lasted for 24 months.

Cyclophosphamide might be preferable to ifosfamide on toxicity grounds.

In patients whose disease was refractory to standard-dose chemotherapy, the long-term results of high dose were inferior. This has been confirmed in a recent retrospective analysis of 283 consecutive patients with relapsed or refractory germ-cell cancers treated in four centres in the USA or Europe [22]. Patients were considered refractory to cisplatin if they have had a response but then progressed within four weeks. If there was not a response to cisplatin therapy, patients were considered absolutely refractory. Overall, the failure-free survival rate was 32% at one year and 30% at two years. On univariate analysis, an extragonadal primary tumour, an unfavourable response to previous salvage treatment, insensitivity to cisplatin, advanced Indiana stage and high levels of HCG or AFP at the time of high-dose chemotherapy, predicted a shorter failure-free survival. However, on multivariate analysis, the dominant prognostic factors just prior to high-dose chemotherapy were an HCG of over 1000 U/litre with a hazard ratio of 2.38, or the presence of absolutely refractory disease with a hazard ratio of 1.98, as shown in Table 14.5. A prognostic index for high-dose chemotherapy salvage can be constructed from this table, as shown in Table 14.6.

As a consequence of early studies, high-dose chemotherapy has now been promoted to be considered a standard second-line chemotherapy approach for germ-cell tumours. A report confined to patients treated with high-dose therapy as their first salvage regimen, and excluding patients with extragonadal primary germ-cell tumours, was based on 23 patients [23]. Two cycles of induction chemotherapy comprising either vinblastine, ifosfamide, cisplatin or platinum with vinblastine, bleomycin were followed by carboplatin (1500–2100 mg/m^2) and etoposide (1200–2250 mg/m^2) over three days followed by ABMT. Eighteen of the 23 patients completed therapy. There was 1 treatment-related death which occurred during the induction phase. The report indicated that 7 patients remained alive and free of disease at a median follow-up of 26 months.

Current studies of high-dose chemotherapy in the salvage of germ-cell tumours include an evaluation of

Table 14.5 Multivariate analysis of failure of high-dose salvage chemotherapy of germ-cell tumours (from Beyer et al. (1996) [22])

Variable*	Hazard ratio	*P* value	Index***
Progressive disease	1.51	0.024	1
Mediastinal primary	1.69	0.029	1
Refractory disease**	1.71	0.004	1
Absolute refractory**	1.98	<0.002	2
HCG >1000 U/litre	2.38	<0.001	2

*at time of high-dose. **Defined in text ***See Table 14.6.

Table 14.6 Prognostic index for high-dose salvage chemotherapy (From Beyer et al. (1996) [22])

Index * score	Salvage patients (%)	Two-year failure-free survival (%)
0	39	51
1 or 2	33	29
>2	28	5

*Derived by adding index scores from Table 14.5.

the role of growth factors after rescue in view of results, suggesting that this leads to a reduced number of infections and toxic deaths [24]. Additionally, the widespread adoption of peripheral blood stem-cell harvesting to replace bone-marrow stem-cell support has facilitated investigation of high-dose regimens. In a German Testicular Cancer Group study using high-dose carboplatin, etoposide and ifosfamide, both ABMT and peripheral blood stem-cell (PBSC) support were used [25]. All 20 patients receiving PBSC rescue plus granulocyte colony-stimulating factor (G-CSF) had a full haematological recovery, which was faster than that in patients receiving ABMT [26].

A second major area of investigation is the comparison of high-dose with standard-dose chemotherapy in first-line salvage conducted as a pan-European multicentre trial coordinated by the trials office of the Institut Gustave-Roussy (Paris). This trial compares, in first-line salvage, four cycles of conventional-dose chemotherapy based on the combination of cisplatin, ifosfamide and either vinblastine or etoposide with an experimental treatment arm, comprising three cycles of this chemotherapy followed by a single cycle of high-dose carbopec (carboplatin, etoposide, cyclophosphamide) [27]. The trial is seeking recruitment of 280 patients based on the need to demonstrate a 15% difference in one-year survival to justify the high-dose approach.

High-dose chemotherapy as primary treatment of patients with advanced germ-cell cancer

The efficacy of standard treatments in curing patients with metastatic germ-cell tumour has obviated the need to consider high-dose chemotherapy in the majority of cases. However, this approach has been evaluated in two patient groups perceived to have a particularly poor prognosis. The first of these is defined by presentation factors such as very high tumour-marker levels, and the second is defined by relatively slow tumour-marker response.

In patients with poor presentation factors, a randomized trial has failed to show therapeutic benefit for high-dose chemotherapy [28]. In this trial, poor-risk patients were randomly assigned to receive three or four cycles of vinblastine, etoposide, bleomycin and double-dose cisplatin (200 mg/m^2) (PVeBV) or two cycles of PVeBV followed by

one cycle of high-dose chemotherapy and ABMT. The high-dose regimen had double-dose cisplatin with high doses of etoposide and cyclophosphamide. A weakness in this comparison was that the total platinum dose was not significantly different in the two arms of the trial, and furthermore, there is evidence that a cisplatin dose of 200 mg/m^2 is no more effective than the standard dose of 100 mg/m2 [29].

The Eastern Co-operative Oncology Group is conducting a trial comparing standard BEP chemotherapy versus high-dose chemotherapy in previously untreated patients with poor-risk germ-cell tumours. Comparison will be between either four cycles of BEP or two cycles of BEP followed by carboplatin (1800 mg/m^2), etoposide (1800 mg/m^2) and cyclophos-phamide (150 mg/kg), each divided over three days. Responding patients on the high-dose arm will receive a second identical cycle of high-dose chemotherapy.

At the Memorial Sloan-Kettering Cancer Center, an analysis of tumour-marker patterns following chemotherapy suggested that a slow or inadequate clearance of tumour markers was associated with poor prognosis after conventional treatment, and a protocol was introduced of switching patients to high-dose treatment if their tumour-marker clearance was slow. The problem with this approach is that the significance of tumour-marker regression rates may depend upon the time at which they are measured [30]. The results of this approach reported from the Memorial Hospital indicated that 9 of 16 patients with slow marker clearance had a complete response to high-dose chemotherapy [31].

Conclusions

High-dose chemotherapy with carboplatin and etoposide with or without an oxazaphosphorine followed by haematopoietic stem-cell support appears to be curative in 20–30% of patients with germ-cell tumours relapsing after cisplatin-based combination chemotherapy regimens. It is more effective in tumours which have retained some sensitivity to conventional-dose chemotherapy. High-dose chemotherapy should be considered as second-line therapy, with the possible exception of patients who relapse late with a limited extent of disease. Currently, the use of high-dose chemotherapy in the first-line treatment is not established, but it is being investigated in defined high-risk patient groups.

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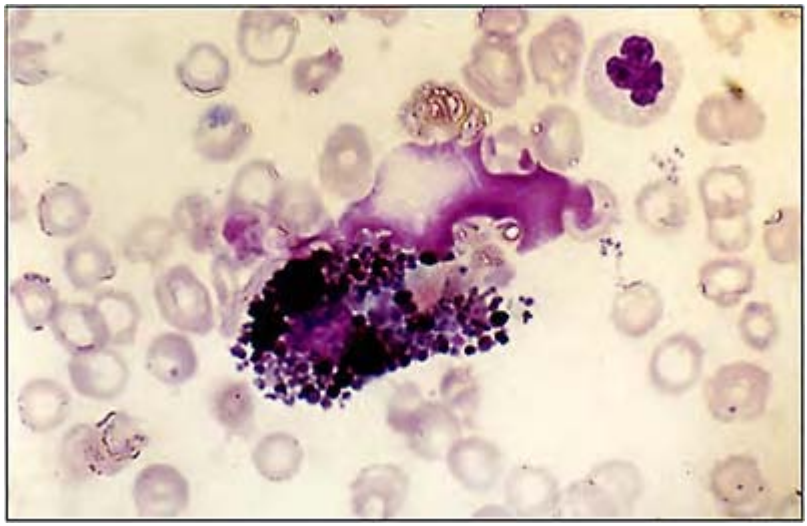

Chapter 15
Immunodeficiency diseases

Alain Fischer

Introduction

Most primary immunodeficiency diseases (PID) occur secondary to intrinsic defects of lymphocytic and/or phagocytic cell lineages. Therefore, replacement of genetically-impaired haematopoietic stem cells by normal haematopoietic stem cells is a logical therapeutic approach. The first reports of successful bone-marrow transplantation (BMT) for PID were published in 1968: Gatti *et al.* described correction of a severe combined immunodeficiency (SCID) case [1] and Bach *et al.* described partial correction of a Wiskott-Aldrich syndrome (WAS) case [2]. Since then, it is estimated that over 1500 patients with PID have undergone allogeneic BMT. About 19 different inherited immune deficiencies have been cured by BMT (Tables 15.1 and 15.2), including the different forms of SCID, other T-cell immunodeficiencies, WAS, various phagocytic cell diseases and more recently, hyper-IgM and X-linked proliferative (XLP) syndromes. BMT has also been used as a source of

Table 15.1 Immunodeficiencies of the lymphoid system curable by allogeneic bone-marrow transplantation

Severe combined deficiencies (SCID)
Reticular dysgenesis
Adenosine deaminase deficiency
X-linked SCID (γc deficiency)
Autosomal recessive SCID with B-cells (mostly *JAK3* deficiency)
Autosomal recessive SCID without B-cells
T-cell immunodeficiencies
Purine nucleoside phosphorylase deficiency
Omenn's syndrome
Defective T-cell activation deficiency including ZAP70
HLA class II expression deficiency
Cartilage hair hypoplasia
Wiskott-Aldrich syndrome
X-L hyper-IgM syndrome (CD40 ligand deficiency)
X-L proliferative syndrome (Purtilo's disease)

Table 15.2 Immunodeficiencies of the phagocytic system curable by allogenic bone-marrow transplantation

Severe congenital neutropenia and similar disorders (cyclic neutropenia, Schwachman's syndrome)
Leukocyte adhesion deficiency
Chronic granulomatous diseases (X-linked and autosomal recessive)
Chediak-Higashi syndrome
Familial haemophagocytic lymphohistiocytosis
Immune deficiency with partial albinism (Griscelli disease)

mature T-cells to correct, at least for the duration of observation, the severe T-cell lymphocytopenia observed in DiGeorge's syndrome [3]. Based on a better understanding of major histocompatibility complex (MHC) molecular biology, alternatives to BMT from HLA genetically identical donors have been proposed, for example, use of related, partially matched donors as well as matched unrelated donors.

Bone-marrow transplantation for severe combined immune deficiencies (SCID)

SCID represent a group of diseases characterized by an inherited defect in T-cells, with or without B-cell differentiation, resulting in the absence of mature T-cells, with or without B-cells. The overall frequency is estimated at 1 in 75000 live births. The main SCID syndromes are:

1. Reticular dysgenesis, characterized by both defective myelopoiesis and lymphopoiesis.
2. Alymphocytosis, where T- and B-lymphocytes are absent while natural killer cells (NK) are present (as in the murine SCID model).
3. Selective blockade of T-cell differentiation which can be inherited either as an autosomal recessive, or more frequently, as an X-linked recessive disorder.
4. Adenosine deaminase deficiency.

In the absence of bone-marrow transplantation, SCID syndromes are fatal, usually within the first year of life. In the recent past, the molecular basis of X-linked SCID has been identified. It consists of mutations of the gene encoding the gamma common chain of the interleukin-2 (IL-2) receptor, which is also involved in the high-affinity receptors for IL-4, IL-7, IL-9 and IL-15 [4], while most autosomal recessive (AR) forms of SCID with B-cells are caused by mutations of the *JAK3* tyrosine kinase gene [5].

As mentioned earlier, Gatti *et al.* first reported the successful correction of SCID by allogeneic BMT [1]. Since then, at least 200 children with SCID have received HLA-identical BMTs worldwide. Results have gradually improved because earlier diagnosis prevents life-threatening complications such as transfusion-induced graft-versus-host disease (GvHD), and more effective antibiotics are now available. In Europe, the cure rate has been greater than 90% since 1983 [6,7].

The most remarkable features of HLA-identical BMT for SCID are the lack of an absolute requirement for conditioning, the rarity of acute and chronic GvHD and the rapid development of T- and B-cell function post-transplant. In most cases, only lymphocytes of donor origin can be identified. In pure T-cell deficiency, donor-derived T-lymphocytes may coexist effectively with host-derived B-lymphocytes [8,9]. It is unknown whether engraftment of stem cells occurs in the marrow with selective lineage differentiation, whether stem cells differentiate in the thymus, or whether only mature donor T-cells expand (as observed after transfer of mature T-cells to athymic nude mice). The latter hypothesis may account for the rapid (within a few weeks) development of full immune function, and the success of BMT as a cure for the ID of DiGeorge's syndrome [3].

The low incidence of GvHD may be related to the absence of a marrow ablative regimen pretransplant, thus possibly sparing natural suppressor cells. In some patients, a partial B-cell immunodeficiency persists, including IgA and sometimes IgG2 and IgG4 production impairment. It is not currently clear whether B-cell immunodeficiency results from intrinsic host B-cell deficiency in these cases, as observed in X-linked SCID and *JAK3* deficiency in which B-cells are unresponsive to IL-2 and IL-15 but responsive in part to IL-4 and to IL-13, or whether another mechanism causes residual B-cell

immunodeficiency. A minority (<10%) of SCID patients who have undergone HLA-identical BMT are supplemented with long-term intravenous immunoglobulin.

Further studies will be required to more accurately correlate B-cell immune function development with chimerism of purified B, and other, cell populations. Whatever form of SCID is diagnosed, BMT invariably carries a good prognosis provided that the donor is an HLA-identical sibling. At present, the only failures are observed in SCID patients who are diagnosed late and have developed severe infections unresponsive to aggressive therapy. In a small number of cases, a dramatic rise in T-cell counts is observed in the second week following BMT, sometimes associated with minor, GvHD-like symptoms. This phenomenon corresponds to abrupt amplification of donor T-cells specific for maternal antigens which eliminates the maternal T-cells frequently present in SCID patients [11]. This so-called 'graft-versus-graft reaction' is transient and does not require major therapeutic intervention.

Only approximately 20% of SCID patients (as is the case with other potential bone-marrow recipients) have an HLA-identical sibling. HLA phenotypically identical BMT from related donors has also been used successfully, although the success rate is significantly lower compared to HLA genotypically identical transplants (65%) (n=23) versus 85% (n=60), P<0.05, in Europe).

Alternatives in the absence of HLA-identical donors

In the 1970s, the use of HLA-mismatched foetal liver cells as a source of haemopoietic stem cells to cure SCID patients without an HLA-identical donor was proposed. This was based on the knowledge that major histocompatibility complex (MHC) disparate stem cells could engraft and then generate T- lymphocytes which differentiated in the host thymus to become tolerant to recipient tissues. In a low proportion of cases (11%), foetal liver transplantation was successful, leading to T-cell, and sometimes B-cell, differentiation without causing GvHD, provided that the foetal liver originated from a foetus not older than 12 weeks [12]. Some patients are alive and well with normal immune function 10–18 years after this procedure. However, the procedure often failed, and it required one to two years before patients could safely leave the protected environment.

Interesting observations have been made concerning chimerism and tolerance in the long-term survivors. In the context where T-cells are of donor origin, while B-cells and monocytes are host, and NK cells are either donor or host [13], it was found that donor anti-host reactive CD4 and CD8 T-cell clones could be derived, and no GvHD occurred. This discrepancy may result from the high level of immunosuppressive IL-10 production by anti-host T-cells and host non-T-cells [14], while anti-host T-cells also produce low levels of IL-2. These surprising findings show that tolerance in this setting is mainly the consequence of a peripheral control mechanism.

The poor results of foetal liver transplantation show that this procedure can no longer be proposed for SCID patients without a family HLA-identical donor. However, they form the basis for modern aspects of HLA non-genetically identical bone-marrow stem-cell transplantation. Nowadays, there are two possible sources of donors in this setting: unrelated donors, as closely matched as possible to the recipient; and HLA partially identical family donors. Given the extension of the pool of potential unrelated donors to

more than 4×10^6 in 1997, the likelihood of finding an acceptable donor, at least for a Caucasian patient, is now above 50%. However, a protracted period is required to find the best donor, on average three to five months. It is generally thought that it is dangerous to wait for such a period of time to transplant a SCID patient, even if a protected environment can be provided.

Nevertheless, BMT from matched unrelated donors has been successfully performed in SCID patients. Of note, the very first success of BMT from an unrelated donor was performed in a SCID patient [15]. Data collected in the USA [16] and in Europe give a success rate for matched unrelated-donor transplants in SCID of approximately 65% (F.Porta, unpublished results).

Besides the issue of time, the key question in this setting deals with the possibility of infusing or not infusing an unmanipulated marrow graft. Injection of mature T-cells, as in BMT from HLA-identical siblings, carries the major advantage of transferring T-cells able to rapidly reconstitute efficient immunity. In contrast, T-cells from unrelated donors may also cause life-threatening GvHD. It is therefore too early to decide which are the optimal strategies regarding choice of donor, and the pertinence of T-depletion of marrow grafts from unrelated donors.

As soon as T-cell depletion methods became available, HLA partially compatible BMT was proposed as an alternative to foetal liver transplantation. It was expected, from animal models, that T-cell-depleted marrow transplantation would give rise to normal lymphoid differentiation in the absence of GvHD. Reisner *et al.* [17] successfully used a physical method (combination of soybean agglutination and sheep erythrocyte rosetting) to remove mature T-cells from the marrow. Results were reproduced in other centres using other T-cell-depletion methods, such as anti-T-cell antibodies, or sheep erythrocyte rosetting alone. From the literature, it appears that at least 300 patients with SCID syndromes have received T-cell-depleted marrow from a related donor, usually a parent. About 60% are alive and have developed immune function.

Some single centres have reported success rates of 70% [7,9,18,19]. Overall, the two-year survival in Europe is 56% [6]. Analyses have been performed to identify prognostic factors of these transplants. It appears that neither the type of SCID syndrome nor the T-cell depletion method used has any influence on outcome. More recently, however, it has been recognized that partially identical HLA BMT has a poorer outcome in B(−) SCID. This may be the consequence of a higher incidence of graft failure (see below). Other major factors were age at BMT or presence of a lung infection prior to BMT (the two variables are not independent), use of a protective environment and use of a conditioning regimen (for patients transplanted since 1986) (Table 15.3).

Results of HLA non-identical T-cell-depleted transplants for SCID syndromes in Europe differ significantly from HLA-identical transplants ($P<0.01$) [6]. No improvement in survival was observed for the former type of transplant in Europe between 1981 and 1988. It was recognized at that time that a combination of favourable factors (protective environment, optimal donor and use of a conditioning regimen) gave a 76% survival rate compared to 42% for all other patients.

In the absence of a conditioning regimen, failure of engraftment occurs in 40% of patients, while the use of busulphan (8 mg/kg) and cyclophosphamide (20 mg/kg) leads to a 95% engraftment rate. It also appears that a conditioning regimen promotes the rate of development of immune function, and that T- and B-

Table 15.3 Factors influencing the outcome of HLA non-identical, T-cell-depleted BMT for severe combined immune deficiencies (after [6])

Factor	Relative risk*	*P*
Lung infection before BMT	9.3	0.002
Absence of protective environment	11.1	0.001
Female donor to male recipient	3.4	0.065
Absence of conditioning regimen (since 1986)	3.3	0.02
*Cox multivariate regression analysis.		

cell function both recover more frequently. The latter observation seems to be correlated with the pattern of chimaerism. In the absence of a conditioning regimen, engraftment of the donor B-cell and myeloid cell lineages occurs in ≤30%, while it is present in 75% of conditioned patients. Moreover, donor B-cell chimaerism is strongly associated with the development of B-cell antibody production [6,20,21]. A high-dose conditioning regimen, however, may be detrimental in profoundly immune-deficient patients. Thus, busulphan 16 mg/kg with cyclophosphamide is associated with significantly poorer survival compared to busulphan 8 mg/kg with cyclophosphamide (54.5% versus 69.5% two-year cumulative survival rate; $P<0.05$). Some SCID patients infected at the time of transplant cannot, however, tolerate conditioning prior to BMT.

Graft failure and GvHD

Several factors have been proposed to account for the surprisingly high graft failure rate of HLA non-identical T-depleted marrow transplants in SCID patients. In the murine SCID model, it has been demonstrated that NK cells contribute to failure of marrow engraftment. This can be prevented by 4 Gy total body irradiation (TBI). NK cells may also prevent engraftment in SCID patients [10,22].

Other studies have shown disparate results [18]. NK-cell activity is absent in some SCID syndromes such as typical X-linked SCID but graft failure occurs in all types of SCID. This suggests that other mechanisms may be involved. However, in a recent European survey, it was found that presence of NK cells in recipients, as found in most cases of B(−) SCID as opposed to most B(+) SCID, had a negative impact on engraftment as previously suggested in single studies. [Bertrand unpublished data, 20,22].

Engraftment of maternal T-cells is frequent (30–50%) in SCID patients. These could contribute to graft rejection but seem actually more frequently associated with occurrence of GvHD (W.Friedrich, unpublished data). It has therefore been suggested that the mother be the marrow donor if a conditioning regimen cannot be used pretransplant. Maternal T-cells, however, are frequently oligoclonal [23] and are likely to have a poor capacity for inducing graft rejection, in the same way as they seldom induce GvHD in SCID recipients. Persistence of maternal T-cells after HLA non-identical BMT may lead to features of chronic GvHD. Finally, the likelihood of T-cell depleted marrow engrafting

in adenosine deaminase (ADA)-deficient patients is reduced, presumably because of the presence of toxic substrates such as deoxyadenosine, which may block donor stem-cell proliferation.

Provided that T-cell depletion of the marrow inoculum is sufficiently profound leading to infusion of less than 1×10^5 T-cells/kg, the risk of severe GvHD is limited. Indeed, recipients of marrow treated by soybean agglutination and sheep erythrocyte rosetting developed GvHD infrequently [7,10].

Recovery of immune function

The kinetics of development of T- and B-lymphocyte function are slower in recipients of T-depleted, HLA-incompatible marrow than in recipients of HLA-identical marrow. We found that full T- and B-lymphocyte-mediated responses were present at day 186 in recipients of HLA-identical transplants, but at day 505 in recipients of HLA non-identical transplants [20,21].

Factors associated with slower development of T-cell function are non-utilization of a conditioning regimen and GvHD. B-cell function fails to develop in approximately 40% of transplanted patients. This is apparently strongly correlated with an absence of donor B-cells, and thus with lack of a conditioning regimen. Van Leeuwen *et al.*, however, found in a recent survey from the Leiden group experience that in 9 long-term survivors of haploidentical BMT for SCID, IgG isotype antibody responses were detectable whether donor B-cells were present or not. Three patients only had IgA deficiency [24]. It is difficult at this time to understand the observed discrepancy. An intrinsic B-cell defect may contribute, although in some cases host B-cells have been shown to produce antibodies following HLA-identical and non-identical [8,24–26] BMT.

Ineffective T-cell stimulation because of donor-derived MHC Class II restriction is excluded since:

- donor and recipient always share at least one HLA haplotype, and
- engrafted class II MHC responsive (‘helper’) T-cells can recognize antigen in the context of host class II HLA antigens as previously found in murine models of bone-marrow transplantation [27–29].

It has been found that patients with selective engraftment of donor T-cells exhibit T-cell ‘autoreactivity’ specific for donor MHC class II molecules [30]. These results might be accounted for by the absence of donor-derived MHC class II expressing cells of the monocyte lineage in host thymus, resulting in a lack of negative selection. The latter cells have been previously shown to be required for negative selection of autoreactive T-cells. As in foetal liver transplant (FLT) recipients, it was demonstrated that in HLA haplo-identical T-cell-depleted marrow recipients, anti-host T-cell clones could be derived. Lack of *in vivo* reactivity appears to be associated with the inability to produce high levels of cytokines including IL-2, IL-4, IL-5, IL-10, γ-interferon and granulocyte colony-stimulating factor (G-CSF) [31].

Bone-marrow transplantation for other immune deficiencies

In 1968, Bach and colleagues were successful in achieving HLA-identical lymphoid engraftment in a patient with Wiskott-Aldrich syndrome [2]. Since then, allogeneic BMT has been found to be effective in at least 14 distinct non-SCID immune deficiencies (Tables 15.1 and 15.2).

Conditioning regimen

The main difference compared with SCID patients is the usual requirement of a conditioning regimen to achieve engraftment [25,32,33]. A few exceptions have been noted, particularly in the treatment of profound T-cell immune deficiency such as Omenn's syndrome. Initially, TBI was used to prepare immune deficiency patients for BMT. It was later recognized that TBI could be safely replaced by busulphan at dosages (16–20 mg/kg) that did not produce adverse late effects as severe as those seen after TBI. It was also found that such doses were sufficient for young patients (below the age of two to four years), despite the reduced bioavailabilitiy of busulphan in this age group [34,35]. There is now a general consensus on the use of busulphan 16–20 mg/kg together with cyclophosphamide 200 mg/kg as a conditioning regimen for patients with non-SCID immune deficiency treated by HLA-identical BMT [36]. One should, however, notice that ten recent studies have demonstrated variability in high-dose busulphan levels among young children (by a factor of six) [35]. These results favour an individual close adjustment of busulfan. This regimen may, however, be insufficient in young patients with phagocytic cell disorders (see below). Alternatively, other myeloablative drugs can be utilized, such as thiotepa [37].

Wiskott-Aldrich syndrome

After the partial correction obtained in 1968, full correction of WAS was achieved in 1978 following the use of an appropriate conditioning regimen [33]. HLA-identical BMT has been found to be efficient in 90% of patients who have an HLA-identical sibling [38]. All aspects of WAS are corrected by marrow transplantation, including the eczema, autoimmune problems and the risk of lymphomas. This indicates that disease complications are related to the immune deficiency. Similarly, thrombocytopenia and bleeding tendency disappear after BMT. HLA-identical BMT is recommended for patients with WAS, and should be performed as early as possible.

T-cell immune deficiencies

Extension of BMT treatment to T-cell immune deficiencies has not been as successful, although a number of patients have been cured. Overall, HLA-identical BMT for various T-cell immune deficiency syndromes, including Omenn's syndrome [39,40], MHC class II deficiency [41], purine nucleoside phosphorylase deficiency [42,43], cartilage hair

hypoplasia [44] and others, cures approximately half the patients [45], a figure significantly inferior to that seen with other immune deficiencies.

In the absence of BMT, likelihood of survival to adulthood is virtually non-existent [46,47]. It appears that failure is often related to viral infections or organ failure. These complications are directly related to the pretransplant clinical status. Since the immune deficiencies are less pronounced than in SCID, they are compatible with life for several years, although infections caused by intracellular micro-organisms develop and are exacerbated following marrow ablative and immune suppressive conditioning. Early diagnosis of these immune deficiencies should lead to BMT as early as possible in order to offer maximal likelihood of cure. Indeed, in a European survey, transplants performed before the age of two years give a 79% success rate, versus 48% over four years of age (including all non-SCID immunodeficiency diseases) [45].

X-linked hyper-IgM syndrome (CD40 ligand deficiency)

Hyper-IgM syndrome (HIGM-I) was considered as a pure B-cell immunodeficiency for a long time, because of the observed defective production of IgG, IgA and IgE. Following the knowledge that CD40 ligand expression deficiency on activated T-cells caused the disease, it was appreciated that many of HIGM-I patients suffered from opportunistic infections caused by microorganisms such as *Pneumocystis carinii*, *Toxoplasma* and *Cryptosporidium*. The latter induces chronic cholangitis in many patients, leading to cirrhosis. A recent survey has shown that likelihood of survival to adulthood is below 25% [48,49]. Thus, BMT currently appears to be the only curative treatment for this disease and has indeed been found to be effective in a small number of cases [50]. Early BMT is recommended, given the severity of secondary organ failure which can require liver transplantation and which has been performed in several patients.

X-linked proliferative (XLP) syndrome

This syndrome is essentially characterized by poor handling of Epstein-Barr (EBV) infection, and results in a fatal illness within a few years after contact with EBV. No patient has been reported to be alive beyond the age of 38 years. Most have died before the age of 20 years [51]. Although the mechanism underlying the disease is unknown, it is reasonable to assume that a deficiency in bone-marrow-derived cells accounts for it. Following a first unsuccessful attempt, several patients have now been cured by either BMT or cord blood transplantation [52–55]. Disease diagnosis is, however, difficult since there is no clear biological marker of the disease in the absence of a family history. The identification of the *XLP* gene, soon to be expected given recent progress in genetic studies of the disease, will be of considerable help in identifying patients who require transplantation.

Phagocytic cell disorders

Some of the inherited phagocytic cell disorders remain lethal, either because of the severity of infections in the absence of alternative therapy, or because of the onset of specific complications such as the 'acute phase' of the Chediak-Higashi syndrome.

Leukocyte adhesion deficiency (LAD) is a rare disease characterized by defective expression of the β_2-integrin subunit shared by the leukocyte adhesion proteins. Its complete absence leads to the severe phenotype of LAD, which predisposes to severe bacterial infections, often causing death within the first years of life. HLA-identical BMT is the treatment of choice for this condition. Aggressive conditioning including the use of etoposide or TBI may be required for appropriate marrow ablation [9]. As shown in Table 15.4, results of allogeneic BMT have been satisfactory.

Similarly, HLA-identical BMT can cure the haematological manifestations of the Chediak-Higashi syndrome [56], of familial haemophagocytic lymphohistiocytosis [57] and of immunodeficiency

Table 15.4 Survival in non-SCID congenital immune deficiency syndromes: proportion surviving to adulthood

Syndrome	No BMT (%)	HLA-identical BMT (%)	Matched unrelated-donor BMT (%)	HLA-non-identcal BMT (%)
T-cell immune deficiencies				
Omenn syndrome	0	80		50
Purine nucleoside phosphorylase deficiency	0	<10–20		–
MHC class II deficiency	0	50–60	~50	30
Others	0	60		50
Wiskott-Aldrich syndrome	30–40	90	70	40
Phagocytic cell disorders				
Agranulocytosis	90–100	>90 (see text)		–
Leukocyte adhesion deficiency				
severe phenotype	0	>70		75
moderate phenotype	50		70	
Chronic granulomatous disease	80–90	90 (see text)		–
Chediak-Higashi syndrome	5	90		

Familial haemophagocytic lymphohistiocytosis	0	70

with partial albinism. In these instances, BMT prevents relapse of lymphohistiocytic activity. It is worth noting that mixed chimaerism is sufficient to achieve disease control [58]. This indicates that an abnormal regulatory process, rather than a malignant process, underlies these conditions. Use of TBI is not necessary; a combination of etoposide, busulphan and cyclophosphamide appears both a safe and efficient conditioning regimen for these diseases.

Most chronic granulomatosis disease (CGD) patients can survive to adulthood because prophylaxis with co-trimoxazole, itraconazole and γ-interferon [59] has considerably reduced the incidence of life-threatening infections. It is estimated, however, that the risk of severe aspergillosis is still 1 in 100 patient-years with a death rate of 30%. Patients also develop debilitating complications such as lung/bladder/colon granulomas requiring long-term steroid therapy. It has therefore recently been advocated that BMT should be considered for patients with severe forms of CGD. Seger has recently reviewed CGD patients transplanted over the last few years (unpublished data). He found that 8 out of 9 patients have been cured by an HLA identical BMT. Remarkably, 2 of the patients were very sick at time of BMT because of restrictive lung disease and active refractory aspergillosis. In the latter case, transfusion of granulocytes may have contributed to the control of the *Aspergillus* infection during BMT. Although it is presently impossible to draw a firm conclusion, BMT can certainly be considered as a therapeutic option in CGD patients who have developed severe complications [60].

The prognosis for severe congenital neutropenias (SCN) has been considerably modified by the use of G-CSF. G-CSF enables almost all of these patients to have virtually normal granulocyte counts and thus few or no infections, so that patients can enjoy a normal life [61]. In a proportion, however, myelodysplasia and overt leukaemia occur. It is possible that malignancies are associated with the presence of acquired clonal heterozygous mutations of the G-CSF receptor [62]. Therefore, detection of such a mutation in marrow cells of a patient with SCN could be a good indicator for BMT, provided that there is a suitable donor [63].

The ten-year outcome of HLA-identical BMT for most immune deficiency syndromes appears reasonably satisfactory. Correction of the underlying deficiency is sustained, despite frequent mixed chimaerism in the absence of TBI usage. Sequelae are generally limited to consequences of the primary disease. Chronic GvHD is of limited frequency in these young patients (<10%) [9].

Over the last decade, increasing numbers of allogeneic transplants have been performed for immune deficiency syndromes. Younger age at time of BMT is associated with excellent outcome, and progress has been made in recent years, particularly in infection prevention (intravenous immunoglobulin, antiviral drugs), and GvHD prophylaxis (combination of ‘short’ methotrexate and cyclosporin). In Europe, rates of success for BMT (performed from October 1985 until March 1991) were 81.5% versus 52% before October 1985 [45].

Alternative donors

Unrelated donors

Following the improved results for HLA non-identical BMT in the treatment of SCID syndromes, a similar approach has been taken for the treatment of the other lethal immune deficiencies. Unfortunately, results have been disappointing because of a high incidence of graft rejection (up to 75%) [9,64] and because of infectious complications. More recently, successes have been obtained using matched unrelated donors [65,66]. In a survey of matched unrelated-donor BMT, Kernan *et al.* [66] indicated that the success rate of BMT for patients with inborn errors was 50%. Filipovich *et al.* have reported success of matched unrelated-donor BMT in 6 of 8 SCID recipients, 2 of 2 patients with WAS and 2 of 2 patients with Chediak-Higashi syndrome [16]. Some success using HLA partially incompatible unrelated donor marrow has also been reported, especially when the degree of incompatibility was restricted to one HLA antigen and provided the marrow was T-cell depleted [16,65].

The most dramatic improvement has been seen in the treatment of WAS by unrelated donor BMT. In reviewing International Bone Marrow Transplant Registry data, Filipovich *et al.* have reported a 73% rate of success, which is remarkable. Similar figures have been noted in Europe in a smaller number of cases (65%; F.Porta). Prognosis appears much better when BMT was performed below the age of five years (89% cure versus 30% for patients over five years).

BMT from unrelated donors appears, therefore, to be a useful option for patients lacking an HLA-identical sibling. It should probably be restricted to diseases which allow a donor search time of several months. Acceptance of some degree of HLA incompatibility and optimal strategy for GvHD prevention, including use or non-use of T-cell depleted marrow, remain open questions, since no clear-cut, differences in results have so far been detected.

Partially incompatible donors

In the mid-1980s, we were surprised by the consecutive success of three HLA non-identical bone-marrow transplants in patients with the severe phenotype of LAD [67]. This has since been confirmed in 9 patients with the severe phenotype of LAD who received HLA non-identical BMT: engraftment occurred in all, and 7 are alive with partial or full correction of the LAD [68]. This is in sharp contrast to the engraftment rate of marrow transplanted under the same conditions to other immune deficiency patients (excluding SCID), where the engraftment rate was 26% [9,64,69].

It was therefore postulated that the defective expression of the LFA-I molecule on graft rejection effector cells (cytotoxic T-cells and NK cells) impaired cell contact with donor marrow cells and thereby permitted engraftment as shown in a murine model [70]. This led to the *in vivo* infusion of a monoclonal anti-LFA-I (CD11a) antibody to inhibit LFA-I-ligand interactions.

In a multicentre trial, we showed that a 10-day course of antibody at 0.2 mg/kg/day reduced the graft failure rate from 74% to 28% following transplantation of T-cell-depleted marrow in patients with immune deficiencies and osteopetrosis [64]. No late

rejection occurred. Acute GvHD was observed in 35.5% of cases and chronic GvHD in 12.9%. The survival rate with a functioning graft was 47% in 42 patients (median follow-up 50 months), rather than 20% in similar patients who did not receive the anti-LFA-I antibody. An update of these data shows a 74% engraftment rate with 45% long-term survival in 38 immunodeficient patients who were treated with anti-LFA-I antibody, while engraftment occurred in only 37.5% and survival in 20.8% of 24 patients treated similarly, but without anti LFA-I-antibody [45]. Recipients of closely matched-related (phenotypically identical or one HLA antigen mismatched) marrow were not found to have a better outcome than recipients of two or three HLA antigen mismatched marrow in a recent survey [45]. These surprising results may be related to the small number of patients (n=22) in this group.

Delay in the recovery of T- and B-cell function (6–40 months) causes significant infectious complications which remain a major concern in recipients of T-cell-depleted HLA non-identical transplants. For example, 16 of 71 patients who underwent two or three HLA antigen incompatible T-depleted BMT for primary immunodeficiency (PID) (excluding SCID) had EBV-induced B-lymphocyte proliferative syndromes, and 10 of them died from this problem [45]. This complication is particularly frequent in WAS patients who undergo HLA non-identical BMT. In a recent European survey, 39% of such patients, compared with 12% of patients with T-cell deficiencies, and 3% of those with SCID, developed a lymphoproliferative disorder after HLA non-identical marrow transplantation (European BMT Registry, unpublished data). This may have been caused by poor T-cell control of EBV replication.

There are few other reports concerning HLA haploidentical BMT in PID other than SCID. Rummelhart *et al.* have described success in 3 of 4 WAS patients [71], while Brochstein *et al.* reported only 1 success in 6 for the same condition [38]. These contrasting results stress the need for careful evaluation of the indications for HLA haplo-identical BMT in these patients who lack both HLA genetically identical and HLA-matched unrelated donors. Indications depend upon clinical status as well as upon prognosis, defined by the type of immunodeficiency, age (results are poorer above two years of age) and degree of HLA incompatibility [45].

Mullen *et al.* recently stressed that splenectomized WAS patients could survive until adulthood [72]. Nevertheless, WAS patients who developed refractory thrombocytopenia and/or severe autoimmune manifestations could be considered for HLA haplo-identical BMT (Table 15.4). Similarly, patients with functional T-cell immunodeficiencies, including HLA class II deficiency, have a poor prognosis (median survival five to eight years) in the absence of BMT [46, 47].

Apart from LAD (see above), HLA haplo-identical BMT for patients with phagocytic cell disorders remains highly experimental, given the high risk of failure (Table 15.4). The simultaneous infusion of another anti-adhesion antibody (anti-CD2), as performed in 43 patients, resulted in an engraftment rate of 80% without additional toxicity, with 55% survival. By using this strategy, 5 out of 9 patients with familial haemophagocytic lymphohistiocytosis have been cured by haploidentical BMT [73]. It is, however, too early to assess the overall benefit of this strategy.

Other approaches aimed at selectively inhibiting graft rejection have been developed experimentally and could be of potential benefit. Anti-CD4 and anti-CD8 antibody infusions promote engraftment of H-2 incompatible marrow in mice [74]. Selective T-cell

depletion of marrow may prevent complications such as EBV-induced B-lymphocyte proliferative disorders [75]. Another option to enhance engraftment consists of delivering a significantly higher number of haematopoietic stem cells. Mobilization of $CD34^+$ cells by injection of G-CSF offers this possibility. Aversa *et al.* reported successes in adults with leukaemia after infusing three- to tenfold more $CD34^+$ cells compared to infusing marrow alone, and achieved a very high rate of engraftment with limited GvHD [76]. However, this method does not prevent the occurrence of long-lasting lymphocytopenia following transplantation. Relevance for immunodeficient patients, mostly young children, may also be limited by the fact that a relatively high number of $CD34^+$ cells/kg recipient weight are infused by using marrow alone from haplo-identical adults. Ideally, infusion of high numbers of $CD34^+$ cells should be combined with acceleration of development of T-cell immunity. This is not yet in hand, although selective alloreactive T-cell depletion of the graft, or addition of cytokines such as IL-7, could contribute to this ultimate goal.

Recently, an alternative source of haematopoietic stem cells for transplantation has been proposed, namely cord blood cell transplantation. Several successes with cord blood transplantation from HLA-identical siblings have been reported, notably in patients with genetic disorders including immunodeficiencies [46,77,78]. GvHD incidence appears low, although the graft failure rate could be increased [77]. It has been advocated that an unrelated cord blood bank could enlarge opportunities for the treatment of patients lacking suitable donors. Extent of acceptable incompatibility has, however, to be determined in addition to cost effectiveness, to ascertain whether this approach is worth pursuing.

Alternative therapies

Alternative treatments to marrow transplantation have been devised for some congenital immune deficiencies over the last decade. For instance, congenital agranulocytosis is efficiently treated by daily subcutaneous injections of G-CSF [61]. The prognosis of chronic granulomatous disease has been profoundly modified by the prophylactic use of antibiotics (co-trimoxazole) and possibly of γ-interferon (the International CGD Cooperative Study Group) [59].

Adenosine deaminase (ADA)-negative SCID can now be treated by a weekly intramuscular injection of bovine ADA covalently coupled to a carbohydrate: polyethylene glycol (PEG-ADA) that stabilizes ADA and diminishes its immunogenicity. Over four patients have currently been treated worldwide [79]. In most, a significant improvement occurred that allowed a normal or almost normal lifestyle. In addition, ADA deficiency appears to be an appropriate candidate for gene-transfer therapy because:

- gene expression is not regulated in a complex manner;
- gene size is compatible with incorporation into available vectors;
- target T-cell (lymphocytes or haemopoietic stem cells) are easily available.

In vitro, *ADA* gene transfer into T-lymphocytes can be achieved, and results in correction of the metabolic defect [82]. At the National Institute of Health, USA, two patients have

been treated by repeated infusions of autologous T-lymphocytes which have been transduced with the *ADA* gene using a retroviral vector. It is presently difficult to assess the efficacy of this therapy because both patients are also maintained on PEG-ADA treatment. Nevertheless, *ADA*-transduced functional lymphocytes can be detected in the blood more than two years after transfer [80].

Stem-cell gene transfer will be the ultimate goal. This has been successfully achieved in mice using retroviral vectors without harmful effects. Early clinical applications in ADA-deficient patients have not yet met with significant clinical improvement [81,83], although some transduced lymphocytes could be detected *in vivo*.

Availability of prenatal diagnosis for several primary immunodeficiencies offers the option of *in utero* treatment. Recently, Flake *et al.* reported the success of *in utero* transplantation of HLA haplo-identical paternal marrow CD34 cells to a patient with X-linked severe combined immunodeficiency [84].

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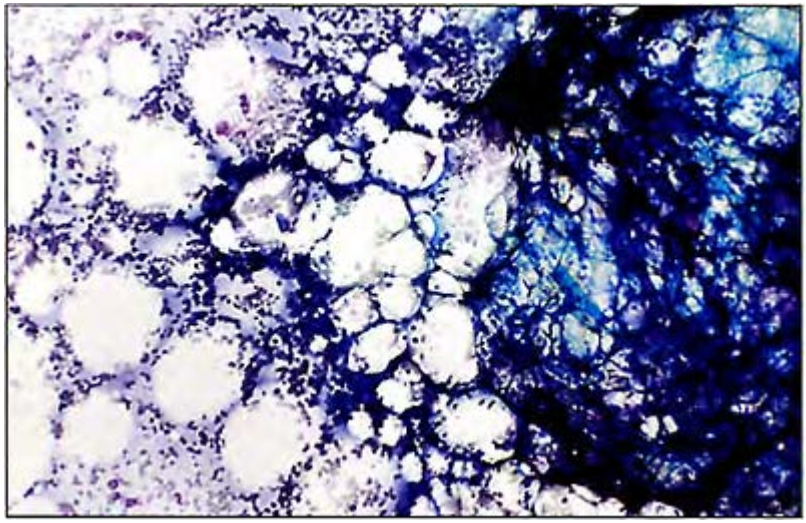

Chapter 16
Aplastic anaemia

Judith Marsh and Ted Gordon-Smith

Introduction

Acquired aplastic anaemia (AA) has been traditionally defined as the presence of peripheral blood pancytopenia associated with a hypocellular bone marrow in the absence of (a) an abnormal infiltrate, (b) an increase in reticulin or (c) morphological abnormalities in the remaining haemopoietic cells. Differentiation of cases of AA from hypoplastic myelodysplastic syndrome (MDS), however, may be difficult on morphological grounds. Special investigations such as cytogenetics on bone-marrow cells, clonogenic cell cultures and clonality studies may be helpful in some, but not all, cases. The association between AA and paroxysmal nocturnal haemoglobinuria (PNH) is more frequent and complex than previously realized. The routine availability of flow cytometric analysis of phosphatidylinositolglycan (PIG)-anchored proteins enables more sensitive detection of a small PNH clone not only among red cells, but also monocyte, neutrophil and lymphocyte populations [1–3]. The demonstration of a PNH clone in 20–50% of patients with AA who have a negative Ham test, has implications not only for the pathogenesis of AA but also possibly for prediction of outcome following

immunosuppressive therapy and prediction of graft rejection in patients who are transplanted using additional immunosuppression in the form of monoclonal antibodies such as Campath-1G which recognizes the CD52 antigen, a PIG-anchored protein [4].

The mechanism of the bone-marrow failure in AA is complex. A defect in the proliferative and differentiative properties of the haemopoietic stem cells can be demonstrated in long-term bone-marrow cultures, and in most instances the stromal cell microenvironment functions normally in terms of its ability to support the proliferation and differentiation of normal stem cells [5–7]. The role of apoptosis as a contributory factor in the stem-cell defect in AA has recently been assessed. In some patients with AA, increased numbers of activated $CD8^+$ T-lymphocyte releasing the inhibitory cytokines γ-interferon and tumour necrosis factor-α can be demonstrated. The latter cytokines upregulate the expression of Fas (CD95) on AA progenitor cells resulting in haemopoietic suppression *in vitro* [8], and in normal bone-marrow result in increased apoptosis of $CD34^+$ cells [9]. A greater percentage of AA bone-marrow $CD34^+$ cells are apoptotic compared with normal marrow $CD34^+$ cells [10]. Cross-linking of Fas by Fas ligand (expressed on activated T-cells) may be the mechanism underlying the process of cell death by apoptosis, although recent work demonstrates that Fas ligand does not appear to be upregulated in AA (F. Gibson, unpublished observations). These recent findings may have implications for the potential use of haemopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF) with immunosuppressive therapy, and post stem-cell transplant to increase haemopoietic cell proliferation as well as differentiation.

Patients with AA die as a consequence of their pancytopenia, usually from bleeding or infection. Improvements in supportive care have had a major impact on clinical outcome. Thus, in any comparative assessment of specific therapeutic modalities such as immunosuppressive therapy or bone-marrow transplantation (BMT) for AA when historical controls are used, it may be difficult to separate the relative contributions of specific therapy and supportive care to improved patient survival.

Spontaneous recovery of AA can occur, the frequency probably depending on disease severity, but may be delayed for months or even years. Recovery can be accelerated using immunosuppressive therapy with antilymphocyte globulin (ALG) and cyclosporin alone or in combination [11–14]. The additional use of G-CSF for three months after ALG and cyclosporin appears to increase not only the neutrophil count but also the platelet count and haemoglobin level in an even greater proportion of patients, although the results of prospective randomized studies are awaited [15]. When haematological recovery does occur, haemopoiesis remains abnormal in terms of defective stem-cell proliferation [6]. Relapse, which includes PNH, occurs in 30% of patients [16] and 5–15% of patients may later develop MDS or acute myeloid leukaemia (AML) [17,18]. The development of PNH more likely reflects the natural history of AA rather than being caused by the immunosuppressive therapy, and it has been suggested that an initial immune attack against normal haemopoietic cells results in the later emergence of PIG- eficient cells [19].

An alternative approach to treatment of AA is the infusion of normal haemopoietic stem cells by allogeneic BMT or peripheral blood stem-cell (PBSC) transplant. BMT for AA carries the problems common to all allogeneic transplants, namely graft rejection, graft-versus-host disease and increased risk of infections. Particular problems specific for

AA include patient selection, and difficulties of conditioning patients who may be infected and who may already be sensitized through multiple blood and platelet transfusions. The latter problem contributes to a higher risk of graft rejection compared with patients who are transplanted for leukaemia. Use of G-CSF mobilized PBSC instead of steady state bone marrow for allogeneic transplantation may be associated with faster haematological engraftment and allows the delivery of a larger number of haemopoietic progenitor cells, possibly including stem cells.

Diagnosis of aplastic anaemia

Morphology

The diagnosis of AA is made by exclusion. There are several causes of a hypoplastic bone marrow with peripheral blood pancytopenia (Table 16.1). It is essential to examine both the bone-marrow aspirate and an adequate trephine biopsy, in order to (a) establish the degree of hypocellularity, (b) that the remaining haemopoietic cells are normal morphologically and (c) that there is no excess of possibly leukaemic blast cells. Some degree of dysplasia affecting the erythroid series and red-cell macrocytosis is very common in AA, and if megakaryocytes are absent, there may be only a few granulopoietic cells remaining to evaluate for possible evidence of MDS. In the very early stages of AA, phagocytic activity may be prominent, sometimes with ingestion of red-cell precursors. An additional difficulty in some patients with AA is the persistence of patches of apparently normal haemopoietic activity within the bone marrow. However, their presence requires cautious interpretation because it is impossible to tell whether such areas or ‘hot pockets’ represent residual surviving haemopoietic cells or evidence of regeneration, unless serial marrow examinations are performed from exactly the same site. Nevertheless, their presence does not alter the prognosis of AA when the degree of peripheral blood pancytopenia is taken into account.

Table 16.1 Causes of a hypocellular bone marrow with pancytopenia

Idiosyncratic aplastic anaemia
Cytotoxic drugs and irradiation
Hypocellular myelodysplasia or hypocellular acute myeloid leukaemia in adults
Hypocellular acute lymphoblastic leukaemia in children
Hairy-cell leukaemia
Other bone-marrow infiltrations, e.g. lymphoma, carcinoma, myelofibrosis
Severe infections, e.g. tuberculosis, overwhelming Gram-negative or Gram-positive sepsis
Anorexia nervosa

Cytogenetics

The major difficulty is excluding hypocellular MDS or hypocellular acute leukaemia, and repeated examination of the peripheral blood and bone marrow over a six-week to three-month period may be necessary. Marrow cytogenetic analysis to look for clones with chromosome abnormalities in haemopoietic metaphases is often hampered by the difficulty in obtaining sufficient metaphases from a hypocellular marrow. The finding of a clonal abnormality is suggestive of a premalignant condition, but it is now apparent that a proportion of patients with otherwise typical AA have a clonal cytogenetic abnormality [20], often in a small proportion of metaphases, which can remain stable for long periods of time without overt transformation to AML, or which may spontaneously disappear [21; J.Marsh and E Gordon-Smith, unpublished observations]. For these reasons we would suggest that the treatment of a patient with AA without morphological evidence of MDS/AML but with a clonal cytogenetic abnormality, should be the same as for a patient with AA and normal cytogenetics, providing that the cytogenetic finding is not one frequently associated with MDS or AML, such as monosomy 5 or 7, for example.

Preliminary data from two centres show that AA patients with a clonal cytogenetic abnormality appear to respond well to immunosuppressive therapy with antithymocyte globulin (ATG) or cyclosporin, and that immunosuppression does not appear to stimulate growth of the abnormal clone [22]. Furthermore, the finding of an abnormal karyotype alone in a patient who does not fulfil the criteria for severe AA, and who does not have good evidence of MDS or AML, would not be an automatic indication for BMT. Careful monitoring of the size of the abnormal clone is essential to detect those who may later evolve to MDS or AML. In such an event, however, the presence of an abnormal clone would indicate that for BMT the conditioning regimen should be myeloablative, incorporating total body irradiation (TBI).

Marrow-cell culture studies

Short-term clonogenic marrow cultures in patients with AA reveal either reduced or absent numbers of colonies, but the correlation of colony numbers with disease severity is poor. The finding of abnormal clusters would indicate a leukaemic or preleukaemic process, but in MDS colonies may also sometimes be absent, reduced or normal in number. Long-term bone-marrow cultures consistently demonstrate a severe defect in haemopoietic stem-cell proliferation and differentiation, regardless of whether the patient is untreated or treated with good haematological response [6], Patients with MDS may either show a similar pattern to patients with AA or a near normal pattern of progenitor cell generation [23]. Thus, marrow-cell cultures have proven somewhat disappointing in their ability to discriminate all cases of AA from preleukaemia.

X-chromosome inactivation studies

It is possible to determine whether haemopoiesis is polyclonal or monoclonal using X-linked DNA probes, such as hypoxanthine phosphoribosyltransferase (HPRT), phosphoglycerate kinase (PGK) and M27β (by Southern blotting, or by the polymerase chain reaction using PGK or the human androgen receptor gene (HUMARA). Earlier

reports suggested that a large proportion of patients with AA had evidence of monoclonal haemopoiesis [24], but more recent studies using strict controls and excluding cases with extreme lyonization have demonstrated that in the majority of AA patients haemopoiesis is polyclonal. However, if cell populations are separated into PIG-deficient cells and PIG normal cells, then it can be shown that the PIG-deficient cells are monoclonal and the PIG normal cells are polyclonal [25]. X-chromosome inactivation studies have contributed to the understanding of the stem-cell abnormality in AA, but for differentiating cases of AA from preleukaemia these studies have not proven useful and are difficult to interpret in isolation. They should be evaluated alongside results of morphology and cytogenetics, since monoclonal haemopoiesis does not necessarily indicate malignancy, and in AA it may reflect stem-cell depletion or preferential selection of a particular cell population, for example PIG-deficient cells (see Introduction).

Demonstration of a PNH clone

A positive Ham test and the presence in the urine of haemosiderin are required for the diagnosis of haemolytic PNH. Approximately 5–10% of patients with AA later develop haemolytic PNH with evidence of a cellular bone marrow and overt haemolysis, and AA may develop in up to 20% of patients with haemolytic PNH. The features of PNH are due to a deficiency of PIG-anchored proteins on the surface of not only red cells but also on neutrophils, monocytes, platelets and sometimes also lymphocytes, resulting from somatic mutations in the *PIG-A* gene which is located on the X chromosome (Xp22.1) [26–27]. The availability of flow cytometry to detect small populations of cells deficient in the expression of PIG-anchored has revealed that between 20 and 50% of patients with AA who have a negative Ham test and no clinical or laboratory evidence of haemolysis show evidence of PIG-anchored protein deficiency in a small proportion of cells, particularly neutrophils and monocytes [1–3]. For patients undergoing BMT, in whom there is a significant PNH clone especially in the presence of haemolysis or a history of previous thrombotic episodes, a myeloablative conditioning regimen using TBI may be more appropriate in order to eliminate the PNH clone. Although there have been rare documented cases of PNH transplanted from an identical twin donor, where simple marrow infusion without conditioning of the recipient has led to suppression of the PNH clone, in almost all cases this was only transient [28–30].

Because the Campath antigen (CD52) is a PIG-anchored protein [4], if Campath monoclonal antibodies are to be used in the conditioning regimen for patients with AA in order to reduce the risk of graft rejection, it is important to determine prior to transplantation whether the donor lymphocytes express CD52 [31]. If they do not, then it is anticipated that Campath monoclonal antibodies would not be effective in this setting, and then alternative immunosuppressive agents such as ALG should be used.

Aetiology of aplastic anaemia

The AA consequent on cytotoxic drugs or radiation is predictable and usually self-limiting, but prolonged and apparently idiosyncratic AA may follow repeated exposure to low doses of cytotoxic drugs, such as busulphan, azathioprine and chlorambucil. Such cases probably represent true idiosyncratic acquired AA and such patients may be

selected for immunosuppressive therapy or transplantation using the same criteria as for other causes of acquired aplasia. The aetiology of AA (Table 16.2) does not affect the outcome of BMT or immunosuppressive therapy, except that graft rejection post-BMT is lower in patients with post-hepatitic AA compared with patients with apparent idiopathic disease [32]. This may reflect earlier transplantation in these patients with lower

Table 16.2 Aetiology of aplastic anaemia

Congenital
Fanconi anaemia
Dyskeratosis congenita
Schwachman-Diamond syndrome
Other rarer types
Acquired
Idiopathic (75% cases)
Drugs: NSAIDs, gold, chloramphenicol, sulphonamides, ?ecstasy (3,4-methylenedioxymethamphetomine)
Chemicals: benzene, paints, organochlorine pesticides
Viruses: hepatitis A or B, non-A, non-B, non-C hepatitis, EBV
PNH
Rarely: SLE, pregnancy

sensitization as the interval between diagnosis and BMT was significantly less in this subgroup of patients.

Although patients with post-hepatitc AA usually have more severe AA, they have the same prognosis as patients with idiopathic AA when allowance is made for the severity of the aplasia. On the other hand, patients with bone-marrow failure associated with other disorders such as MDS, acute leukaemia or inherited AA such as Fanconi anaemia, have a different prognosis from acquired AA, and establishing the correct diagnosis is an important part of the preparation of patients for BMT.

Diagnosis of inherited aplastic anaemia

It is essential not to miss a diagnosis of inherited AA, the most common type of which is Fanconi anaemia. The syndrome is associated with somatic abnormalities, but up to 30% of patients do not have any of these features [34]. The diagnosis of Fanconi anaemia is made by cytogenetic analysis carried out on peripheral blood lymphocytes which are cultured in the presence and absence of a clastogenic agent such as diepoxybutane (DEB) or mitomycin C, to establish the increased incidence of spontaneous and induced chromatid breakages, specific for Fanconi anaemia [35]. Because some patients with Fanconi anaemia, who usually have no or very subtle somatic anomalies, can present in their early twenties or thirties, it is advisable for all new patients presenting with

apparently acquired AA under the age of 30 years to be routinely screened for Fanconi anaemia. The importance of making the diagnosis of an inherited AA is that (a) the transplant conditioning regimen needs to be modified, (b) the proposed donor must also be screened for inherited AA and (c) genetic counselling can be instigated. For a detailed review of Fanconi anaemia and its treatment, see Chapter 17.

Skeletal abnormalites in Fanconi anaemia are usually obvious clinically but in some cases they may be subtle and detectable only on radiology of the hands and forearms. A condition associated with late-onset marrow failure and proximal radio-ulnar fusion has also been described, the importance of which lies in the need to test potential family donors for the same condition, since bone marrow from such donors may not repopulate the host's haemopoietic system normally [36].

Assessment of patients with aplastic anaemia prior to BMT

Most of the investigations described above are carried out on all patients with AA prior to transplantation in order not only to confirm the diagnosis and determine the severity of the disease, but also to help decide whether to perform a transplant and what conditioning regimen should be employed. Additional tests necessary prior to planned BMT are discussed below.

Reassessment of bone-marrow morphology, cytogenetics and PNH status, and ensuring that an inherited form of aplasia has been excluded

It is important to reassess the bone-marrow morphology, cytogenetics and PNH status of the patient prior to BMT in order not to miss a change in the nature of the disease from time of diagnosis, since evolution to a premalignant or malignant condition would influence the choice of transplant conditioning regimen, as discussed above. Likewise, because the conditioning regimen for patients with Fanconi anaemia needs to be modified, compared to the regimen for patients with acquired AA, in order to avoid serious toxicity, and also because the potential donor for a patient with inherited AA must be screened for the same condition, it is essential to ensure that a possible diagnosis of inherited AA even in young adults has been definitely excluded pretransplant.

Human leukocyte antigen (HLA) antibody screening

Repeated exposure of patients with AA to blood and platelet transfusions increases the risk of HLA alloimmunization resulting in resistance to random donor platelets, and also contributes to the risk of graft rejection post BMT. Early observations from Seattle showed that AA patients who had received few transfusions had a lower risk of graft rejection compared with multiply transfused patients. Hence the recommendation for early rather than late BMT in eligible patients [37].

Although there have been very few reported studies on the risk of alloimmunization in AA, the risk appears to vary between 34 and 100% [38,39], but the more recent routine use of leukocyte-depleted blood products from diagnosis has resulted in a lower incidence of alloimmunization [40]. Screening for HLA antibodies, preferably by not only lymphocytotoxicity but also enzyme-linked immunosorbent assay (ELISA) prior to BMT, will indicate the potential need for HLA-matched platelets in those patients already alloimmunized. It is fairly common for patients with HLA antibodies to show later disappearance of the antibodies despite continued need for transfusional support especially if leukocyte-depleted blood products are used, and so regular monitoring for HLA antibodies even in patients with pre-existing antibodies is useful, especially as disappearance of HLA antibodies has been reported to be associated with loss of resistance to random donor platelets.

Virological studies

The cytomegalovirus (CMV) status of the patient and any potential family donors should be determined as soon as possible after diagnosis. The patient should receive only CMV-negative blood products until their CMV status is known, and be maintained on CMV-negative products if he and any potential donor is also CMV-negative. The routine use of leukocyte-depleted blood products will also provide good, but may not provide absolute protection against CMV infection. Our current practice is to use CMV-negative, leukocyte-depleted blood products if both the patient and donor are CMV-negative, but to use CMV-random, leukocyte-depleted products for all other cases.

All patients must also be screened for hepatitis A, B and C and human immunodeficiency virus (HIV), as with other BMT candidates. Additionally, evidence should be sought for recent infection with Epstein-Barr virus (EBV) and parvovirus, since the former and possibly the latter may be associated with transient pancytopenia and marrow hypoplasia. In the presence of recent infection, BMT should be delayed for up to three months to allow time for spontaneous recovery.

Post-hepatitic AA is usually severe and may require urgent transplantation if there is a HLA-compatible sibling donor. The results of immunosuppression in post-hepatitic AA appear to be the same as for idiopathic AA if the severity is equal [32,33]. The hepatitis usually precedes the aplasia by four to six weeks, but persistently abnormal liver-function tests complicate the management of the aplasia [41]. It is advisable to postpone BMT while the liver-function tests continue to improve, but if some abnormality remains following initial improvement, then BMT may still be considered. Most cases of post-hepatitic AA are non-A, non-B, non-C hepatitis.

Supportive care

The principal aim of management of a patient presenting acutely with severe AA is to keep the patient alive and to stabilize his clinical condition. At the same time it is important to consider aspects of supportive care that are unique to patients with AA and which will have an impact on subsequent proposed treatment for their aplasia, be it BMT or ALG therapy. In the acute early presentation of the illness, bleeding is usually a more

common problem than infection, and death from cerebral haemorrhage still occurs. Several aspects of blood-product support should be considered as soon a diagnosis of AA is suspected.

Blood-product support

Transfuse to maintain a safe blood count

During the late 1970s and early 1980s, results of transplantation for severe AA indicated that superior results were obtained for relatively untransfused patients (less than ten transfusions) compared with multiply transfused patients (more than ten transfusions) [37]. Following this observation, a policy of not transfusing newly diagnosed patients unless absolutely necessary was advocated so that they would not become sensitized prior to planned BMT. As a consequence, vital platelet and red-cell transfusions were sometimes withheld in patients with severe anaemia and thrombocytopenia, and fatal or near fatal haemorrhages occurred before patients ever got to transplant. In practical terms, the finding of retinal haemorrhage, for example, is not just an indicator of high risk of cerebral haemorrhage but may be considered a manifestation of central nervous system bleeding. Avoiding unnecessary transfusions is clearly sensible, but unfortunately the practice of withholding clearly indicated transfusion in this situation has still not completely died out.

Debate remains as to whether patients should receive regular prophylactic platelet transfusions or only when bleeding episodes occur. We tend to favour prophylactic transfusions with the aim of maintaining the platelet count above 15×10^9/litre to prevent cerebral haemorrhage. In the presence of infection, we aim for a higher platelet count of $20–30\times10^9$/litre. The rationale for prophylactic platelets is that the great majority of patients with platelet counts below 10×10^9/litre develop significant bleeding or purpura. They need platelet transfusions and it is better to give them at a preplanned time than as an emergency.

When transfusing red-cell concentrates to AA patients who are significantly thrombocytopenic, platelets should also be given before red cells, since theoretically, subclinical red-cell reactions may activate the complement system and lead to platelet consumption and bleeding. Red-cell concentrates as well as platelets should be leukocyte-depleted to help reduce the risk of alloimmunization.

Prevention of alloimmunization

The majority of patients with severe AA (SAA) require regular transfusion support and are therefore at risk of becoming sensitized to HLA and non-HLA (minor histocompatibility) antigens. HLA alloimmunization is the commonest cause of immune platelet refractoriness resulting in poor increments in the peripheral blood-platelet count following transfusion with random donor platelets. This usually necessitates a requirement for HLA-matched platelets. Sensitization to minor histocompatibility antigens results in a high risk of graft rejection following allogeneic BMT [42].

A recent prospective randomized study in patients with haematological malignancy showed that the use of in-line bedside filters did not significantly reduce the risk of HLA alloimmunization in patients requiring intensive red-cell and platelet transfusion support [43]. In contrast, the use of pre-storage leukocyte depleted red cells and platelets with $<5\times10^6$ residual leukocytes significantly reduced the incidence of HLA antibodies in thrombocytopenic patients, the majority of whom also had haematological malignancies [44]. We are aware of only two reported studies of alloimmunization risk in patients with AA receiving unfiltered blood products, of 34% and 100% respectively [38,39]. This prompted us to perform a study assessing the use of pre-storage leukocyte-depleted blood products (PLDBP) in 24 patients with AA in whom all but one had previously received multiple unfiltered or bedside-filtered blood products [40]. Out of 16 with no detectable HLA antibodies at study entry, only 2 (12%) formed HLA antibodies, compared with 50% among a control group. In 3 out of 8 patients with HLA antibodies at study entry, HLA antibodies could no longer be detected after transfusion with leukocyte-depleted HLA-matched platelets, allowing a change to random donor platelets with loss of subsequent platelet refractoriness. Thus, PLDPB appear to reduce the risk of alloimmunization in this high-risk group of patients, and we suggest that all newly presenting patients should receive exclusively PLDPB from the outset. Even if HLA antibodies develop, patients should continue to receive PLDBP and be regularly screened for HLA antibodies, since a proportion may later lose their antibodies and respond well to random donor platelets [40].

Irradiation of blood products

As for all transplant recipients, all platelet and red-cell concentrates must be irradiated in order to prevent transfusion-associated graft-versus-host disease (GvHD). Irradiation of blood products should begin when the pretransplant conditioning regimen commences. There have been no reported cases of transfusion-associated GvHD following treatment with ALG so routine irradiation of blood products in this situation or prior to plannned BMT is not currently indicated.

CMV-screened blood products

At initial presentation, for patients <45 years of age who may be eligible for BMT at some time, only CMV-negative blood products should be given until the CMV status of the patient is known. If the patient and their HLA-identical sibling are both CMV-negative, then the patient should continue to receive only CMV-negative blood products. Leukocyte-depleted blood products may provide protection against CMV infection but as this may not guarantee complete protection, we currently prefer to use only CMV-negative blood products for a CMV-negative patient with a CMV-negative donor. If either the patient or potential donor are CMV positive, we give CMV-random, leukocyte-depleted blood products.

Prevention and treatment of infection

Because of the prolonged period of neutropenia that many patients with severe AA may have while waiting for BMT or following a course of ATG, the issues of prophylaxis against bacterial and particularly fungal infection, and specific treatment of prolonged febrile episodes are of paramount importance for the survival of the patient.

Infection prophylaxis

For patients with a neutrophil count $<0.2\times10^9$/litre, a case could be made for continuous prophylaxis with non-absorbable antibiotics such as neomycin and colistin. Concern has been raised about emerging resistance of Gram-negative and Gram-positive organisms with the use of prophylactic ciprofloxacin instead of non-absorbable antibiotics. Very severely neutropenic patients should also regularly use an antiseptic mouthwash and take antifungal prophylaxis with either fluconazole or itraconazole. The latter may help to prevent colonization with *Aspergillus*, which is usually impossible to eradicate in very severely neutropenic patients even with therapeutic intravenous amphotericin, unless the patient regenerates neutrophils.

Treatment of febrile episodes

A policy for treatment of febrile neutropenia in AA patients would be similar to that for patients with haematological malignancy, except that intravenous amphotericin should be introduced earlier, rather than waiting to progress through third-line antibiotics, and should even be considered with the first episode of fever if fungal infection is suspected clinically.

Granulocyte transfusions

The use of irradiated buffy coats as a source of neutrophils to treat infective episodes that are not responding to intravenous antibiotics and antifungals has been largely superseded by the use of haemopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Granulocyte transfusions may still be useful to treat severe localized or deep-tissue infections that have failed to respond to the addition of G-CSF or GM-CSF. This practice, however, is associated with a high chance of alloimmunization and transmission of CMV. Bensinger and colleagues at Seattle have evaluated the use of irradiated G-CSF mobilized neutrophils from allogeneic donors, which resulted in significantly higher levels of peripheral blood neutrophil counts in neutropenic recipients, compared with using standard granulocyte transfusions from historical donors [45]. However, family donors should not be used in this situation if the patient is eligible for sibling BMT as the recipient may become sensitized to the non-HLA antigens on the haemopoietic cells of the potential donor.

Haemopoietic growth factors

Haemopoietic growth factors such as G-CSF or GM-CSF may be useful in the management of patients with AA in the following situations:

- to treat systemic infection that is not responding to intravenous antibiotics and antifungals;
- for patients with graft rejection following transplantation;
- following treatment with ALG and cyclosporin as part of an ongoing multicentre European trial [15];
- to mobilize PBSC for allogeneic PBSC transplant.

Although there have been no prospective randomized studies examining the efficacy of G-CSF or GM-CSF in the treatment of febrile neutropenia in AA patients, it would be reasonable to consider a short course of such a growth factor for one to two weeks in a patient with severe systemic infection which is not responding to conventional treatment. Since the best effect of G-CSF in AA in terms of increasing the neutrophil count is seen in those patients with some residual marrow granulopoietic activity, continuation of G-CSF for longer than one to two weeks in patients showing no rise in the neutrophil count would confer little benefit. G-CSF is very well tolerated clinically. In contrast, GM-CSF may precipitate haemorrhage in AA patients who are thrombocytopenic [46]. Studies in dogs have demonstrated that GM-CSF induces destruction of platelets in the liver and spleen possibly mediated by monocyte-macrophage activation, resulting in thrombocytopenia in all treated animals [47]. If fungal infection is suspected or proven, and G-CSF has produced no increase in the neutrophil count, it is reasonable to change to GM-CSF in an attempt to stimulate both neutrophil and monocyte counts, but a lower starting dose should be considered in order to avoid the risk of haemorrhage in these patients.

Patient selection for BMT

When planning specific treatment for a newly presenting patient with AA, once their clinical condition has been stabilized with appropriate supportive care, the major decision is whether to treat with BMT or immunosuppressive therapy (see Table 16.3 for summary of results of treatment). Successful BMT without the long-term complication of chronic GvHD offers the only chance of cure of the disease, and approximately 75–90% of patients survive long term [48–51]. If irradiation is avoided in the conditioning regimen, growth and fertility are not affected. On the other hand, a failed BMT usually results in death of the patient, although autologous haematological recovery may still occur providing irradiation has not been used. Furthermore, a second transplant is a possible option if the patient is clinically stable and free from infection. Death may also occur from acute GvHD or pneumonitis (although the latter

Table 16.3 Results of treatment for severe aplastic anaemia

HLA-identical sibling BMT (%)		Immunosuppressive therapy (%)	
Survival	75–90	Survival	70–90
Graft rejection	5–15	Relapse	30
Acute GvHD	15–40	MDS/AML	5–10
Chronic GvHD	10–50	Ham test—positive	10
		PIG-protein deficiency with Ham test—negative	15–50

is seen less frequently in HLA-identical sibling transplants using a non-irradiation-based regimen compared with patients transplanted for leukaemia).

In contrast, immunosuppressive therapy is associated with much lower procedure-related mortality with survival rates of 70–90% [11–15], but this must be balanced against a lack of cure with frequent partial responses, relapse of aplasia and emergence of premalignant or malignant clones. Response to immunosuppressive therapy is slow and usually takes two to three months or longer. During this period, the patient may die not directly from treatment-related causes, but from the effects of the disease itself. Fortunately supportive care continues to improve and such events are less frequent. For severe AA, the combination of ALG and cyclosporin is frequently employed [14]. Results from a European pilot study indicate that the additional use of G-CSF for three months with ALG and cyclosporin appears to increase the response rate further compared with using ALG and cyclosporin alone [15]. However, recent reports particularly from Japan have highlighted a seemingly frequent occurrence of MDS/AML in association with monosomy 7 in patients treated with immunosuppression (in most cases with cyclosporin but without ALG) and long-term G-CSF [52,53], although this has not been seen in patients treated with ALG/cyclosporin/G-CSF in Europe [15]. Clearly, further long-term follow-up is important, and a currently ongoing European multicentre, prospective, randomized study should confirm whether G-CSF significantly improves response with immuno-suppressive therapy in AA or not.

One possible solution to the dilemma of whether to transplant or treat with immunosuppression might be to treat the patient conservatively with immunosuppression in the first instance and only to use BMT for patients who do not respond. However, historically this is complicated by the effect of multiple transfusion on the outcome of BMT, with the best results for patients who are only minimally transfused and proceed to BMT early. The problem of sensitization in multiply transfused patients has been reduced to some extent with (a) the routine use of leukocyte-depleted blood products in newly presenting patients promising to reduce the risk of alloimmunization and (b) the use of additional immunosuppression incorporated into the conditioning regimen in order to reduce graft rejection, along with the routine use of cyclosporin.

Indications for BMT in aplastic anaemia

HLA-identical sibling BMT

Indications for HLA-identical sibling BMT in AA are dependent on the following factors: it should be offered as first line-treatment if the patient (a) has severe AA, (b) is <45 years old and (c) has an HLA-identical sibling donor. These factors require further discussion, however.

Definition of severe aplastic anaemia

Traditionally, SAA has been defined by criteria drawn up by the International Aplastic Anaemia Study Group in 1976 [54] (Table 16.4). These criteria are still widely used and have prognostic value, although the neutrophil count is the most important parameter in terms of predicting outcome after treatment with immunosuppression and BMT (Figure 16.1). A more recent subclassification of SAA defined such patients as those who fulfill the above criteria for severe AA but in addition have a neutrophil count $<0.2\times10^9$/litre [54]; often, in practical terms, the neutrophil count alone is used to define those patients with severe and very severe AA.

Patients with very severe AA respond less well to immunosuppressive therapy with ALG and cyclosporin, but response rates were not significantly different from response rates for patients with neutrophil count $0.2–0.5\times10^9$/litre when patients were treated with ALG, cyclosporin and G-CSF [15].

Some patients with a neutrophil count $>0.5\times 10^{9/}$litre may not fulfil the criteria for SAA, but may have problems with platelet transfusion support with early development of HLA antibodies and major bleeding episodes, for example. Since in such patients the AA is phenotypically severe, it would be very reasonable to consider early BMT if there is an HLA-identical sibling donor.

Table 16.4 Criteria far severe aplastic anaemia

Bone-marrow cellularity
<25% of normal, or 25–50% normal and <30% residual haemopoietic cells
Peripheral blood counts
neutrophils $<0.5\times10^9$/litre
platelets $<20\times10^9$/litre
reticulocytes <1% (corrected for haematocrit)
For severe AA, bone marrow and two of three blood criteria. For very severe AA, criteria as for severe AA except neutrophils $<0.2\times10^9$/litre

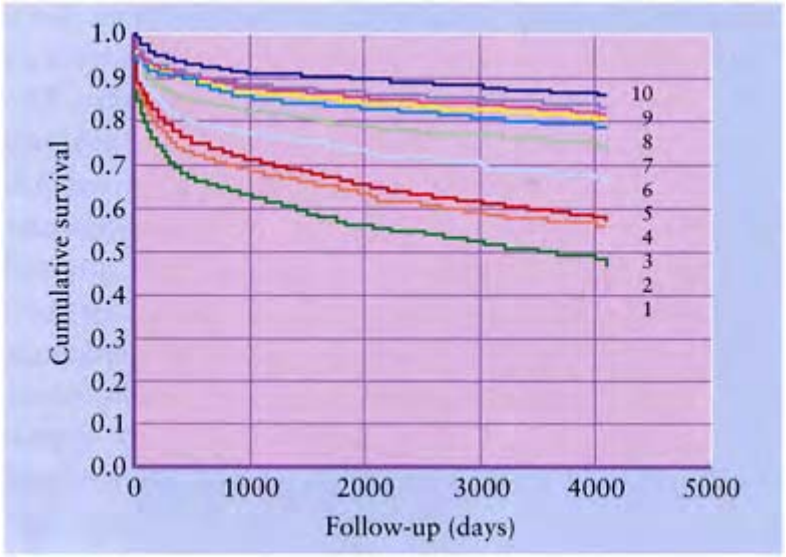

Figure 16.1 Survival of patients with aplastic anaemia following treatment with antilymphocyte globulin according to percentiles of neutrophil count (with kind permission of A.Bacigalupo on behalf of the EBMT Severe Aplastic Anaemia Working Party).

Age

There has been much debate concerning the upper age limit for HLA-identical sibling BMT for patients with AA. Many centres would take an upper limit of around 45 years although if the patient is fit and clinically well it may be possible to extend this to nearer 50 years in patients who have failed to respond to one or two courses of ALG. This needs to be balanced, however, against an increased risk of acute GvHD with increasing age, so additional anti-GvHD prophylaxis should be considered. In the mid-1980s, the EBMT reported that survival after ALG was identical to that following HLA-identical sibling BMT in patients over the age of 20 years [54], but this was reported before the long-term complications of clonal disorders occurring in patients followed up for ten years or more after ALG treatment were appreciated. Because of the time taken to develop MDS, AML or PNH may be longer than the natural lifespan of a patient who is 50 years or older, there is probably little point in advocating transplantation much above this age.

Results of BMT in children are generally better than in adults. Conversely, children appear to respond less well to immunosuppressive therapy especially those under six

years of age, but the reason for this is not known [56]. However, data from the recent European pilot study using ALG, cyclosporin and G-CSF indicate that children under 16 years old fare as well as adults over 16 years of age [15].

HLA phenotypically identical non-sibling BMT

In approximately 6% of patients it is possible to find a phenotypically matched parent, or extended family testing may reveal a phenotypically matched family donor either by chance or due to consanguinous marriage, emphasizing the importance of routinely typing the whole family including all siblings and the parents. Reports of small numbers of such transplants from the EBMT and Seattle using standard high-dose cyclophosphamide alone as conditioning, suggest that results may be comparable to HLA genotypically identical transplants. Beatty and colleagues reported that all of 6 patients survived following BMT from a phenotypically matched parent [57] and 6 of 9 similar transplants were performed successfully in Europe [58].

Alternative donor BMT

The use of mismatched family donors for transplantation in AA, whether one or more HLA antigen mismatched, can no longer be recommended because of the universally poor survival rates of <30% at ten years.

The use of volunteer unrelated donors for marrow transplantation continues to be an unsuccessful approach to the treatment of SAA in most centres. It had been hoped that the poor results seen during the early and mid-1980s could be improved with more rapid and more accurate HLA typing, better patient selection, and more intensive conditioning regimens to reduce graft rejection. However, the donor search process using DNA-based techniques and cellular assays such as cytotoxic T-lymphocyte precursor (CTLP) frequencies can still take several months or longer, during which time the patient is likely to have received multiple transfusions thus increasing the risk of alloimmunization. Furthermore, transplants for AA using volunteer unrelated donors are still associated with a higher rate of graft rejection, acute GvHD and infections than transplants from HLA-identical siblings.

Reasonably successful results of unrelated donor BMT for severe AA have been reported from Wisconsin [59]. Margolis and colleagues have transplanted 28 children and young adults up to the age of 24 years from unrelated donors in 13 of whom there was a one-antigen mismatch and in 6 at least a two-antigen mismatch. An intensive conditioning regimen of cyclophosphamide, cytosine arabinoside and TBI was combined with additional GvHD prophylaxis comprising T-cell depletion of donor marrow using an anti-CD3 antibody, and cyclosporin. Eight patients also received ALG to help reduce the likelihood of graft rejection. Graft rejection occurred in 3 (11%), all of whom had at least a one-antigen mismatch. Acute GvHD grade II or more occurred in 28% and extensive chronic GvHD in only 2 patients. Although the incidence of graft rejection and GvHD were succesfully reduced, and 54% of patients survive with a median follow-up of 2.7 years, B-lymphoproliferative disease was observed in 4 (14%) patients. Additional potential long-term complications such as second malignancies following such

transplants where a very intensive conditioning regimen has been used require evaluation after further follow-up.

Successful engraftment in a small series of children transplanted from unrelated donors has been reported from Nagoya using cyclophosphamide, ALG and TBI [60], whereas the combination of cyclosporin and ALG alone appears to be insufficient for successful engraftment [61]. However, using a non-irradiation-based regimen, as for sibling transplants, means that there remains the potential for autologous haematological recovery, as seen in 3 patients transplanted at our centre with cyclophosphamide and Campath-1G *in vivo.* Two patients failed to engraft and the third lost his graft at six weeks, but all are now transfusion-independent with rising blood counts and Karnovsky scores of 100% (J.Marsh, unpublished observation).

Apart from the above reports, most centres have found that transplantation using unrelated donors is in most cases unsuccessful, particularly in adults. The EBMT have recently performed a retrospective analysis of 142 alternative donor transplants for acquired AA and Fanconi anaemia, 88 of whom were transplanted between 1989 and 1994 [62]. The ten-year survival rate for all patients was only 20% and there has been no improvement in survival for those transplanted after 1989. There was no difference in survival between (a) unrelated and mismatch family donor transplants, (b) patients with Fanconi anaemia and acquired AA and (c) patients aged under 15 and over 15 years old.

In conclusion, transplantation using volunteer unrelated donors still cannot be recommended as first-line treatment for patients with SAA who lack an HLA-identical sibling donor. The encouraging improvement in response rate with immunosuppressive therapy and G-CSF for patients with severe and very severe AA argues further against a standard practice of alternative donor transplantation in these patients. If a patient fails to respond to an initial course of ALG, it would be reasonable to consider a second course of ALG since approximately 50% will respond, before contemplating unrelated donor BMT. Furthermore, knowledge that a slow late response after ALG (up to six months or longer) occurs occasionally, may encourage a more conservative approach of careful observation with good supportive care rather than subjecting a patient who is clinically well to a procedure which currently still carries a very high risk of mortality.

Syngeneic twin BMT

The occurrence of AA in one of a pair of monozygotic twins is a unique event because it provides an opportunity to investigate the pathogenesis of the disease. Because of genetic identity between the donor and recipient, if AA is due to an intrinsic stem-cell abnormality, immuno-suppression should not be necessary to prevent graft rejection. However, this has proven not to be the case in a large proportion of reported syngeneic transplants [28,63], and indicates that graft rejection due to alloimmunization to minor histocompatibility antigens is not the only possible mechanism involved.

Other mechanisms include:

- rejection mediated through recipient lymphocyte recognition of viral and other neo-antigens;
- re-emergence of the abnormal population of T-lymphocytes responsible for the original disease [64];

• inhibition of donor stem cells by recipient natural killer cells [65];
• a stromal-cell microenvironmental defect in the recipient [66].

In 1989, Champlin and colleagues on behalf of the IBMTR reported results of 19 syngeneic twin transplants for AA [62]. Six were transplanted with cyclophosphamide with or without irradiation and all engrafted and survived. Thirteen recipients were transplanted with bone marrow alone and no immunosuppression and 9 of these had either no or transient engraftment and went on to receive a second BMT. The second BMT was performed with immunosuppression (cyclophosphamide alone in 6 cases, cyclophosphamide and irradiation in 2 and nitrogen mustard in 1). Out of the 13 patients who recieved one or more transplants, 12 were alive at the time of the report. A recent update of the IBMTR data by Hinterberger and colleagues with follow-up on 40 patients confirmed that the likelihood of haematological recovery is greater in patients whose first transplant is with cyclophosphamide conditioning (Figure 16.2) [67].

Thus, only about 30% of recipients transplanted without immunosuppression will achieve sustained engraftment. It may therefore be reasonable to suggest that AA patients with an identical twin and requiring BMT should perhaps receive cyclophosphamide conditioning with the initial marrow infusion.

Graft rejection

Types and incidence

Early graft rejection (‘no take’) results in either no evidence of engraftment following the transplant with persistent severe pancytopenia, or early engraftment with peripheral blood-donor neutrophils and monocytes evident but followed by pancytopenia when recipient T-lymphocytes with prominent cytoplasmic granules suddenly appear in the blood. Late graft failure occurs after a period of established engraftment and may be associated with CMV infection or reactivation, acute GvHD, drugs such as ganciclovir or Septrin, and later, at six to nine months, at the time of cyclosporin withdrawal. The latter situation is sometimes associated with re-emergence of host cells, and may respond to reintroduction of cyclosporin therapy. It is important to know if the graft failure is due to failure to sustain donor haemopoiesis or recurrence of AA with re-establishment of recipient haemopoiesis, because this will influence the need or not for further immunosuppression prior to a possible second transplant.

Graft-rejection rates following sibling BMT for AA vary from 5 to 15% [32,68], although data from the EBMT Registry indicate that there has been a reduction in graft rejection with time. Several factors influence the rejection rate (Table 16.5).

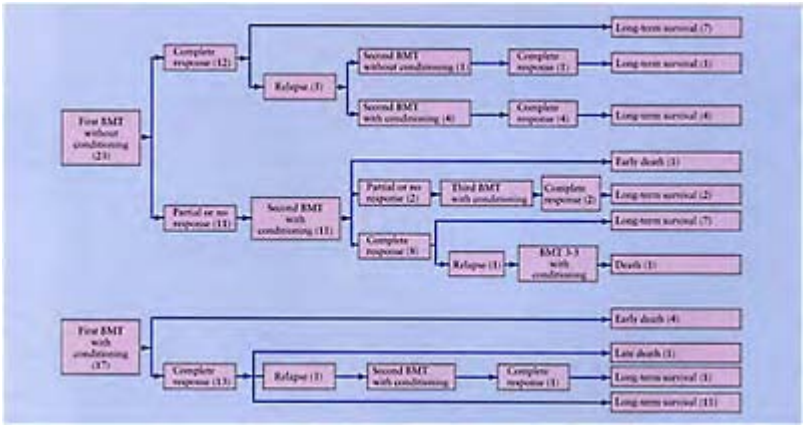

Figure 16.2 Outcome of syngeneic twin transplants in patients with aplastic anaemia ([67] and with kind permission of M. Horowitz on behalf of the IBMTR). The data presented here were obtained from the Statistical Center of the International Bone Marrow Transplant Registry.

Factors affecting graft rejection

Nature of the disease

Compared to patients with haematological malignancy who have received multiple courses of chemotherapy, patients with AA exhibit intact cellular immunity which allows generation of a large rejection potential. This may be compounded by the underlying immune pathophysiology of the disease.

Table 16.5 Factors influencing graft rejection in patients transplanted for aplastic anaemia

Number of transfusions pretransplant
Degree of HLA disparity between donor and recipient
Cyclosporin
Additional pretransplant immunosuppression, e.g. ATG, Campath-1, irradiation
CMV infection/reactivation
Donor marrow/blood stem-cell dose

T-cell depletion of donor marrow/stem cells
Aetiology of aplasia
Graft-versus-host disease
Drugs, e.g. ganciclovir, co-trimoxazole

Number of transfusions prior to BMT

Historical data from Seattle demonstrated the superior survival of relatively untransfused patients (under ten transfusions) compared with multiply transfused patients (over ten transfusions), due to less graft rejection following allogeneic BMT [37]. However, a separate survey showed that transfusion of <40 units had a less marked effect on graft-rejection rates [63]. Multiple transfusions result in sensitization of recipient T-cells to minor histocompatibility antigens present on cells of the donor but lacking in the recipient, and this is thought to mediate graft rejection [42]. The incidence of graft rejection even in multiply transfused patients has fallen, however, as discussed below. In addition, it is possible that the routine use of leukocyte-depleted blood products may reduce the incidence of sensitization to HLA and non-HLA antigens [40].

Aetiology of the disease

Interestingly, patients with post-hepatitic AA have a low incidence of graft rejection (4%) compared with 20% for patients with idiopathic AA. This may reflect earlier referral for BMT and therefore a lower incidence of sensitization because of the usually very severe nature of post-hepatitic AA [32].

Cyclosporin

Cyclosporin was introduced as an alternative to methotrexate to prevent GvHD, and it was also shown to reduce the incidence of graft rejection [69,70]. Continuation of cyclosporin to 12 months post-BMT appears to be important in the prevention of late graft failure.

Additional immunosuppression in the pretransplant conditioning regimen

Standard conditioning regimens for transplantation in AA solely employ the powerful immunosuppressive drug cyclophosphamide at a higher dose than is used for transplantation for leukaemia. However, the use of irradiation as extra immunosuppression successfully reduced graft rejection but this was offset by increased toxicity from GvHD and pneumonitis, resulting in no improvement in survival [68], as well as the complications of second malignancies, delayed growth in children and sterility. Storb and colleagues reported excellent survival rates with a very low incidence of graft rejection following the combination of ALG and cyclophosphamide, although

chronic GvHD remained a problem [50]. At St George's Hospital we have evaluated the use of the monoclonal antibody Campath-1G *in vivo*, which recognizes the CD52 antigen and is directed against T- and B-cells and monocytes [51]. When used either pre-BMT to prevent graft rejection, or pre- and post-BMT to help reduce GvHD, it resulted in a very low incidence of GvHD. Although graft rejction remained a problem, autologous haematological reconstitution occurred in some patients. A high incidence of mixed chimaerism seen in these patients may explain these clinical observations.

Donor haemopoietic stem-cell dose

A large dose of donor stem cells is important in the prevention of early graft rejection. Because the incidence of graft rejection increases progressively as the cell dose falls below 3×10^8 nucleated cells/recipient body weight [71], it is necessary to collect at least this number of cells. The rationale for giving unirradiated donor buffy cells in the immediate post-graft period was to give additional stem cells to help prevent graft rejection. This manoeuvre successfully reduced graft rejection but the practice was abandoned on account of an increased risk of chronic GvHD [72]. The use of mobilized peripheral blood stem cells as an alternative to steady-state bone-marrow cells offers the opportunity to administer a larger number of $CD34^+$ cells, and to achieve faster engraftment [73] although it is uncertain whether this will also be associated with an increased risk of chronic GvHD.

T-cell depletion of the donor bone marrow

The IBMTR had previously reported that T-cell depletion of the donor bone marrow was associated with a very high risk of graft rejection [63]. This problem can be overcome by incorporating irradiation into the conditioning regimen [74]. However, the early and late complications of irradiation are considerable, and T-cell depletion of the donor marrow cannot currently be recommended for HLA-identical sibling transplants.

Treatment of graft failure

Haemopoietic growth factors and supportive care If there is no evidence of engraftment by four weeks it is reasonable to use a growth factor such as G-CSF or GM-CSF for one to two weeks, but if this has no effect on the blood count a second transplant should be planned, providing the patient is free from infection and clinically stable. As the patient may have been severely neutropenic for a prolonged period of time, including prior to the transplant, the quality of supportive care is important, particularly the prevention and treatment of possible fungal infections.

Second transplant

Second transplants performed for graft rejection carry a high risk of mortality if performed while the patient is infected and within 60 days after the first transplant [32]. Usually there is only one HLA-compatible sibling available so the same donor stem cells

are used. If there is a choice of donors, then transplantation with stem cells from the other compatible sibling would be favoured since the recipient may have become sensitized to minor histocompatibility antigens present on the original donor cells. It may also be preferable to collect G-CSF mobilized PBSC rather than steady-state bone marrow cells for the second transplant since it is possible to collect a larger dose of $CD34^+$ cells and there may be more rapid engraftment [73]. It is important to assess the chimaeric status of the patient using sensitive PCR-based DNA techniques to determine whether the graft rejection is due to re-establishment of recipient haemopoiesis or failure to maintain adequate donor haemopoiesis. If haemopoiesis is partly or completely of recipient origin, then reconditioning of the recipient prior to second transplant is necessary. This will require a more intensive conditioning regimen probably incorporating irradiation. Graft rejection which is associated with inadequate donor haemopoiesis may benefit from re-infusion of more donor stem cells without the need for further immunosuppression.

Further immunosuppression for late graft rejection

Late graft rejection may respond to reintroduction of therapeutic doses of cyclosporin if the rejection occurred after inadvertent discontinuation or withdrawal of cyclosporin before nine months [69]. Additional immunosuppression with methylprednisolone may be of benefit. If there is still no response, then a second transplant may be necessary.

Assessment of haematological engraftment

Previous studies of chimaerism using cytogenetic analysis in transplant recipients with AA demonstrated that mixed chimaerism occurred frequently but was usually transient [75]. The more sensitive technique of hypervariable minisatellite probes (sensitivity 2–5%) detected recipient T-cells in 21% of transplanted patients [76], but if irradiation was used in the conditioning regimen, all patients had complete long-term donor engraftment [77]. Thus, non-irradiation-based conditioning regimens for AA permit a mixed chimaeric state post-BMT. Lawler and colleagues [78] on behalf of the EBMT SAA Working Party have employed an even more sensitive technique using PCR of short tandem repeats in 85 recipients of BMT for AA [79]. Complete donor chimaerism was seen in 39 (46%) of patients at all times and only one of these rejected the graft. Transient mixed chimaerism was seen in 18 patients with no cases of graft rejection. Persistent recipient cells were detected in 9 cases with under 10% recipient cells and no graft rejection, in 13 cases with 15–80% recipient cells associated with graft rejection in 11, and in 6 cases a rising level of recipient cells with early rejection in all cases. Late graft rejection in the 11 cases with 15–80% recipient cells occurred while cyclosporin was being tailed off in 7 of these patients. Thus monitoring of chimaeric status during cyclosporin withdrawal may predict those at risk of graft rejection and allow appropriate preventative measures such as re-establishing therapeutic doses of cyclosporin.

Hill and colleagues had earlier noted a low incidence of acute GvHD as well as a high incidence of graft rejection in patients with mixed chimaerism as detected by sequential cytogenetic analysis [75], and the recent studies using PCR short tandem repeats have confirmed that a high incidence of mixed chimaerism is associated with a significantly lower incidence of both acute and chronic GvHD [78].

The conditioning regimen

The following conditioning regimen, which describes the practical difficulties that may be encountered, applies to BMT from HLA-identical sibling donors for acquired AA. Possible modifications for volunteer unrelated donor transplants are also discusssed. The conditioning regimen for patients with Fanconi anaemia is different from that for patients with acquired AA, including major dose reduction of cyclophosphamide and incorporation of low-dose irradiation. For full details of BMT for Fanconi anaemia see Chapter 17.

Cyclophosphamide

The most widely used conditioning regimen for BMT for AA using HLA-identical sibling donors was based on the pioneering animal work of Thomas and colleagues [80]. Cyclophosphamide is given as a single immunosuppressive drug. The dose is 50 mg/kg/day for four days.

Major side-effects include haemorrhagic cystitis, fluid retention on account of its antidiuretic hormone effect and cardiotoxicity. Nausea and vomiting may occur but usually are not marked, and mucositis usually does not occur. Total hair loss is inevitable, this occurring about 10–14 days post-treatment. Haemorrhagic cystitis is caused by acrolein, a metabolite of cyclophosphamide, and can be a major problem when cyclophosphamide is used at such high doses. For this reason, it is essential to prevent this either by using mesna or forced alkaline diuresis. The former is much easier to administer and requires a daily fluid intake of 3 litres but forced alkaline diuresis is not necessary when mesna is used. Mesna is administered as an intravenous bolus at 40% of the cyclo-phosphamide dose, at 3, 6, 9, 12, 16 and 20 hours following cyclophosphamide. It is continued on the day following the last dose of cyclophosphamide. Mesna has no serious side-effects but gives a false-positive test for urinary ketones. Late haemorrhagic cystitis, occurring at three to six weeks after the transplant is usually due to viral infection of the urinary tract. Following the four days of cyclophosphamide, a further day is allowed to elapse before the infusion of harvested bone marrow or PBSC.

Cyclophosphamide is not fat-soluble and the dose is usually modified for obese patients based on their ideal or lean body weight. If a second transplant is required because of graft rejection, cyclophosphamide can safely be given again without apparent toxicity [64]. If only cyclophosphamide is used for the transplant, both male and female transplant recipients retain their fertility, and patients should receive appropriate contraceptive advice post-transplant. Pregnancy outcome after BMT for AA is discussed later.

Cyclosporin

Cyclosporin was introduced to prevent graft rejection, and compared with an historical group who were given methotrexate to prevent GvHD, the rejection rate was significantly lower in the cyclosporin group [68]. Cyclosporin also has a powerful anti-GvHD effect. In multivariate analyses, cyclosporin has been shown to be a significantly important

factor in the improvement in survival of patients transplanted for AA during the last decade [49]. It should be given for a minimum of six months and preferably for a year, with gradual tapering of the dose beginning at nine months, in order to prevent late graft failure. It is commenced on day −1 at a dose of 5 mg/kg/day in two divided doses given as an intravenous infusion over 2–3 hours, with careful monitoring of blood pressure, serum creatinine, liver function tests and cyclosporin blood levels. Whole blood trough levels of 200–300 ng/ml should be the aim. Oral cyclosporin can be introduced provided that the patient is not vomiting and does not have marked diarrhoea which influence the absorption of cyclosporin. The recent availability of Neoral, a lipid emulsion formulation of oral cyclosporin, results in increased bioavailability and more constant and predictable blood levels. Because cyclosporin is highly fat-soluble, the dose should not be modified for obese patients.

Common side-effects of cyclosporin include tremor, hypertrichosis and some loss of appetite. Cyclosporin is nephrotoxic and hepatotoxic and drug interactions are common. Nephrotoxicity is cumulative with the side-effects of nephrotoxic antibiotics and intravenous amphotericin. Fluid retention may occur often associated with marked hypertension, especially if corticosteroids are also used, and this situation, particularly in children, may precipitate seizures. Rigorous control of fluid balance and the use of antihypertensive agents prevent these complications.

Additional pregraft immunosuppression

In order to reduce further the risk of graft rejection, additional immunosuppression with ALG or Campath-1G, for example, can be used. Irradiation should be avoided for reasons discussed earlier.

Possible modifications for volunteer unrelated donor transplantation

There is currently much debate concerning not only an appropriate conditioning regimen for unrelated donor transplants, but also concerning the indications for such transplants in patients with AA. This is discussed in some detail earlier.

Acute graft-versus-host disease (GvHD)

This section considers only the prevention and treatment of acute GvHD in relation to transplantation for acquired aplastic anaemia. It is well recognized that there is a reciprocal relationship between acute GvHD and graft rejection, and the presence of acute GvHD may be associated with late graft failure. This relationship is particularly striking in BMT for AA, probably because the immune mechanisms responsible for graft rejection play an important role in the original pathogenesis of the aplasia.

Prevention of acute GvHD

Methotrexate was the original prophylactic regimen for GvHD, but this was associated with a high incidence of graft rejection in patients transplanted for AA. Introduction of cyclosporin to reduce graft rejection also led to a reduction in the severity but not the incidence of acute GvHD [81]. The Seattle group reported that a combination of methotrexate given for a short time on days 1, 3 and 5 together with cyclosporin resulted in a reduction in the incidence and severity of acute GvHD compared with methotrexate or cyclosporin alone, in patients transplanted for acute leukaemia [82]. For patients with AA, there are no data to determine whether the combination of cyclosporin and methotrexate is superior to cyclosporin alone, although there is currently an ongoing prospective, randomized EBMT study hoping to answer this question.

GvHD can also be prevented by T-cell depleting the donor bone marrow, but this led to an unacceptably high incidence of graft rejection in patients transplanted for AA. This incidence of graft rejection can be reduced by increasing the immunosuppression, particularly by using irradiation. However, long-term survival is not improved. An alternative approach is to use a monoclonal antibody such as Campath-1G post-BMT as well as pre-BMT, for its anti-GvHD effect [51].

Treatment of acute GvHD

The treatment for established acute GvHD in AA patients is similar to that for patients transplanted for acute leukaemia.

Chronic GvHD

Chronic GvHD occurs in 15–50% of patients transplanted for AA [48], although the distinction between limited and extensive chronic GvHD is not clear in all published series. In 20% it appears to occur as a serious complication. Risk factors for chronic GvHD include those associated with transplants for leukaemia, namely the presence of acute GvHD, increasing recipient age [82], and specifically for AA, irradiation and the use of unirradiated donor buffy-coat cells. In AA patients transplanted without irradiation, the presence of mixed chimaerism is associated with a lower incidence of acute and chronic AA [51,78]. In contrast to patients transplanted with the Seattle regimen of cyclophosphamide and ALG where chronic GvHD occurred in 35% of cases, the *in vivo* use of Campath-1G monoclonal antibody was associated with a very low incidence of chronic GvHD, which was limited in each case [51]. Treatment of chronic GvHD for patients transplanted for AA is similar to the treatment given to patients transplanted for leukaemia.

Fertility and pregnancy outcome following transplantation

Since a large proportion of patients transplanted for AA survive long term, the issue of future fertility in children and adults is of great importance, and emphasizes the need to avoid irradiation-based regimens or other myeloablative protocols with the potential to damage germ-cell chromosomes and gonadal function.

Until recently, there has been a paucity of data on pregnancy outcome following transplantation for AA, although earlier reports from Seattle indicated that successful pregnancies occurred in patients transplanted for AA using high-dose cyclophosphamide alone [83]. The same group, however, has recently reported on 56 patients transplanted with cyclophosphamide alone [84]. Among the 28 female patients there were 56 pregnancies which resulted in 44 live births and 28 male patients fathered 62 pregnancies of which 51 resulted in live births. The spontaneous abortion rate was not increased above the expected rate for the general population; and preterm labour and delivery occurred in 18% of women compared with 6% expected and 62.5% for patients transplanted for haematological malignancy using cyclophosphamide with either TBI or busulphan. There was no excess of congenital anomalies among babies born to AA patients.

Second malignancies after transplantation

Long-term follow-up of patients in Europe indicated that second malignancies do occur following transplantation for AA [85]. Since AA is a non-malignant disorder with a high chance of long-term cure of the aplasia following BMT, the occurrence of later malignancy is a particularly devastating event.

The European study indicated that irradiation as part of the conditioning regimen may be an important factor, and also suggested that chronic GvHD particularly affecting the mouth was a potential risk factor for squamous-cell carcinoma [85]. The study from Hôpital St Louis in Paris reported that 4 out of 107 patients developed a solid tumour, namely epidermoid carcinoma of the tongue, lip and oral cavity respectively in 3 patients and 1 case of mucoepidermoid carcinoma of the parotid gland. All 4 patients, of whom one had Fanconi anaemia and the rest acquired AA, had received irradiation and 2 had oral chronic GvHD. The eight-year cumulative incidence rate of solid tumours after BMT was 22%. In contrast, Witherspoon and colleagues in Seattle [86] reported that only 5 out of 330 patients transplanted with cyclophosphamide alone developed secondary tumours, squamous cell in all cases, affecting the tongue, maxillary sinus, oral mucosa, skin and vulva, respectively. All 5 patients had chronic GvHD and were treated with prednisolone and azathioprine. The cumulative incidence of second malignancy was 0.4% at 5 years, 1.4% at 10 years and 4.2% at 15 years. None of the patients in this series had received irradiation. More recently the same two groups of workers have performed a combined analysis of risk factors for second malignancy in 700 patients with AA transplanted at Seattle and Hôpital St Louis [87]. A malignancy developed in 23 patients with 20 years of follow-up. There were 5 cases of acute lymphoblastic leukaemia or

lymphoproliferative disorders and 18 solid tumours of epithelial origin (17 squamous cell and 1 mucoepidermoid carcinoma) and these predominantly involved the head and neck. For patients with acquired AA, lymphoid malignancies occurred during the first post-transplant year, while solid tumours developed later, with two peaks at 8 and 17 years, respectively, after BMT. There were no cases of myeloid malignancies. Risk factors for developing a malignant tumour in acquired AA were irradiation, and chronic GvHD and its treatment with azathioprine. Since all patients with chronic GvHD received azathioprine, it was not possible to analyze the impact of chronic GvHD and azathioprine separately. A diagnosis of Fanconi anaemia was also a separate risk factor.

Thus, better prevention of GvHD and/or avoidance of azathioprine, and the use of non-irradiation-based conditioning regimens may reduce the tumour risk in patients transplanted for acquired AA.

Conclusions

The outcome of patients with AA, whether treated with HLA-identical sibling BMT or immunosuppressive therapy, continues to improve with time, such that the majority will be ‘cured’ by BMT or will achieve a haematological response to immunosuppression [11,88]. The quality of haematological recovery is superior after BMT in that the peripheral blood count returns to normal in most cases which is unusual following immunosuppressive therapy, and patients post-transplant do not appear to be at risk of the clonal disorders such as MDS, AML or PNH that can occur post-ALG [89], However, there is still a lack of data regarding *in vitro* clonogenic cell cultures and long-term marrow cuture/long-term culture initiating cell frequency post-BMT to know yet if transplantation results in complete correction of the stem-cell defect in AA. It is difficult to quantify the true impact of improved quality of supportive care on improvements in survival for patients treated with BMT or immunosuppression, but this has contributed to a significant degree to improved survival. Graft rejection post-transplant for AA still remains a problem for some patients despite increasing the intensity of the conditioning regimen, although the incidence has fallen. Acute GvHD also occurs less frequently now, as in marrow transplants performed for other haematological disorders.

The quality of life post-transplant in terms of chronic GvHD is a major issue especially as patients have a good chance of long-term cure of their aplasia. Chronic GvHD is still seen, although this may potentially be reduced by additional post-transplant therapy with agents such as ALG or Campath-1 monoclonal antibody.

The role of volunteer unrelated donor transplants in AA remains controversial since such transplants are still associated with a higher risk of acute GvHD and graft rejection compared with sibling transplants. The use of increased pretransplant conditioning and additional anti-GvHD prophylaxis has been of benefit in some centres, but the long-term consequences in terms of second malignancies are as yet unknown. The encouraging improvements in haematological response seen with the combination of immunosuppression and G-CSF may represent a safer option for those patients with severe AA and who lack an HLA-identical sibling donor.

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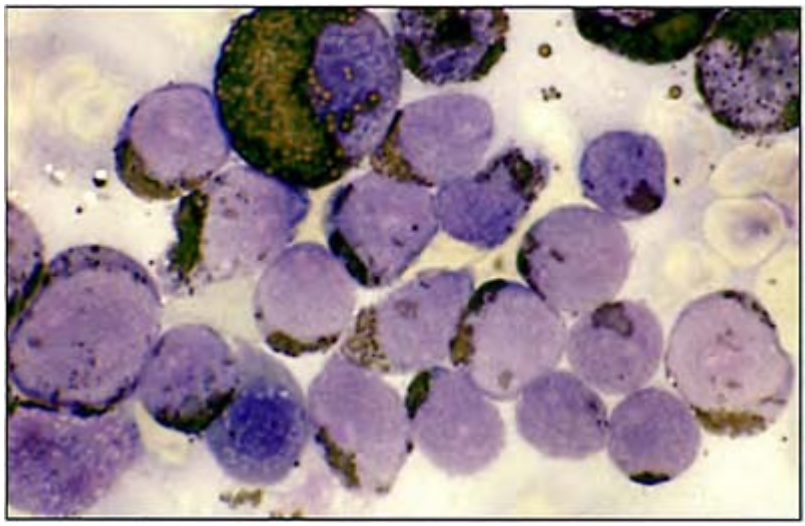

Chapter 17
Fanconi anaemia

Eliane Gluckman

Introduction

Fanconi anaemia (FA) was originally described as an autosomal recessive disorder, characterized by progressive pancytopenia, diverse congenital abnormalities and increased predisposition to malignancy [1]. FA cells show a high level of chromosomal breakage, both spontaneously or when induced by cross-linking agents such as mitomycin C, nitrogen mustard, diepoxybutane or photoactivated psoralens [2–3]. At least five genetic complementation groups (A–E) have been described [4]. In Europe, the frequency of each complementation group has been determined: FA-A 66%; FA-B 4.3%; FA-C 12.7%; FA-D 4.3%; FA-E 12.7%. The cDNA for group C has been cloned and located on chromosome 9q22.3 [5]. Six disease-associated sequences were found; one mutation IVS4 >T is observed mostly in Ashkenazi Jews and is associated with poor prognostic features. The function of the *FAC* gene is, however, still unknown [6–8]. More recently, the gene for group A has been localized to chromosome 16q24.3 [9] and the group D gene has been localized to chromosome 3 p22–26 [10].

Fanconi anaemia is an heterogeneous disorder with both genetic and phenotypic variability. This variability can be due to different genetic mutations or to genetic reversion and mosaicism. Bone-marrow failure is the most frequent haematological

abnormality, occurring typically around five years of age, but aplasia can appear later. Clonal abnormalities including a high frequency of monosomy 7 and duplications involving 11q can be observed on marrow cytogenetic analysis as a sign of transformation to myelodysplastic syndrome or acute myeloblastic leukaemia [11,12]. Without curative treatment, survival is poor, death occurring during the second decade of life from aplastic anaemia, leukaemia or cancer.

Bone-marrow failure seems to occur early in life, and bone-marrow studies including clonogenic assays and long-term marrow cultures show a decrease of the haematopoietic stem-cell pool without gross microenvironmental defects [13]. It is not known if normal haematopoietic stem cells can persist for long periods. Although the molecular expression of the c-*kit* proto-oncogene and kit ligand in long-term marrow culture is normal [14], there is evidence of a subtle microenvironmental defect consisting of dysregulation of cytokines such as interleukin-6 (IL-6), tumour necrosis factor (TNF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), which may contribute to marrow failure [15].

Treatment with androgens, steroids or haematopoietic growth factors can give a transient improvement but cannot cure the patients [16].

Results of human leukocyte antigen (HLA)-identical sibling transplants

Bone-marrow transplantation is the only treatment which definitively restores normal haematopoiesis. Cyclophosphamide, which is used for the conditioning of patients with idiopathic aplastic anaemia at the total dose of 200 mg/kg, has been proven too toxic, leading to a high rate of transplant-related mortality [17]. This was shown *in vitro* as well as *in vivo.* Radiosensitivity toxicity studies have shown a delay in radio-induced skin lesions, an increased intensity of skin damage, and an absence of repair after fractionated irradiation [18,19]. For this reason, the conditioning regimen for bone-marrow transplantation was modified by our team in 1980; it includes cyclophosphamide 20 mg/kg given i.v. over 4 days and 5 Gy thoraco-abdominal irradiation (TAI), followed by cyclosporin alone for prevention of graft-versus-host disease (GvHD) [18,21] (Table 17.1). In the study performed by our centre, which included 45 patients who had received an HLA-identical sibling bone-marrow or a cord blood transplant, the five-year actuarial disease-free survival was 75.6% [20]. The causes of death observed in 15 patients were: bone-marrow failure (2), infection (1), haemolytic uraemic syndrome (1), interstitial pneumonitis (1), chronic GvHD and infection (5), secondary tumour (3) and post-hepatitis B cirrhosis (1). Chimerism studies showed that all engrafted patients were of donor type with no residual host cells [21]. This confirms that decrease of the dose of immunosuppression was sufficient to induce long-term tolerance and absence of autologous reconstitution.

Table 17.1 Protocol for Fanconi conditioning

Cyclophosphamide 20 mg/kg over 4 days, −5 to −2 TAI, 5 Gy day −1
Cyclosporine day −1→6 months

A recent report from the International Bone Marrow Transplantation Registry (IBMTR) analyzed the outcome of 151 patients who underwent transplantation from an HLA-identical sibling and 48 patients who received transplants from an alternative related or unrelated donor [22]. The two-year probability of survival was 66% after HLA-identical sibling transplantation and 29% after alternative donor transplantation. Younger age (P=0.0001), higher pretransplant counts (P=0.04), use of antithymocyte globulin (P=0.0005) and use of low-dose cyclophosphamide (15–25 mg/kg), plus limited field irradiation (P=0.009) for pretransplant conditioning and cyclosporin for GvHD prevention (P=0.002) were associated with increased survival.

In patients who received HLA-identical transplants, the probability of graft failure was 8% (95% confidence interval [CI], 5–14%), of grade I to IV GvHD 42% (95% CI, 34–50%) and of chronic GvHD 44% (95% CI, 34–53%). Results of unrelated donor transplantation gave poor results because of an increased risk of rejection (24%) and GvHD (51%).

This study, as well as publications from other centres, shows that low-dose cyclophosphamide is better tolerated and allows better survival than high doses [23–25]. However, some centres report that doses of cyclophosphamide in the order of 100–140 mg/kg with or without anti thymocyte globulin (ATG) and without irradiation gave a long-term survival of about 50–80% [25,26]. Small numbers of patients were involved, and the overall toxicity was reported as high, but these results raise the possibility that sensitivity to alkylating agents may vary according to the type of genetic defect which is reflected *in vivo* by the heterogeneity of the phenotypic expression and *in vitro* by the variability of the number of chromosomal breaks. Some mutations might be more severe than others.

The good results of allogeneic bone-marrow transplantation with an HLA-identical sibling raise several questions concerning the optimal timing of BMT and the best conditioning regimen. As far as the timing of BMT is concerned, there is general agreement that BMT from an HLA-identical sibling should be used as first-line therapy, without trying androgens or steroids which have side-effects. When blood counts show the criteria of severe aplastic anaemia (haemoglobin <8 g/100 ml, polymorphonuclear leukocytes <500/mm^3 or platelets <20000/mm^3), transfusions are necessary and therefore these levels are a suitable indication for BMT. During the waiting period, it is very important to perform regular bone-marrow aspirations and cytogenetic analysis for detection of clonal abnormalities or signs of leukaemic transformation. Results show that transplants performed late, after a long period of aplasia or during leukaemic transformation, yield poor results.

Deciding upon the best conditioning regimen is more difficult for several reasons. Firstly, because the number of patients is so small, a prospective randomized multicentre study is not feasible. Secondly, *in vitro* tests for predicting the sensitivity of a given patient to the conditioning regimen are not currently performed. There is no information about the sensitivity of cell subsets to alkylating agents. The sensitivity of leukaemic cells seems to be increased, as some remissions have been obtained after low-dose

chemotherapy or after conditioning with low-dose cyclophosphamide without any attempt to induce remission, but these cases are rare and most of the patients treated for acute leukaemia do not tolerate standard-dose chemotherapy and have a very poor prognosis. It is possible that the genetic diagnosis, which will be elucidated in the future, will delineate criteria for disease severity. For this reason, it is highly important to report all the new cases to registries. In Europe, the European Fanconi Anaemia Registry (EUFAR) group has begun to register cases of familial aplastic anaemias including Fanconi's anemia [27].

Finally, it will be very difficult to prove further improvement, when most survival curves show more than 75% long-term survival. Furthermore, absence of irradiation from the conditioning regimen does not seem to diminish the risk of secondary tumours which are also related to the genetic defect and to the environment, as shown by the observation of different phenotypic expressions of the disease in homozygous twins (unpublished observations).

Alternative donor transplants

During recent years, the development of large volunteer bone-marrow donor registries, including more than three million donors throughout the world, has led to the possibility of finding donors for patients without HLA-identical siblings. In Fanconi anemia, the results of mismatched family transplants or transplants performed with HLA-identical unrelated donors have been disappointing. In our series of 18 patients, only 2 are surviving. The causes of death have been: multi-organ failure (6), acute GvHD (5), rejection (2) and interstitial pneumonitis (2).

Of 41 patients with alternative donors reported to the IBMTR, the actuarial two-year survival was 29%; the probability of rejection was 24%; the probability of grade II–IV GvHD 51%; of chronic GvHD 46%; and of interstitial pneumonitis 25%. The number of patients was too small to analyze risk factors (unpublished data from Minneapolis Workshop, November 1995).

A recent survey in six major centres confirmed these poor findings. Forty-two cases were analysed. Many had poor prognostic indices: the mean age was 13.4 years, and 7 patients had leukaemia. Conditioning included 20–80 mg/kg cyclophosphamide (42 patients), 2.4–8 g/m^2 cytosine arabinoside (Ara-C) (12 patients) and 400–620 cGy TBI/TLI/TNI (41 patients). In addition, ATG, Campath, methylprednisolone, VP-16, busulfan and procarbazine were given to some patients (Table 17.2). Thirty-three received a conventional

Table 17.2 Alternative donot transplants in Fanconi anaemia in Europe: analysis of 42 cases from six centres

[Mean age 13.4 years, leukaemia present in 7]
Conditioning:
• 20–80 mg/kg CTX n:42

- 2.4–8g sqm ARA-C n:12
- 400–620 cGy TBI/TLI/TNI n:41

Other approaches;

- ATG
- VP16
- CAMPATH
- Methylprednisolone (MP)
- Busulfan
- Procarbazine
- T-cell depletion

bone-marrow transplant; 9 had T-cell depletion. Thirty-two patients engrafted, 2 were non-evaluable, 8 failed initial engraftment, 1 had late graft failure and 8 patients received a second bone-marrow transplant (Table 17.3) Graft failure was associated with T-cell depletion: 5 of 9 patients (56%). The incidence of grade II–IV GvHD was 66.6% with 15 grade II–IV (50%). Major causes of death were GvHD 43%, graft failure 16.7%, multi-organ failure 23.3% and infection 10%. (Unpublished data from Minneapolis Workshop, November 1995) (Table 17.4).

Several modifications of the transplant procedure can be used experimentally to overcome the risks of toxicity, GvHD and rejection. To overcome GvHD, T-cell depletion can be used either by positive selection of $CD34^+$ cells on columns or magnetic beads, or by depletion with elutriation or lysis with monoclonal antibodies. This approach is likely to increase the frequency of rejection. As intensifying the conditioning regimen will certainly increase the risk of toxicity, it is possible to use agents not yet tested such as melphalan or thiotepa in the conditioning regimen, or to increase the number of stem cells infused by adding G-CSF-mobilized peripheral blood $CD34^+$ cells to the $CD34^+$ cells taken from the donor's marrow. The add-back of lymphocytes after transplant might also improve

Table 17.3 Engraftment

32 Engrafted
2 non-evaluable
8 failed initial engraftment
1 late graft failure
8 had second BMT

Table 17.4 Principal causes of death

GvHD	:	43.4%
Graft failure	:	16.7%
Multi-organ failure	:	23.3%
Infection	:	10.0%

engraftment in patients without increasing the risk of GvHD.

Secondary tumours

The observation of secondary tumors after transplant is of major concern. The probability of developing malignant tumours is very high in this population because of the presence of several risk factors including method of conditioning with or without irradiation, environmental exposure and the chromosome instability characteristic of the disease [28]. Risk factors for the development of a new malignancy after bone-marrow transplantation were analyzed in 700 patients with severe aplastic anaemia including Fanconi anaemia treated with allogeneic BMT at the Fred Hutchinson Cancer Center in Seattle and Hôpital Saint Louis in Paris [29]. The chance of developing a solid tumour by 20 years after transplantation was estimated at 42% (95% CI, 10–74%) among patients with Fanconi anaemia and 13% (95% CI, 3–23%) in idiopathic aplastic anaemia. All secondary tumours were observed in males at an interval of 6–11 years after transplant. They were all head and neck squamous-cell carcinomas. Risk factors for developing solid tumours, identified in univariate analysis, included the underlying disease of Fanconi anaemia (P=0.0002), azathioprine therapy for chronic GvHD (P=0.0001), irradiation (P=0.0002), chronic GvHD (P=0.0099), acute GvHD (P=0.0135) and male sex (P=0.0499). In multivariate analysis stepwise proportional hazards models, azathioprine therapy ($P<$ 0.0001) and the diagnosis of Fanconi anaemia ($P<$ 0.0001) were significant factors for all patients.

Table 17.5 Secondary tumours after BMT for Fanconi anaemia [30]

- Incidence 20 years after BMT for Fanconi anaemia 42% and after BMT for aplastic anaemia 13%
- All tumours occurred 6–11 years after BMT
- All tumours were head & neck squamous-cell carcinomas
- Risk factors included male sex, irradiation, c-GvHD or a-GvHD, azathioprine therapy

Irradiation was a significant factor (P=0.004) only if the time-dependent variable azathioprine was not included in the analysis (Table 17.5). The fact that, in Fanconi anaemia patients, only solid tumours and no lympho- or myeloproliferative malignancies

were observed post-transplant is consistent with the fact that transplantation provides patients with a normal haematopoietic system, but does not affect the congenital defect in other tissues.

New strategies for therapy

Cord blood transplants

The first successful HLA-identical cord blood transplant was performed in a patient with Fanconi anaemia [30]. The donor was known, before birth, to be HLA identical to the patient and not affected by Fanconi anaemia. His cord blood was collected at birth and transplanted after thawing to his HLA-identical brother who had severe aplastic anaemia due to Fanconi anaemia. The patient had received low-dose cyclophosphamide and TBI before transplant, and after transplant he received cyclosporin for prevention of GvHD. This patient had complete haematological and immunological reconstitution with complete donor chimaerism. He has now been followed-up for more than seven years, is doing well and is apparently cured of his disease.

Since this first successful transplant, the numbers of cord blood transplants have increased worldwide and more than 200 adults and children with various haematological diseases have been reported. Currently, cord blood banks are being developed, mostly in the USA and in Europe, for unrelated matched or mismatched cord blood transplants. Several patients with Fanconi anaemia have been transplanted successfully with partially matched unrelated cord blood. It seems that one of the advantages of using cord blood instead of bone marrow is a reduction in the severity of GvHD, due to the relative immaturity of the immune system of the newborn.

Gene therapy [31]

Autologous transplantation is of very limited value in Fanconi anaemia where the few $CD34^+$ cells isolated from the blood after G-CSF mobilization do not grow in long-term culture and are unlikely to give short- or a long-term engraftment. Collection of cord blood at birth, or of peripheral blood $CD34^+$ stem cells can be performed at an early stage in the disease for gene transfer. The recent localization of two new genes and the demonstration *in vitro* that transfected cells have a selective growth advantage over Fanconi anaemia cells has increased interest in designing gene-therapy protocols in Fanconi anaemia. Several issues remain unresolved, including integration of the gene in primitive haematopoietic stem cells, the level of integration necessary for correction of the disease, the long-term expression of the transfected gene, the selective growth advantage of transfected cells, and the function of the Fanconi anaemia gene proteins.

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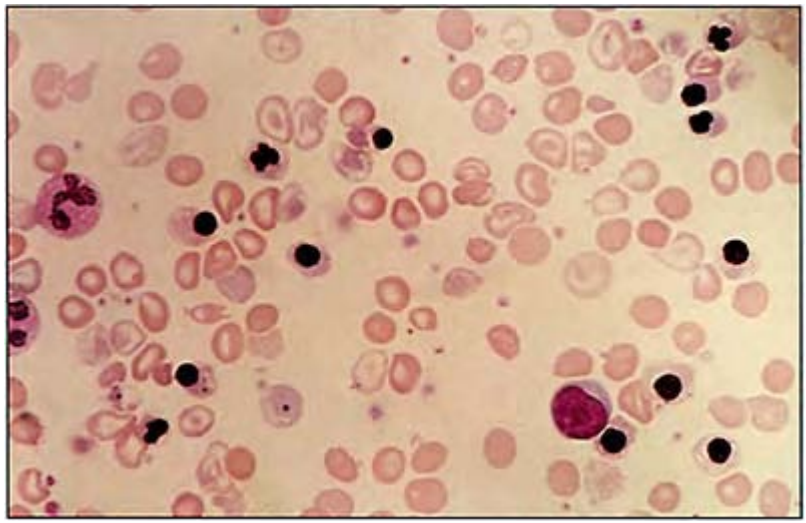

Chapter 18
Thalassaemia

Caterina Borgna-Pignatti

Introduction

Thalassaemia major was first described in 1925 [1] in 7 children of Italian origin. At the same time, in Italy, a disease called ‘haemolytic jaundice with increased red cell osmotic resistance’, with a clinical picture corresponding to what is called ‘thalassaemia intermedia’ today, was described in 2 patients from Ferrara [2]. This disorder, considered the most common monogenic disease, is transmitted as an autosomal recessive and is due to abnormal synthesis of one or more haemoglobin polypeptide chains. Underlying genetic defects include total or partial deletions of globin chain genes and nucleotide substitutions, deletions and insertions leading tos decreased, absent, or functionally defective mRNA. In addition, haemoglobin E, a β-globin structural variant, when inherited together with β-thalassaemia produces the phenotype of thalassaemia major. Ineffective erythropoiesis and haemolysis give rise to severe anaemia, that usually appears, in the case of homozygous β-thalassaemia, in the first year of life. The thalassaemia mutations occur in high frequency in areas of past endemic falciparum

malaria. Although community screening and genetic counselling have decreased the births of new patients to very low levels in the Mediterranean area (Figure 18.1), it has been estimated that in the Indian subcontinent and South-East Asia many thousands of thalassaemics will be born in the near future [3]. In Iran, registered thalassaemia patients number more than 20000 [4],

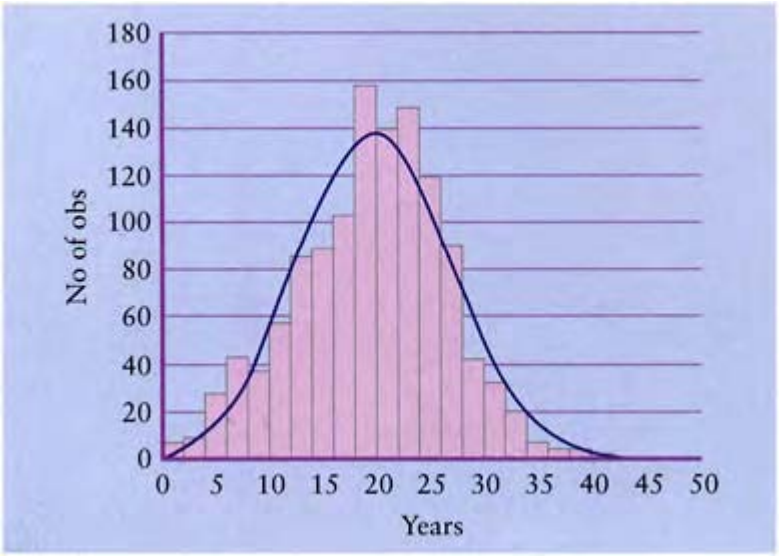

Figure 18.1 Age distribution of thalassaemic patients in Italy in 1996.

Table 18.1 Clinical forms of β-thalassaemia

Thalassaemia minor (heterozygous β-thalassaemia)
Thalassaemia intermedia (various molecular assets) Transfusion-independent
Thalassaemia major. Homozygous or double heterozygous
Transfusion-dependent

Table 18.2 Clinical forms of α-thalassaemia

Silent carrier: (α/α)
a thal trait: (−α/αα, α/−α, − −/αα, non-deletion α thal)
HbH: one functioning gene out of four
Hb Bart's: Foetal hydrops

Table 18.3 Conventional treatment of thalassaemia

Transfusion to maintain mean Hb=11–12 g/dlitre
Chelation with subcutaneous desferrioxamine during 8–10 h/day with battery-operated pump
Splenectomy when transfusion requirement >1.5 baseline or 200 ml/kg/year. Leuko- or thrombocytopenia

while in India there are 10000 births a year [5]. β- and α-thalassaemias can be clinically classified as in Tables 18.1 and 18.2. Conventional treatment includes red cell transfusions, chelation therapy to control the transfusional iron overload, and, in most cases, splenectomy (Table 18.3).

Bone-marrow transplantation

Despite the great improvement in survival induced by modern therapy, and in particular by intensive subcutaneous chelation (Figure 18.2), complications are still very common (Table 18.4) [6].

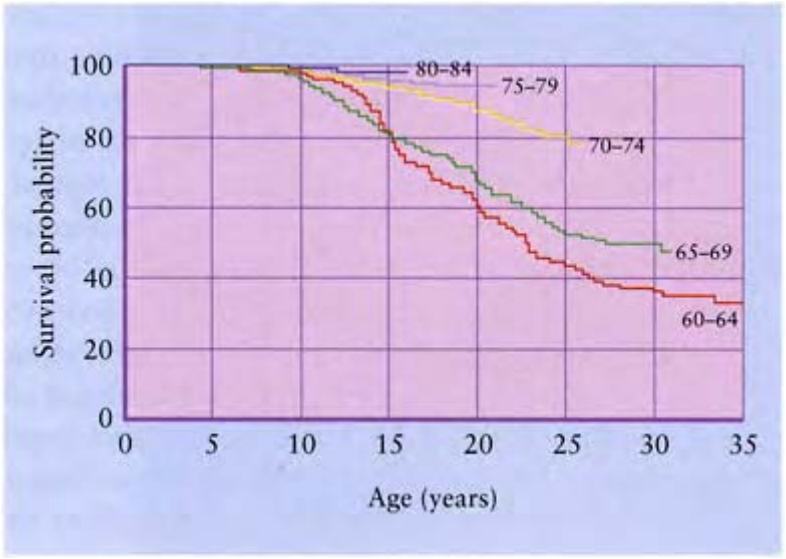

Figure 18.2 Survival probability (January 1996) of 1079 transfused and chelated Italian patients according to year of birth.

Table 18.4 Complications in a series of Italian thalassaemia patients (*n=700*) *and age at appearance*

Complication (years)	Percentage	Mean age
Diabetes	7	17.7
Hypothyroidism	12.5	15.8
Heart failure	8.5	15.4
Arrhythmias	7	17
Thrombosis	1	19
HIV	1.9	12.5

Bone-marrow transplantation represents the only curative approach to the treatment of thalassaemia to date. The first patient was transplanted in Seattle in 1981 [7] from his human leukocyte antigen (HLA)-identical sister and he is now completely cured. More than a thousand patients have since been transplanted worldwide. The vast majority were affected by thalassaemia major, while a few had thalassaemia intermedia, HbE/β-thalassaemia, or HbH disease (where three of four chain genes are affected).

The largest experience has been obtained in Pesaro, Italy, where, to date, more than 800 patients have undergone transplantation [8]. Reports from several countries have appeared, in recent years, testifying to the use of the procedure worldwide. At first, in most centres, only young, minimally transfused children were considered candidates for transplantation. Subsequently, patients of all ages have been grafted, including several adults [9,10]. A few hundred patients have been transplanted in Asiatic countries where conventional therapy is not available for most patients. Bone-marrow transplantation may represent the only available opportunity for survival in that setting.

It has become customary, as suggested by the Pesaro group, to classify the patients according to three levels of transplantation risk [11] (Table 18.5) Age does not seem to directly influence prognosis, but it is rare for an older patients to have a normal liver biopsy or a history of early and constant chelation. This classification, however, is not universally accepted, as other authors have not confirmed the influence of liver histology and of chelation compliance on prognosis [10,12].

Donors

Donors have usually been chosen among HLA-identical siblings, but a few partially mismatched siblings or parents, phenotypically identical parents and other relatives have also donated marrow. Very rarely, donors have been HLA-matched, unrelated volunteers. In fact, the high risk of death and of chronic GvHD reported in patients grafted for leukaemia limits this choice to an extremely restricted number of thalassaemia patients.

As expected, two-thirds of donors had thalassaemia trait. It has been observed that, in recipients of marrow from heterozygous donors, activation of HbF was more marked and protracted [13], but the results of the procedure have not otherwise been different.

Table 18.5 Pesaro classification transplantation risk

Class	Hepatomegaly (>2 cm)	Hepatic fibrosis	Irregular chelation
Class I	No	No	No
Class II		One or two	
Class III	Yes	Yes	Yes

Conditioning regimens

In general this has included busulfan 14 mg/kg and cyclophosphamide 200 mg/kg. In some centres melphalan, antithymocyte globulin or total lymphoid irradiation (TLI) have been added, or the dose of busulfan has been increased to 16 mg/kg, and cyclophosphamide to 440 mg/kg, in an attempt to minimize the risk of rejection. In a small percentage of patients, myeloablation has been attempted with total body irradiation (TBI), in doses varying from 300 to 1200 cGy, alone or in combination with busulfan 8–16 mg/kg (Table 18.6). No advantage seems to be apparent by adding irradiation, which should probably not be used, as it affects growth, and can induce growth hormone deficiency, thyroid and gonadal failure, and can further increase the risk of post-transplant malignancies.

Prophylaxis against graft-versus-host disease (GvHD) has included methotrexate and, more recently, cyclosporin A, alone or in combination with methotrexate, cyclophosphamide or T-cell depletion of the marrow graft. It has been suggested that more immunosuppressive conditioning may be necessary to prevent rejection in Chinese patients [14]. GvHD prophylaxis has been performed by most centres with cyclosporin A alone or associated with methotrexate.

Results

The rates of survival vary according to the centre performing the transplant, and to the patient's

Table 18.6 Conditioning regimens used for BMT in thalassaemia

Bulsufan 3.5–5 mg/kg×4
Cyclophosphamide 60–100 mg/kg×2
ALG or ATG
TLI or TBI 300–1200 cGy

Melphalan 140 mg/kg×1

Thiotepa 5 mg/kg×2

ALG=antilymphocyte globulin. ATG=antithymocyte globulin. TLI=total lymphoid irradiation. TBI=total body irradiation.

condition and age at the time of grafting. In 1992 we reported an 80% survival and a 67% thalassaemia-free survival in 548 patients transplanted up to December 1989 [15]. More recently, the prognosis of bone-marrow transplantation for thalassaemia has improved. The most recent results from Pesaro [8] are reported in Table 18.7 and are similar to results obtained in other centres in Italy. In this series, class III patients seem to benefit from a regimen containing less than 200 mg/kg of cyclophosphamide, with or without the addition of antilymphocyte globulin (ALG) [9]. The European Group for Bone Marrow Transplantation has collected data on 80 additional patients from 16 centres in Europe, Asia and South America. Survival at two years was 88%, and disease-free survival 80%. In contrast, a collection of data from several centres in the USA [16] has shown a probability of survival of 80%, but a probability of disease-free survival of only 57%, due to recurrence of thalassaemia in 24% of the patients. Adult patients have mainly been transplanted in Italy. The Pesaro group has reported probabilities of survival, event-free survival and rejection of 65%, 63% and 3% respectively, in 86 patients aged from 17 to 32 years, who had received between 0 and 310 transfusions [9]. In this group, conditioning regimens containing less than 200 mg/kg of cyclophosphamide significantly reduced transplant-related mortality, but at the price of an increased risk of rejection. Sixteen percent of patients developed chronic GvHD. Twenty-six patients transplanted in Pescara, with ages ranging from 15 to 25 years, and selected because of good liver and cardiac

Table 18.7 Result of the Pesaro group: BMT for thalassaemia, 802 patients (as of July 1996)

Class	Survival (%)	Disease-free survival (%)	Rejection (%)
Overall	73	64	12
Class I	95	90	5
Class II	84	82	4
Class III	55	53	9
Adults (17–35 years)	68	65	3

function [10], were reported to have a probability of survival of 92%, and of disease-free survival of 87%, similar to results for patients in other age groups.

Rejection

It was feared from the beginning of the era of bone-marrow transplantation that graft rejection would represent a major problem in multiply transfused patients. It appears that several factors may facilitate rejection (Table 18.8). In one study [17], patients who had received less than 100 red blood cell transfusions before transplantation had a risk of rejection three times higher than patients who had received more transfusions. Rejection may present in different configurations [18]. Patients either fail to take, remaining aplastic, or promptly regrow their thalassaemic marrow. Alternatively they show delayed graft failure normally with autologous recovery. Approximately two-thirds of these events were observed within the first 100 days from transplant. In no series was rejection observed after two years.

Engraftment can be documented by β-chain synthesis, by chromosomal studies or by the polymerase chain reaction of the DNA polymorphisms.

In Thailand, a substantial number of patients treated with allogeneic bone-marrow transplantation have had transient engraftment and survive with recurrent thalassaemia [19]. These patients had, in general, been poorly transfused and not chelated, had considerable hepatosplenomegaly and very active medullary and extramedullary erythropoiesis, in addition to iron-induced organ damage.

The presence of mixed chimaerism two months after transplant has been reported to represent a risk of rejection [20]. In fact, two years after transplant, none

Table 18.8 Risk factors for graft failure

HLA disparity
Class III disease
Less than 100 transfusions
No cyclosporin
Busulfan dose <14 mg/kg

of the patients whose engraftment was complete had rejected, as compared with 29% of those who still had residual host cells. If the percentage of host cells present at two months was higher than 25%, the risk of rejection at two years reached 90%. In three patients with long-term, stable mixed chimaerism, host haematopoiesis has been observed to fluctuate between 25 and 75%. Despite these high percentages of residual host haematopoiesis, the patients produced sufficent donor β-globin chains and haemoglobin.

Good results have also been reported in patients who had developed mixed chimaerism, with the infusion of donor peripheral blood stem cells [21].

Autologous harvesting and cryopreservation prior to bone marrow ablation is regularly performed in some centres and it has been used as a rescue in a few patients who have developed aplasia.

Both the International Bone Marrow Transplantation Registry [22] and the Pesaro group [18] found a higher rate of rejection in patients who received methotrexate alone as GvHD prophylaxis. In addition, a dose of busulfan lower than 14 mg/kg was associated

with an increased risk of rejection in patients reported by some of the transplant groups [15].

Infectious complications

Transfusion-dependent thalassaemia patients are exposed to the life-long risk of bloodborne infections. The prevalence of cytomegalovirus (CMV) seropositivity in thalassaemia patients is high and increases with age. Its clinical impact on the post-grafting course has been variable, causing severe problems in some centres at the beginning of the transplant era. At present, most transplant teams monitor the presence of the CMV antigen phosphoprotein pp65 in the granulocytes of the peripheral blood and institute early therapy with intravenous immunoglobulins, ganciclovir or foscarnet when necessary [23]. In some cases, CMV-negative blood products are used for CMV-negative recipients.

In thalassaemia patients, chronic, persistent or active hepatitis is a frequent event, especially in the older age groups [24]. The infectious process, together with iron overload, contributes to the pathological changes. The presence of pretransplant liver disease is known to be an important risk factor for developing veno-occlusive disease of the liver in the early post-transplant period [25]. Hepatitis B has been reported to have reactivated after transplant [26].

Approximately 1.5% of the European patients regularly transfused for thalassaemia had been infected with the human immunodeficiency virus before blood donations were systematically screened. No seroconversion occurred among previously seronegative patients after screening was introduced [27].

Chronic GvHD

Between 6% [8] and 24% [16] of the patients surviving with a functioning graft for at least 150 days developed chronic GvHD, of moderate or severe grade. This complication is poorly tolerated by patients with thalassaemia, who undergo transplantation to remove the constraints of a chronic disease upon their lifestyle.

Second transplants

Second transplants have occasionally been performed in patients who have rejected their graft. Conditioning protocols for second transplant include ALG with or without TLI, cyclophosphamide, and in a few cases, thiotepa, busulfan or melphalan. Again, the largest experience has been collected in Pesaro [28] where 22 second transplants have been reported. A median time of 400 days had elapsed between rejection and second transplant. Survival was 52%, with a rejection rate of 50% and an event-free survival of approximately 30%. The non-rejection mortality was 34% and attributable largely to infections. Interestingly, the second rejection always evolved toward aplasia, without regrowth of the thalassaemic bone marrow.

Growth, development and fertility

Retarded or absent puberty and reduced fertility are common problems in patients with thalassaemia treated with transfusions and chelation, and are a consequence of transfusional haemosiderosis. Secondary amenorrhoea is also common. Damage has been shown to involve the pituitary, the gonads or both. Pregnancy has been reported in a few patients [29] and several males have fathered children. A large multicentre study showed that approximately 50% of thalassaemia patients undergo spontaneous pubertal development [30]. Gonadal and thyroid dysfunction and decreased growth velocity have been observed in patients transplanted for haematological malignancies after TBI [31], while children transplanted for aplastic anaemia following cyclophosphamide had very few abnormalities [32].

Of 68 patients transplanted for thalassaemia, after a mean of 7.5 years from the procedure, only 32% had reached mid-advanced puberty [33]. Sterility from pretransplant conditioning is considered a possible side-effect of the procedure, although several reports have now appeared showing that pregnancy is possible after bone-marrow transplantation for aplastic anaemia, preconditioned with cyclophosphamide, and even with thoraco-abdominal irradiation or TLI. Reported cases in which pregnancy ensued in patients transplanted for haematological malignancies prepared with carmustine or busulfan with or without total body irradiation, AraC, daunorubicin and 6-thioguanine are less numerous. To date, only one ex-thalassaemic patient has become pregnant and delivered a full-term, normal infant, despite late pubertal maturation and oligomenorrhoea. She underwent bone-marrow transplantation at 10 years of age after ablative therapy with busulfan and cyclophosphomide [34].

Adolescent or adult thalassaemic males who have reached puberty should probably be offered sperm banking before transplantation, since it is likely that the majority of them will become severely oligospermic or azoospermic as an effect of the alkylating agents used in conditioning.

Malignancies

Secondary malignancies, including lymphoproliferative diseases and solid tumours, have been reported to develop with higher frequency in transplant recipients than in the general population. In one study [35], the relative risk of developing a secondary non-Hodgkin lymphoma after BMT for conditions other than thalassaemia, was 355. Lymphomas which appear within the first 300 days are usually Epstein-Barr virus (EBV)-related, are more frequent in HLA partially matched grafts and tend to have a poor prognosis. Non-Hodgkin lymphomas have been observed in 5 patients transplanted for thalassaemia [23,36], between 40 and 210 days after grafting. In all cases, the phenotype was B, and in 3 the histology was immunoblastic. One patient is alive and well after surgery and radiotherapy; the remaining 4 have died. Only two solid tumours have been reported. In one case, a Kaposi sarcoma of the skin regressed after immunosuppressive therapy was discontinued [37].

We have reported an excess of malignancies in a population of thalassaemia patients receiving conventional therapy [38]. This excess could be due to chance alone, but the

possibility that a link exists between haemoglobinopathies and haematological malignancies must be considered [39].

Causes of death

The most common cause of death has been infection, often in patients who have remained aplastic after graft rejection. A few splenectomized patients have died of post-splenectomy sepsis after transplant. Other causes of death are depicted in Table 18.9. The potential complications of BMT are presented in Table 18.10.

Iron depletion

Iron overload represents the main cause of morbidity and mortality in transfusion-dependent thalassaemia. After BMT, the serum ferritin is found to increase, reaching a peak around the third month after grafting, probably as a consequence of a shift of iron from the erythron to the storage compartment during the period of bone-marrow aplasia. Subsequently, the ferritin stabilizes to pretransplant levels, as does the liver iron concentration. Sequential liver biopsies have demonstrated that, in the absence of therapeutic intervention, iron concentrations remain stable, and hepatic fibrosis, often present before transplantation, may increase and even progress to cirrhosis. In recent years, phlebotomy has been adopted by most centres for patients with high ferritin levels and/or high liver iron concentrations measured before transplant. In one study, 42 patients underwent bimonthly venesections starting at least 18 months after grafting. After a mean of 36 months, the median ferritin had decreased from 2587 to 417 mg/litre and the median liver iron concentration from 20.8 to 4.2 mg/g dry tissue [40]. In addition, a decrease in the transaminase levels and in the inflammatory lesions on liver biopsy was observed [41].

An alternative to phlebotomy is monthly erythrocytapheresis, which has the advantage of saving plasma and mononucleated cells, but it is more time-consuming and requires a blood cell separator [42].

Desferrioxamine can be used in patients in whom low levels of haemoglobin after transplantation or difficult venous access prevent phlebotomy. However, its use is best avoided if possible, because desferrioxamine has been reported to interfere with grafting. Chelation should therefore not be started early after transplant if its use is unavoidable.

Cord blood transplants

The first successful transplant in a thalassaemic patient using cord blood stem cells was reported in 1995 from Thailand [43]. Subsequently, a few more such transplants have been performed in Thailand [19], in China [44] and in Europe. In some cases, combined cord blood and bone-marrow cells have been infused [45]. Theoretical advantages of cord blood with respect to bone marrow include a superior potential for engraftment, a lower risk of GvHD, including after

Table 18.9 Causes of death after BMT for thalassaemia

Infection
Acute GvHD
Idiopathic interstitial pneumonia
Cardiac failure
Thrombocytopenia
Cardiac tamponade
Acute liver failure
Lymphoma

Table 18.10 Long-term post-transplant complications

Due to transplantation
GvHD
Malignancies
Hormonal dysfunction
Sterility
Due to thalassaemia
Haemosiderosis
Hormonal dysfunction
Malignancies?

use in mismatched recipients [46], a lower risk of transmission of viral infections, and the avoidance of general anaesthaesia to the donor sibling. The main disadvantage is represented by the longer time necessary to reach safe platelet counts. The administration of growth factors promises, however, to overcome this problem. In addition to its use in transplantation, cord blood may in future have an application in gene therapy [47].

In utero *transplants*

Early in gestation, the foetus is immuno-incompetent and therefore unable to reject allogeneic cells. Several attempts at *in utero* stem-cell transplantation have been performed [48]. None of those performed for thalassaemia have, however, to our knowledge been successful.

Gene therapy

Thalassaemia represents an ideal disease model for gene therapy [49]. In fact, the globin genes are small and haematopoietic stem cells can be obtained easily from peripheral blood, from cord blood or from bone marrow. Once genetically modified, the cells can then be grafted. Studies of mixed chimaerism suggest that small quantities of transformed cells may be sufficent to correct the genetic error. Experience gained with bone marrow and cord blood transplantation will be very useful in this context.

Cost

In the USA, the cost of chelation and transfusion has been calculated to average $30000 per year per patient, before the appearance of complications. The high initial price of BMT, therefore, corresponds to no more than five or six years of conventional treatment.

Conclusions

The decision concerning whether a patient with thalassaemia should undergo BMT is often not easy, at least in developed countries where chelation and relatively safe, compatible blood are available to all. Elsewhere, the conventional treatment of large numbers of thalassaemic patients poses an additional strain on already limited medical resources, and BMT may represent the only chance of survival for such patients.

It is now clear that well chelated patients in good general condition have a higher disease-free survival after bone-marrow transplantation [50], but these are precisely the patients who have superior long-term survival and a lower percentage of complications when treated conventionally. For older patients in whom iron overload has already produced some damage, both treatments pose a high risk of death in the short or medium term.

The yearly increase in the number of patients transplanted around the world indicates that there is now a wide acceptance of the procedure and its incumbent risks.

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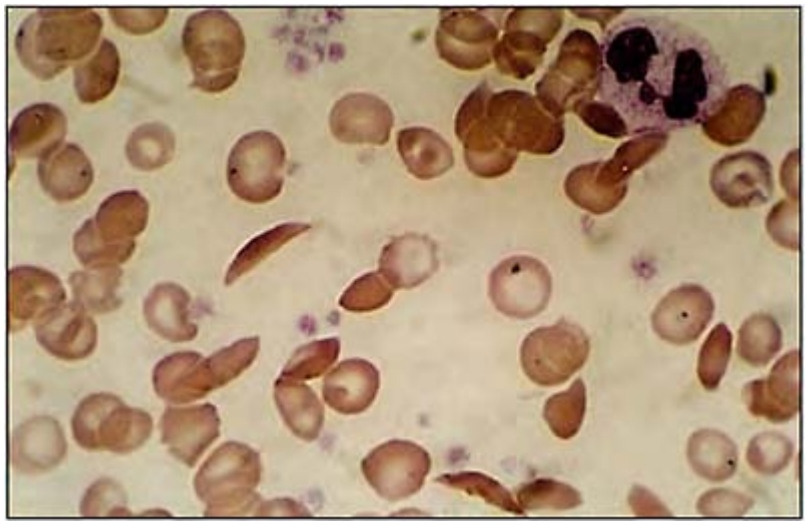

Chapter 19
Sickle cell anaemia

Christiane Vermylen and Guy Cornu

Introduction

Sickle cell anaemia is a hereditary autosomal recessive disorder characterized by chronic haemolytic anaemia, painful episodes due to the occlusion of small vessels and an increased susceptibility to severe, often fatal infections. Vaso-occlusive episodes are related to the tendency of deoxygenated HbS to undergo polymerization, a phenomenon inducing red cell sickling, membrane alterations, haemolysis and changes in the rheology of the red cells. As a consequence, episodic attacks of ischaemic pain and infarcts occur in various organs: bones and joints, the abdomen, the chest, the central nervous system, the kidneys and the eyes, to mention only the organs most frequently involved. Table 19.1 summarizes the primary causes of death from sickle cell disease.

Sickle cell is a disease commonly seen in equatorial Africa, particularly in the west, where 40–50% of the members of certain ethnic groups are carriers of the sickle cell gene. The gene is not restricted to blacks however, as it appears in parts of Turkey, Saudi Arabia, southern India, Sicily, Cyprus and Greece.

The β-globin gene is located on the short arm of chromosome 11. The abnormal β5 globin gene is due to a point mutation which substitutes thymine for adenine in the sixth codon of the gene *GAG/GTG*, thereby encoding valine instead of glutamine in the sixth

position of the β-chain and resulting in the formation of an abnormal haemoglobin, HbS. The β*S* gene is flanked by several distinct polymorphic patterns as part of the β-gene cluster on chromosome 11. This polymorphism gives rise to different haplotypes. Specific haplotypes in African countries are consistently associated with the ethnic groups of the region. Therefore, the haplotypes have been given the name of the geographical area in which they are most frequently found: Benin (Ben), Central African

Table 19.1 Primary causes of death in childhood

• pneumococcal septicaemia
• acute splenic sequestration
• acute chest syndrome
• CVA

Republic (CAR), Senegal (Sen), Saudi Arabia (Sau), Asia or Cameroon (Cam) [1]. The geographical distribution of the β*S* gene coincides with the distribution of malaria, the presence of HbS being associated with protection against the lethal effect of malaria.

Thirty years ago, most internists would have been surprised to encounter an adult with sickle cell anaemia. At the present time, early diagnosis and comprehensive supportive care result in a better survival, although morbidity from vaso-occlusive crises and mortality from progressive organ failure persist especially for people living in Third World countries.

Current therapy includes preventive measures such as population screening, health education and prevention of infections, analgesia in case of vaso-occlusive crises, transfusions or exchange transfusions and hydroxyurea to increase the level of foetal haemoglobin. Other drugs such as butyrates stimulate the production of foetal haemoglobin and trials are ongoing to evaluate long-term efficacy and tolerance. Attempts to perform gene therapy are being made in laboratory animals to replace the defective gene in haematopoietic stem cells but further work is required to increase the level of expression of the inserted gene. Table 19.2 summarizes approaches to the treatment of sickle cell disease.

At the present time, bone-marrow transplantation is the only way to cure a patient with sickle cell anaemia. The first attempt was made by Johnson in a young girl with sickle cell anaemia and acute myeloid leukaemia. The patient had minimal complications following transplantation and haemoglobin electrophoresis confirmed eradication of sickle cell anaemia [2]. Even

Table 19.2 Therapeutic strategies

Modification of underlying pathophysiology:
1. Hb modification—‘anti-sickling agents’
2. Erythrocyte modification—decrease MCHC
3. ‘Genetic modification’
a. augment HbF expression

b.	bone marrow transplantation
c.	gene therapy

though it is very effective, the enthusiasm for bone-marrow transplantation in sickle cell anaemia is restrained by the unpredictable course of the disease [3]. In developed countries and with optimal care, the disease can occasionally be symptom-free for years, but it is not uncommon to see a young patient in whom a devastating stroke is the first symptom of the disease. Genetic factors, environmental factors, poor socio-economic status and problems in obtaining adequate medical support have a determining impact on the disease [4]. Furthermore, patient characteristics such as age or the presence of organ damage and the possibility of using new alternative therapeutic approaches must be put into perspective when discussing bone-marrow transplantation with a patient with sickle cell anaemia.

In our department, bone-marrow transplantation is offered to young patients with symptomatic homozygous sickle cell anaemia, before the occurrence of chronic organ damage and before transfusion-related complications [5,6]. All our patients intended to return to Africa in the near future.

Results of bone-marrow transplantation in sickle cell anaemia

In Europe, 101 patients have undergone bone-marrow transplantation for sickle cell anaemia: 50 in Belgium, 26 in France, 14 in the UK, 6 in Italy, 3 in Germany and 2 in Switzerland [7–11]. In the USA, a further 22 sickle cell patients have undergone bone-marrow transplantation [12,13]. The largest single-centre study includes 32 patients transplanted at the University Hôpital Saint Luc, UCL, Brussels, between April 1986 and January 1996. All our patients came from Central Africa and received bone marrow or cord blood from a human leukocyte antigen (HLA)-identical relative [14]. Patients were between 11 months and 23 years of age (median: 7 years) at the time of transplantation. The conditioning regimen included busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg). Antithymocyte globulin (ATG) was given in patients above the age of 10 years. Graft-versus-host disease (GvHD) prophylaxis included cyclosporin and ‘short’ methotrexate. Prophylaxis of neurological complications included anticonvulsant drugs as well as close monitoring of platelet counts, blood pressure, magnesium and cyclosporin levels.

In all 32 patients, there was a good engraftment. Thirty-one patients are alive with a follow-up ranging from two months to 9.5 years (median: four years). One patient died three months after bone-marrow transplantation of acute GvHD grade III, cytomegalovirus (CMV) and *Aspergillus* infections and lung fibrosis.

In the 31 patients who are doing well, symptoms of vaso-occlusion and haemolysis disappeared soon after transplantation. They have a haemoglobin electrophoresis similar to that of the donor. Five patients have stable mixed chimaerism with predominance of donor cells and are asymptomatic.

Six patients suffered from chronic GvHD: 1 patient has an extensive form of the disease with severe skin involvement, lichenoid changes of the tongue and mild liver disease, 3 have mild involvement of skin, tongue and liver, and the 2 other patients had a localized and transient form of the disease involving only the liver.

As far as long-term side effects are concerned, among the 31 patients, 27 have a follow-up of longer than one year and 9 patients have gone back to their country of origin, the first one in 1988. All of these patients appear to be healthy according to information received in writing or orally.

Among the evaluable patients, bone-marrow transplantation has had no influence on growth and thyroid function. Two patients with chronic GvHD had a transient decrease in growth velocity while on steroid therapy but catch-up growth was observed later. As far as gonadal function is concerned, 2 out of 19 girls underwent bone-marrow transplantation after puberty, and developed secondary amenorrhoea, requiring hormone replacement therapy. All the other girls underwent bone-marrow transplantation before puberty and 3 have delayed pubertal development if we consider that the median age of menarche in sickle cell anaemia is 15 years. The others are still too young to be evaluated.

Among the boys, 3 out of 12 are older than 13 years and have normal pubertal development.

As far as malignancies are concerned, the patient with severe chronic GvHD developed myelodysplasia while receiving intensive immunosuppressive therapy. He recently developed acute myeloid leukaemia. A complete remission was obtained with low doses of AraC.

In our series of 32 patients, the overall survival and disease-free survival are 96%; the event-free survival is 93%. Data from the Collaborative Group Study of Transplantation for Sickle Cell Disease give a survival rate of 88% and an event-free survival of 75% [13]. These differences in the results are mainly due to different selection criteria among the medical teams. In our series, we tend to propose bone-marrow transplantation early, even more so if patients plan to go back to Africa, which is almost always the case.

In other European centres, patients are only considered for transplantation if it has already been shown that their disease is severe [10,11,15]. These patients will therefore have received more blood transfusions and may suffer from chronic organ damage. This is even more true in the USA, where, because of the high standard of conventional care for the majority of patients, selection criteria for transplantation are even more restricted and many patients have already suffered from strokes.

Benefits of bone-marrow transplantation

For the sickle cell patients who do well after bone-marrow transplantation, there is no doubt regarding the benefits of the procedure. Painful crises disappear and haemolysis is no longer present. It is unlikely that patients transplanted early will develop chronic organ damage. Issues that remain unresolved are whether progression of damage in organs including the brain, lungs, liver and kidneys will be halted, or whether lesions will improve or even disappear. It is not known how long patients should wait for bone-

marrow transplantation and how much damage can take place before it becomes irreversible.

The most common complication to occur is splenic infarction, leading to asplenia and an increased susceptibility to infections. So far, it has been shown that recovery of splenic function can be expected after bone-marrow transplantation in a few patients [16].

Preliminary results from the Collaborative Group Study of Transplantation for Sickle Cell Disease show stabilization of lung function and central nervous system vasculopathy [13].

As far as osteonecrosis is concerned, an improvement has been described in 3 patients [17] (Darbyshire, personal communication).

Ferster *et al.* have shown an increase in fetal haemoglobin above 22%, 14, 16 and 39 months after transplantation in 3 patients with graft failure. These previously severely affected patients remain free of symptoms [18]. Table 19.3 summarizes the aims of bone-marrow transplantation in sickle cell disease.

Table 19.3 Aims of bone-marrow transplantation

• Stable Hb with no haemolysis
• No veno-occlusive crises
• Some recovery of splenic function
• Orthopaedic improvement
• Normal growth

Medical problems relating to bone-marrow transplantation

Only a minority of candidates for bone-marrow transplantation are eligible because of the lack of a compatible donor. In sickle cell anaemia, only a fully HLA-matched related bone-marrow transplantation is considered in order to reduce the risks related to the procedure. The theoretical chance of having an HLA-compatible donor not affected by sickle cell anaemia or another major haemoglobinopathy among the siblings, is 1 in 5, whereas for transplants for non-genetic diseases it is 1 in 4 [19].

The large experience in bone-marrow transplantation for thalassaemia and the increasing experience in sickle cell anaemia indicate that the risk of death or severe morbidity is about 10% for bone-marrow transplantation in haemoglobinopathies. Acute GvHD and CMV infection, often associated, require meticulous prophylaxis or treatment.

Patients with sickle cell anaemia are at increased risk of neurological complications after transplantation. In a recent study, the overall incidence of neurological complications after transplantation for sickle cell anaemia was 50% in patients with prior stroke and 23% in those without prior stroke. Seizures were the most common complications, patients with prior stroke being at increased risk for intracranial

haemorrhage [20,21]. The use of busulfan and cyclosporin in the presence of hypertension and thrombocytopenia are adverse risk factors. In our experience, we observed no increase in severe neurological complications, probably because transplantation was carried out at an early stage of the disease. Anticonvulsant prophylaxis, antihypertensive treatment and platelet support are recommended to diminish the frequency of seizures and other neurologic complications.

Long-term complications include chronic GvHD, the possibility of impairment of gonadal function and secondary malignancies [22].

Selection of patients

Several factors must be considered when selecting patients with sickle cell anaemia for bone-marrow transplantation. These include genetic factors, the age of the patient, clinical manifestations of the disease, whether the patient lives in a country where he or she can benefit from optimal care and alternative therapeutic approaches, the feelings of the parents and, of course the presence of a healthy, HLA-identical relative (Table 19.4).

Genetic factors include the presence of α-thalassaemia, the β-globin gene haplotype and hereditary persistence of fetal hemoglobin. The CAR haplotype has low levels of foetal haemoglobin and consequently experiences a more severe clinical outcome [1,4].

The risks related to bone-marrow transplantation increase with age, and the incidence of GvHD is minimal in young children. Clinical manifestations and the presence of chronic organ damage are also more likely to increase with age. In 1991, E.D.Thomas [23] wrote:

> in sickle cell anaemia, we really do not want patients with renal failure, severe neurologic impairment, or pulmonary failure. If we are going to do this [bone marrow transplantation], we ought to do it correctly. We ought to take young patients in whom the risk of GvHD is minimal. Patients who have end-stage complications of vasculopathy are going to cloud the issue.

Access to proper medical care is an important selection factor. The situation is therefore very different in the USA than in Africa. In Third World countries, sickle cell anaemia is, and remains, a very serious condition with a low percentage of patients reaching adulthood. In 1963 in Zaire, only 1% of patients was alive at the age of 18 years. The situation

Table 19.4 Paediatric Haematology Forum, UK criteria for BMT

1	Informed family and patient consent
2	Less than 16 years with HLA matched donor
3a	SS related neurological deficit (CVAs; subarachnoid)

3b	Two or more episodes of acute chest syndrome—not infections, or stage I–II sickle lung disease
3c	Recurrent severe pain
4	Problems regarding future medical care

in 1995 is difficult to evaluate but probably not very different from 30 years ago. In 1989, in Nigeria, 70% of the patients died before the age of five years [24]. In these countries, basic care should be available to all, and the option of bone-marrow transplantation only considered once this goal has been attained. However, if bone-marrow transplantation does have a place in sickle cell anaemia, it is primarily the children in whom the morbidity and mortality are very high and who cannot have the benefit of optimal care who should be considered. The 9 patients who underwent bone-marrow transplantation and went back to Africa, continue to do well and lead a normal life.

The new therapeutic approaches, such as hydroxyurea, require close follow-up and in Third World countries have limited applications. In contrast, in the USA, hydroxyurea may well be considered as an alternative therapy.

Kodish *et al.* has reviewed the factors influencing parental decision with regard to bone-marrow transplantation for sickle cell anaemia. Sixty-seven parents were asked to state the highest mortality risk they would accept before consenting to the procedure to cure their children. Sixteen (24%) were unwilling to accept bone-marrow transplantation with 100% probability of cure and 0% short-term mortality. A further 15 parents (22%) would only consent if mortality did not exceed 5%. Twenty-five (37%) were willing to accept a 15% or greater risk of short-term mortality. Eight parents (12%) were willing to accept a 50% or greater risk of short-term mortality due to transplantation in exchange for a cure for sickle cell anaemia. The parental decision was not related to the severity of their child's disease, but rather reflected socio-economic factors. Parents were most likely to consider bone-marrow transplantation if they had had a high school education, were in full-time employment or had more than one child with sickle cell anaemia [25]. This demonstrates the problem of surrogate decision-taking by parents for children, and we are all aware that the best results are obtained in the youngest children, unable to give their own informed consent.

Conclusion

Bone-marrow transplantation offers a high probability of cure to patients with sickle cell anaemia, but whether this option should be offered to all patients or parents remains unclear. The controversy persists. On one hand, we could follow the guidelines of E.D. Thomas and give the best chances to young children. S. Piomelli recommends can active intervention program in patients with sickle cell syndromes, starting very early in life, possibly no later than 2 or 3 years after birth' [26]. On the other hand, we are reluctant to propose bone-marrow transplantation to patients with mild disease. R.L.Nagel [27] said that:

Table 19.5 Criteria for eligibility for transplantation in children with sickle cell disease in the USA [28]

Criteria for inclusion
Sickle cell disease (sickle cell anaemia, sickle cell haemoglobin C disease, or sickle cell- b-thalassaemia)
Age under 16 years
HLA-identical related donor
One or more of the following:
Stroke or central nervous system event lasting longer than 24 hours
Acute chest syndrome with recurrent hospitalizations or previous exchange transfusions
Recurrent vaso-occlusive pain (more than two episodes per year for several years) or recurrent priapism
Impaired neuropsychological function and abnormal cerebral MRI scan
Stage I or II sickle lung disease
Sickle nephropathy (moderate or severe proteinuria or a glomerular filtration rate 30–50% of the predicted normal value
Bilateral proliferative retinopathy and major visual impairment in a least one eye Osteonecrosis of multiple joints
Red-cell alloimmunization (more than two antibodies) during long-term transfusion therapy
Criteria for exclusion
Age over 15 years
Lack of availability of HLA-identical donor (patients with HLA-matched related donors with the sickle cell trait were not excluded)
One or more of the following:
Karnofsky or Lansky functional performance score <70 (the Lansky performance score is a measure of functional status in children)
Acute hepatitis or evidence of moderate or severe portal fibrosis or cirrhosis on biopsy
Severe renal impairment (glomerular filtration rate <30% of the predicted normal value)
Severe residual functional neurological impairment (other than haeimiplegia alone)
Stage III or IV sickle lung disease
Demonstrated lack of compliance with medical care
Seropositivity for the human immunodeficiency virus

it seems balanced and reasonable to limit marrow transplantation [at the present time] to those patients with sickle cell anaemia who have clinical

evidence of severe and potentially mortal complication(s), but who are not yet so severely affected as to reduce significantly the chances of the procedure to succeed.

In the USA, a collaborative study was initiated by Sullivan to evaluate the results of bone-marrow transplantation in sickle cell anaemia. Patients are only included if they have already shown that they have a severe disorder. The results are encouraging as long as optimal neurological supportive care measures are taken during and after transplantation [28]. Bone-marrow transplantation is still considered on investigational treatment for patients who live in developed countries who are able to benefit from other therapeutic approaches, and inclusion and exclusion criteria are rigorous (Table 19.5). In our series, as the majority of the patients intend to return to Africa, we tend to propose transplantation in a more systematic way and our inclusion criteria are therefore less restrictive. At the present time, it does not seem reasonable to propose hydroxyurea treatment for patients living in Africa, if long-term close follow-up is impossible. Furthermore, the high risk of transfusion-related complications in Third World countries is another factor in favour of a curative approach.

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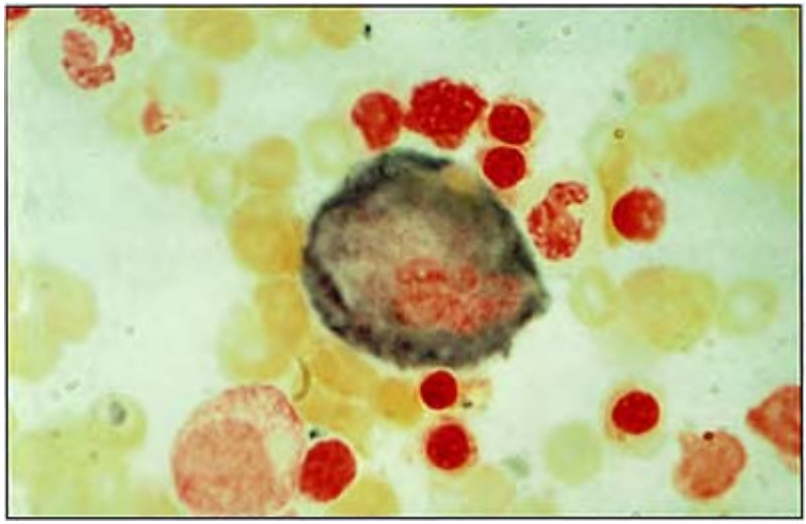

Chapter 20
Lysosomal storage diseases

Peter M.Hoogerbrugge and Dinko Valario

Introduction

Following allogeneic bone-marrow transplantation, the blood cells and tissue macrophages of the patient are gradually replaced by those of the donor. Because of this, allogeneic bone-marrow transplantation is the treatment of choice for diseases involving the haemopoietic system such as aplastic anaemia and severe combined immune deficiency. Since the engrafted haemopoietic stem cells give rise to progeny of all haemopoietic lineages (including the monocyte lineage, as precursors of tissue macrophages in the skin, lung and liver tissue, for example), Hobbs *et al.* [1] proposed that a bone-marrow graft might serve as a permanent source for the missing enzyme α-iduronidase in the treatment of patients with Hurler's disease.

Hypothetically, at least four different mechanisms may contribute to a beneficial effect of allogeneic bone-marrow transplantation in the treatment of lysosomal storage disorders (Table 20.1).

1. In the first place, replacement of the enzymatically deficient cells by enzymatically normal cells may be important, especially in those diseases in which the mononuclear phagocytic cell system is primarily affected, e.g. Gaucher's disease.

2. A second mechanism by which bone-marrow transplantation may be effective is the transfer of enzyme from the enzymatically normal bone-marrow-derived cells to deficient cells by direct cell-cell contact. *In vitro* studies have shown that macrophages and lymphocytes are able to transfer lysosomal enzyme by direct cell-cell contact to enzymatically deficient cells [2,3]. It can be expected that this effect of enzyme transfer by cell-cell interaction will be limited to tissues rich in

Table 20.1 Mechanisms which contribute to improvement after bone-marrow transplantation

Replacement of tissue macrophages and blood cells
Intercellular enzyme transfer
Uptake of donor-derived enzyme in plasma
Clearance of accumulated substrate

bone-marrow-derived cells, including the spleen, liver, lung and skin.

3. Thirdly, bone-marrow transplantation may be effective by releasing enzyme into the plasma by effecting disintegration of donor-derived white blood cells, which then may be taken up by enzymatically deficient cells.
4. Finally, a concentration gradient of storage product between the tissues and the plasma compartment may result from the breakdown of circulating substrate by the lysosomal enzymes present in white blood cells and tissue macrophages of donor origin. Such a gradient may improve clearance of the storage product.

One of the major issues concerning the role of bone-marrow transplantation in metabolic diseases is whether metabolic correction in brain tissue occurs and whether this results in alleviation or prevention of neurological symptoms. Circulating enzyme cannot pass the blood-brain barrier to enter brain tissue, but infiltration of donor-derived cells into brain tissue may contribute to metabolic correction [4]. Circulating enzyme can reach the leptomeninges, resulting in a decrease of storage in these structures, which may lead to amelioration of hydrocephalus and neurological impairment [5].

Although potentially beneficial in a variety of metabolic diseases, two major drawbacks for the widespread use of allogeneic bone-marrow transplantation exist: First, in order to achieve stable engraftment, myeloablative and immunosuppressive treatment prior to bone-marrow transplantation is necessary. This so-called ‘conditioning treatment’, usually with the cytostatic agents cyclophosphamide and busulfan, has significant early and late toxic side-effects. Second, a human leukocyte antigen (HLA)-identical sibling donor is only available for 25–30% of the patients. Therefore, haplo-identical or matched unrelated bone-marrow transplantation must be performed in other patients, and these procedures carry a much higher complication rate. In the future, somatic cell gene therapy of the haematopoietic stem cell may be applicable. As for somatic cell gene therapy, autologous stem cells can be used, this treatment modality is available for all patients, including those lacking an HLA-identical sibling donor. In addition, less intense conditioning treatment may be required to allow engraftment of the genetically repaired, autologous stem cells as compared to the conditioning that is required to achieve engraftment of an allogeneic transplant.

In this chapter, the results of allogeneic bone-marrow transplantation in the treatment of patients with metabolic diseases will be discussed, followed by a review of the current status of stem-cell gene therapy.

Bone-marrow transplantation procedure

In a report of data from the European Group for Bone-marrow Transplantation (EBMT), it was shown that the outcome of the transplant procedure largely depends on the type of bone-marrow transplant performed: if an HLA-identical sibling donor was available, mortality from the procedure was approximately 10%, whereas mortality from non-HLA-identical transplant procedures was 20–25% [6]. A relatively high rejection rate of 15% after HLA-identical transplantation was shown in these studies. Further analysis revealed that this high rejection rate was due to the incidence of rejection in patients transplanted for the various types of muco-polysaccharidosis: 8 of 31 patients who received an HLA-identical transplant for mucopolysaccharidosis rejected the graft (26%) versus none of 21 patients with other diseases. The high rejection rate in patients with mucopolysaccharidoses may be due to a change in metabolism of busulfan in these patients [7]. In patients transplanted with non-HLA-identical sibling grafts, the rejection rate in patients with mucopolysaccharidosis was also slightly higher (7 of 9) than in other patients (5 of 9).

Biochemical data

Detailed data on enzyme distribution after bone-marrow transplantation are available from studies in mice and larger animals with lysosomal storage diseases. Generally, the increase in enzyme level is related to the amount of donor-derived cells present in the various organs of the recipient (Figure 20.1). For example, in bone-marrow and spleen tissue, a rapid increase to donor enzyme levels occurs, as the majority of the cells in these organs will be replaced by those of the donor. Similarly, in patients with lysosomal storage diseases, engraftment generally results in donor enzyme levels in white blood cells within one to four months after bone-marrow transplantation. In a few patients, enzyme levels in leukocytes initially exceeded donor levels for two to three months [6].

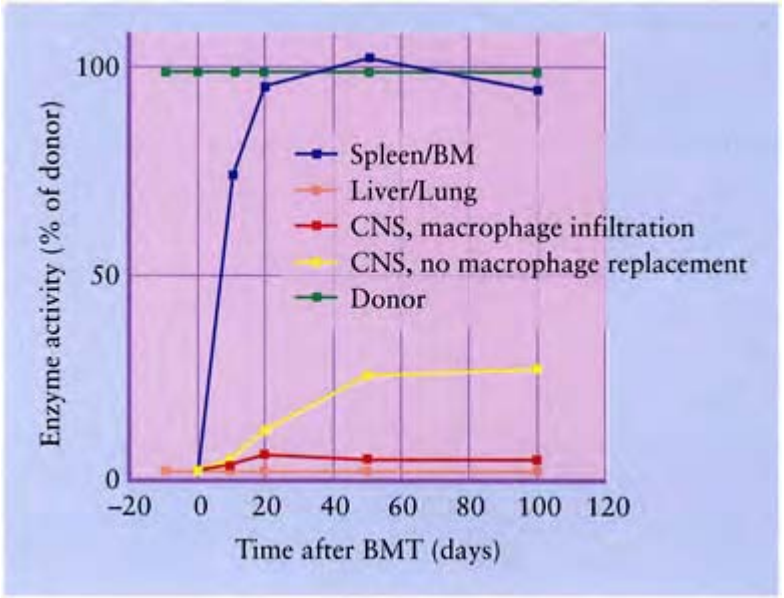

Figure 20.1 Schematic overview of enzyme activities in various organs of mice with lysosomal enzyme deficiencies after bone-marrow transplantation (BMT). Data are adapted from reference [69].

In organs in which only a proportion of the cells are replaced by those of the donor, for example, liver and lung, an increase in enzyme activity to levels between those of donor and recipient are seen (Figure 20.1). Following full or partial enzymatic correction, a decrease in storage product has been reported in a variety of diseases, both in animal models and in human patients. In dogs with mucopolysaccharidosis I, for example, a decrease in accumulation of storage product was reported in liver and brain tissue [8,9]. In human patients, the urinary excretion of storage product is a good measure for the degree of metabolic correction. In a variety of patients with mucopolysaccharidoses and metachromatic leukodystrophy a significant reduction of excretion of storage product occurred.

On the other hand, in the central nervous system, an organ in which little or no infiltration of donor cells occurs and which is protected from the blood circulation by the blood-brain barrier, no or minimal increase in enzyme activity is observed. A significant rise in enzyme activity above background level was absent in the central nervous system of mucopolysaccharidosis VI cats at one year [10] and in mice with a partial deficiency for the enzyme β-glucuronidase at six months after bone-marrow transplantation [11].

In the central nervous system of mucopolysaccharidosis I dogs, only enzyme activity in small amounts, i.e. 1–3% of donor value, was reported, together with a decrease of

glycosaminoglycans storage in cerebrospinal fluid and meninges at more than one year follow-up [8,9]. In transplanted twitcher mice [4,12–14], mannosidosis kittens [15] and fucosidosis dogs [16,17], the enzyme activity in the central nervous system gradually increased to 15–25% of that found in control animals. In twitcher mice, this was explained by infiltration into the brain tissue of enzymatically competent, donor-derived cells [4]. Data from twitcher mice and fucosidosis dogs illustrate the contribution of the time point of transplantation to the final outcome: animals transplanted early, i.e. before the onset of symptoms, showed prolonged survival after bone-marrow transplantation, in contrast to animals transplanted in disease progression. Few data on the enzyme activity in human brain tissue following bone-marrow transplantation for metabolic diseases are reported: In a patient transplanted for Gaucher's disease, no increase in enzyme activity in brain tissue was found at death, two years after bone-marrow transplantation [18].

Following bone-marrow transplantation in patients with adrenoleukodystrophy, a peroxisomal disorder, reduced levels of very long-chain fatty acids have been reported [19].

Clinical data

Although the effect of bone-marrow transplantation on the biochemical parameters shows a relatively uniform pattern as described above, the effect of bone-marrow transplantation on the clinical course of the disease is often less clear. Analysis of clinical follow-up is complicated by the heterogeneity of the patient group with respect to the type of disease and disease progression at the moment of bone-marrow transplantation. Despite these caveats, some general conclusions can be drawn.

Gaucher's disease

In Gaucher's disease (glucocerebrosidase deficiency), the tissue macrophages are not able to metabolize glucocerebroside, a major breakdown product of cell membranes, for example, membranes of degraded blood cells. This results in accumulation within the tissue macrophages of glucocerebroside [20]. As macrophages are gradually replaced after bone-marrow transplantation, it is logical that Gaucher's disease was one of the first diseases to be treated by bone-marrow transplantation. Following bone-marrow transplantation for the non-neurological forms of the disease, successful engraftment has resulted in the disappearance of all symptoms [21]. In forms of the disease with neurological involvement, clinical improvement has not been reported. Tsai *et al.* reported that in a patient who died two years after bone-marrow transplantation, no rise in enzyme level occurred in the central nervous system [18]. Improved results have been reported in patients who underwent splenectomy prior to bone-marrow transplantation as compared to patients without splenectomy [22].

Purified or recombinant glucocerebrosidase also results in reduction of hepatosplenomegaly and improvement in haematological parameters [23]. Life-long treatment with this drug may be an alternative to allogeneic bone-marrow transplantation, especially if no HLA-identical donor is available.

Mucopolysaccharidoses

The majority of patients who have received a bone-marrow transplant for metabolic disease have suffered from mucopolysaccharidoses, predominantly mucopolysaccharidosis IH, or Hurler's disease, caused by α-iduronidase deficiency. The first patient with a lysosomal storage disease in whom a bone-marrow transplant was reported suffered from this disease [1]. In patients with mucopolysaccharidoses, glycosaminoglycans accumulate in connective tissue, heart muscle, liver and the central nervous system. Bone deformities, corneal clouding, cardiac symptoms and mental retardation may develop in these patients. Long-term follow-up data have been published by Downie *et al.* [24] and Whitley *et al.* [5] for patients with Hurler's disease. They showed that 'visceral symptoms' including hepatosplenomegaly, respiratory problems and cardiac involvement [25] improved following engraftment. Generally, it can be concluded that the progression of bone lesions diminishes or even stops; improvement in bone lesions has not been reported. No improvement of neurological symptoms occurs, although long-term follow-up data suggest a stabilization of neurological symptoms. For example, data on patients reported in the registry of the EBMT show stabilization or improvement of the developmental quotient (Figure 20.2). These data are in agreement with studies in dogs with mucopolysaccharidosis I, where increased enzyme and decreased storage product in the central nervous system were observed after bone-marrow transplantation [8,9,26]. Based on these data, it can be recommended that patients with Hurler's disease who have an HLA-identical sibling donor be transplanted before the age of two years. In children lacking an HLA-identical sibling donor, a decision on whether or not to perform a bone-marrow transplant with marrow from a matched unrelated donor should be made on an individual basis.

Broadly speaking, two forms of Hunter disease (mucopolysaccharidosis II, iduronate sulfatase deficiency) can be identified: a patient with the milder form of the disease, in whom neurological defects were absent, received a bone-marrow transplant at the age of 14 years. Following engraftment, he remained neurologically normal and his visceral symptoms improved [27]. After bone-marrow transplantation in patients with severe neurological symptoms at bone-marrow transplantation, progression of neurological symptoms has occurred [28,29].

A variety of patients has been transplanted for Sanfilippo's disease (mucopolysaccharidosis III). Klein *et al.* reported the neuropsychological testing in 8 of 11 successfully engrafted patients, all of whom progressed, even if bone-marrow transplantation was performed very early in the course of the disease [30]. Similar disease progression after bone-marrow transplantation has been reported by others [6,31]. Based on these data, it can be concluded that bone-marrow transplantation does not alter the disease process in Sanfilippo's disease.

In patients transplanted for Morquio's disease (mucopolysaccharidosis IV), the skeletal abnormalities did not improve despite proper donor-cell engraftment [32].

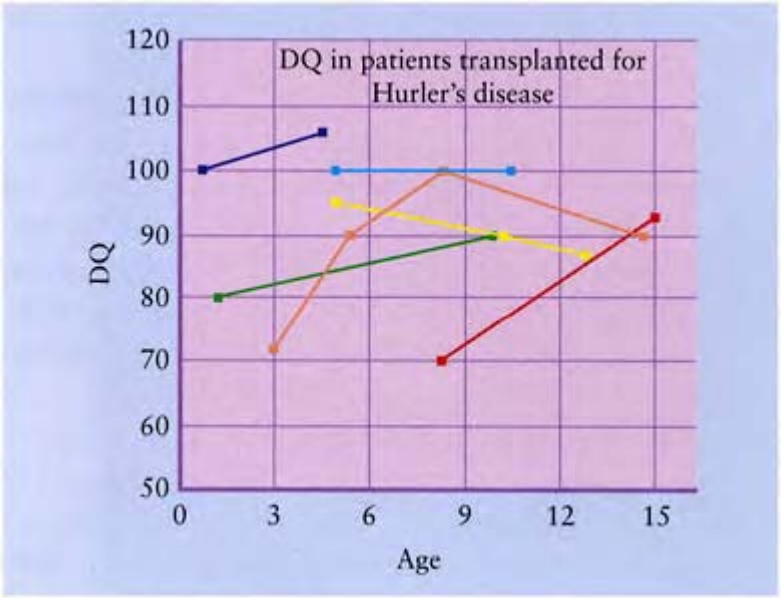

Figure 20.2 Developmental quotients (DQ) in patients with Hurler's disease at bone-marrow transplantation (left end of curves) and at various time points after bone-marrow transplantation. Data are adapted from reference [6].

One well-documented patient transplanted for Maroteaux-Lamy disease (mucopolysaccharidosis VI) with progressive, life-threatening disease, showed dramatic improvement in cardiopulmonary function [33]. Remodelling of skeletal lesions was not observed. This patient is currently doing very well with normal intelligence [34]. At least 6 other patients have been transplanted, but the disease stages varied considerably and conclusions concerning outcome are hard to draw yet.

Lysosomal storage diseases primarily affecting the central nervous system

Metachromatic leukodystrophy (arylsulphatase-A deficiency) can be divided into three clinical disease patterns: late infantile, juvenile and adult type, depending on the age at which the disease becomes manifest. The late infantile form shows a very rapidly progressive disease pattern, which appears not to be beneficially influenced by bone-marrow transplantation [35], although some stabilization of the disease may occur [36].

Stabilization of the disease has been reported following bone-marrow transplantation in adult type metachromatic leukodystrophy [6,37].

In a patient with late onset globoid cell leukodystrophy (Krabbe's disease; galactocerebrosidase deficiency) who presented with rapidly progressive impaired visual acuity and mild neurological symptoms and neuroradiological abnormalities, bone-marrow transplantation was performed four months after diagnosis. After bone-marrow transplantation, stabilization of the disease process occurred [6]. Similar data have been reported by Shapiro *et al.* [38], whereas data on bone-marrow transplantation in the infantile form of the disease are disappointing [38].

In a recent report from the EBMT, it was shown that in patients suffering from Tay-Sachs disease, Farber's disease and Sandhoff disease with significant neurological symptoms, bone-marrow transplantation did not alter the progressive course of the disease [6]. Similar failures have been reported earlier for heavily affected children with GM1 gangliosidosis [39] and dogs with Batten's disease [40].

Other lysosomal storage diseases

So far, two patients have been reported in detail following transplantation for Niemann-Pick disease. One patient with Niemann-Pick type-B disease had hepatosplenomegaly with no other symptoms. Following bone-marrow transplantation, the size of liver and spleen decreased [41], but with longer follow-up, signs of neurodevelopmental regression have been reported [42]. A patient with Niemann-Pick type-IA disease had minimal developmental delay at transplantation, performed at four months of age. One year later, neurodevelopmental impairment had progressed, and no decrease in hepatosplenomegaly had occurred. This patient died 30 months after bone-marrow transplantation. Enzyme activity was virtually undetectable in brain and liver tissue at autopsy [43].

Following bone-marrow transplantation in dogs with fucosidosis (α-L-fucosidase deficiency), enzymatic correction of brain tissue occurred, and prevention of clinical symptoms was seen if the transplant was performed early in the disease process. Vellodi *et al.* [44] reported a patient treated at the onset of clinical symptoms, where the disease progressed, but at a much slower rate than in an affected sibling.

Bone-marrow transplantation failed to alter the disease course in a patient with glycogen storage disease type IIa [45], and follow-up was too short to evaluate the effect of bone-marrow transplantation for mannosidosis [46], as the patient died with transplant-related complications.

Although this review focuses on the role of bone-marrow transplantation in the treatment of lysosomal storage diseases, it has to be stressed that allogeneic bone-marrow transplantation has also been beneficial in a variety of patients treated for the peroxisomal disorder adrenoleukodystrophy [19,34]. The optimum time to perform a transplant in this disease is still a point of debate. On the one hand, bone-marrow transplantation is most effective if performed early, but on the other hand, the disease process can be variable even within one family, so that it is sometimes unclear how severely affected a patient will become if untreated. Details concerning the potential of bone-marrow transplantation in adrenoleukodystrophy have been reviewed recently [34].

Summary

In the past 15 years, more than 200 patients with lysosomal storage diseases have been treated with allogeneic bone-marrow transplantation. Caution in evaluating the possible effects of bone-marrow transplantation in individual patients is warranted, as the natural course of the disease in humans suffering from lysosomal storage diseases is variable, sometimes even within one family, and patients have been treated at different disease stages. Despite these caveats, some general conclusions about the effects of bone-marrow transplantation on disease progression seem justified:

1. A reduction of storage material in visceral organs (heart, lungs, tonsils, liver and spleen) can be expected in the majority of patients. This may result in clinical improvement in mucopolysaccharidosis patients with severe cardiac or respiratory disease, and in patients with non-neurological forms of Gaucher and Niemann-Pick disease, but is insufficient to improve the quality of life of patients with severe musculoskeletal or neurological involvement.
2. Improvement of skeletal deformities in mucopolysaccharidoses is absent following bone-marrow transplantation, but stabilization of the symptoms has been reported.
3. Neurological involvement is a major problem of the majority of lysosomal storage diseases. To date, clear improvement of neurological symptoms in heavily affected patients has not been observed, but stabilization or prevention of neurological lesions by early bone-marrow transplantation is reported, stressing the relevance of performing bone-marrow transplantation early in course of the disease.

Stem-cell gene therapy

In principle, patients with metabolic diseases that improve after allogeneic bone-marrow transplantation will also benefit from reinfusion of autologous stem cells in which a correct version of the gene causing the disease is expressed. Obvious advantages of successful

Table 20.2 Bone-marrow transplantation versus stem-cell gene therapy in the treatment of lysosomal enzyme deficiencies

Parameter	Allogeneic marrow transplantation	Stem-cell gene therapy
Donor availability	25–30%	100%
Conditioning	Myeloablative	Non-myeloablative
Complications	Graft-versus-host disease; take failure	None to date
Current state of art	Success reported in animals and humans	Success reported in animals; not yet in humans

gene therapy over allogeneic bone-marrow transplantation are summarized in Table 20.2. As autologous stem cells are reinfused in gene therapy, the procedure can be performed in any patient, in contrast to allogeneic bone-marrow transplantation, in which a donor is available in 25–30% of patients. So far, no immune-mediated rejection of the transduced stem cells has been reported, but recently, an antibody response in dogs with mucopolysaccharidosis I against human iduronidase was observed [47]. Conditioning prior to reinfusion of the transduced stem cells is only needed to 'create space' for the incoming stem cells. Figure 20.3 shows that engraftment of transduced cells

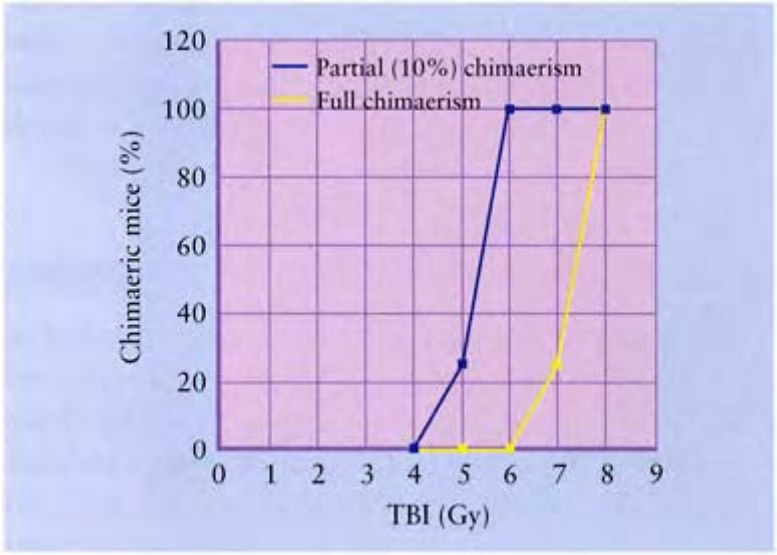

Figure 20.3 Effect of conditioning with total body irradiation (TBI) on engraftment of genetically transduced haematopoietic stem cells. Male bone marrow cells were transduced by co-cultivation with virus-producing cells; 10^6 bone-marrow cells were injected in female recipient mice. Chimaerism was determined by Y-chromosome analysis at one year after transplantation.

can be obtained after sublethal, mild conditioning, in contrast to engraftment of allogeneic stem cells, for which more intense conditioning is needed. Most studies indicate that some conditioning is needed [48–50], although one study reported efficient engraftment of genetically modified cells in the absence of conditioning [51].

Successful stem-cell gene therapy is dependent on an efficient gene-transfer system which has to result in:

- efficient gene transfer into haematopoietic stem cells;
- stable integration of the introduced gene;
- lasting expression of the introduced gene;
- safety for both the patient and its environment.

At present, retrovirus-mediated gene transfer is the only technique that fulfils these requirements, although still at limited levels in primates. Therefore, the majority of the current approaches towards stem-cell gene therapy make use of retroviral vectors. To date, initial studies suggest that expression of introduced genes can, at least *in vitro*, also be obtained after adenovirus and adeno-associated virus-mediated gene transfer. Details about current gene-transfer methods have been reviewed recently [52,53].

In a retrovirus vector, the retroviral genes *gag, pol* and *env*, which are essential for the replication of the virus, are replaced by the cDNA of the gene of interest, the gene encoding glucocerebrosidase in patients with Gaucher's disease, for example (Figure 20.4). This viral construct is introduced into so-called 'packaging cell lines'. Packaging cell lines provide the viral proteins encoded by the genes *gag, pol* and *env*, which are required for the production of virus particles. Packaging cells themselves are incapable of releasing infectious virus because the packaging signal (ψ) has

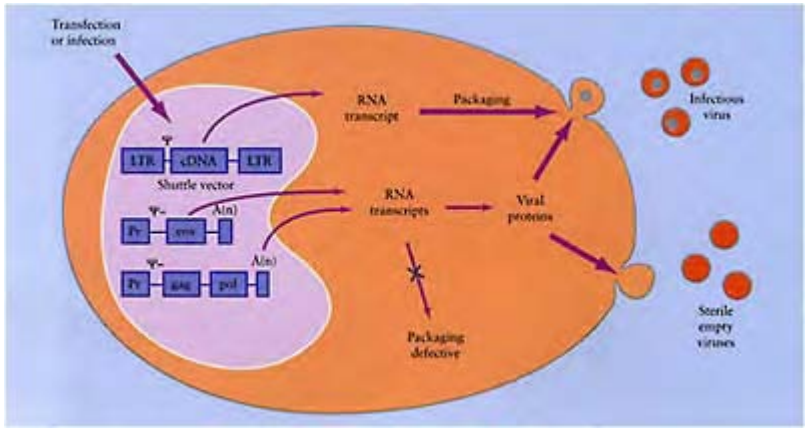

Figure 20.4 Principle of production of retrovirus vectors by virus producer cell line: the producer cell line produces viral proteins, but the RNA transcripts which are essential for the production of viral proteins are not packaged in the viruses. As the packaging signal Ψ is present on the RNA transcripts of the shuttle vector,

these transcripts will be packaged in the virus. The shuttle vector carries the cDNA of the gene of interest.

been deleted from the genes encoding *gag, pol* and *env*, so that the RNA cannot be packaged in the virus (Figure 20.4). Subsequently, a retroviral vector, which carries the gene of interest and the packaging signal ψ, will be introduced into the packaging cell line. This results in a so-called 'virus-producing cell line' that can transcribe the recombinant retroviral genome which can be packaged into virus particles. These virus particles will be released into the culture medium and are able to infect cells that carry receptors for the retroviruses. As the virus does not contain the *gag, pol* and *env* genes, it is replication-defective. By culturing the target cells in virus supernatant-containing medium, gene transfer can be accomplished. Stable integration of the gene only occurs in dividing cells [54]. Therefore, culture conditions have to be developed in which replication of haemopoietic stem cells occurs, without induction of differentiation. Various methods have been described to attain this goal (e.g. adding haemopoietic growth factors and cytokines, culturing in the presence of stroma cells. etc.). To date, efficient expression of the newly introduced gene has been reported after gene transfer into murine stem cells [55–58]. However, after transfer into haemopoietic stem cells of larger animals and human patients, gene transfer efficiency is much lower.

Gene therapy for metabolic diseases

Stem-cell gene therapy has been reported in a variety of murine models of metabolic diseases (for review, see [53]). These include mice with mucopolysaccharidosis VII, where disappearance of lysosomal storage product was described after reinfusion of genetically repaired haemopoietic stem cells. An important additional observation in these studies is the finding that after milder conditioning (4.5 Gy total body irradiation) than necessary for allogeneic bone-marrow transplantation (9–10 Gy), partial 'chimaerism' occurred, which was sufficient to effect metabolic correction of the disease [49]. In addition to stem-cell gene transfer, implantation of so-called 'neo-organs' containing genetically engineered fibroblasts have also been used successfully in mice and dogs [59,60].

Until now, metabolic correction of cultured human fibroblasts by retrovirus-mediated gene transfer has been reported for a variety of diseases including Niemann-Pick disease [61], Gaucher's disease [62], metachromatic leukodystrophy [63] and adreno-leukodystrophy [64], indicating that successful stem-cell gene therapy is a therapeutic option in these diseases.

In various preclinical studies, efficient gene transfer has been reported into haemopoietic progenitor cells cultured in various *in vitro* systems (reviewed in [65,66]). A major concern in these studies is that the gene-transfer efficiency into progenitor cells determined in these assays is generally 1–2 logs higher than gene-transfer efficiency into stem cells that repopulate myeloablated recipients: *in vitro* assays show gene transfer into 10–50% of progenitors, whereas stem-cell gene transfer in *in vivo* studies in larger

animals and humans is generally less than 1–5% [67,68]. Despite these caveats, the first clinical gene-therapy studies for patients with lysosomal storage diseases have started in patients with Gaucher disease. With the current vectors, high levels of expression can generally be achieved if the vector integrates with the cellular genome. Therefore, at present the main challenge for *ex vivo* stem-cell gene therapy is to find ways to trigger the stem cells into cycle, without inducing differentiation.

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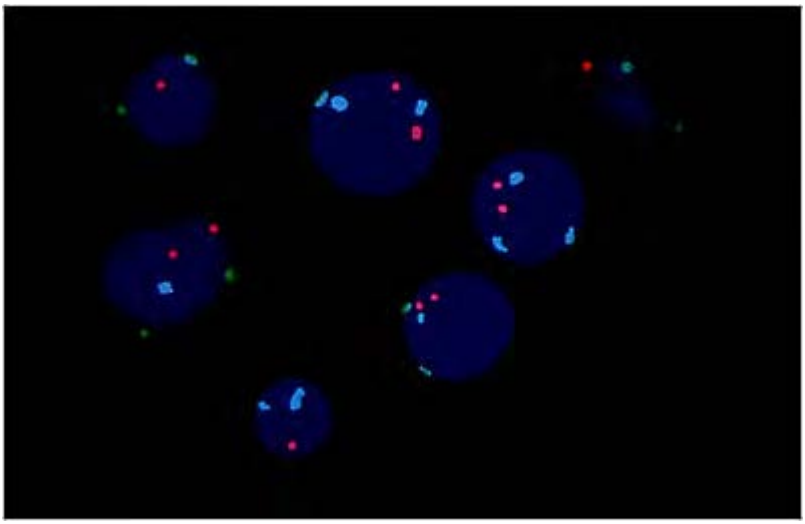

Chapter 21
Chronic lymphocytic leukaemia

Mouricette Michallet

Introduction

Chronic lymphocytic leukaemia (CLL), the most frequent type of adult leukaemia in Western countries, is a clonal haematopoietic disorder with proliferation and accumulation of small lymphocytes usually of B-cell lineage [1–3]. Use of innovative dose-intensive therapies requires justification, and careful evaluation of patients should be made if these treatment approaches are being considered.

Age

Although CLL is usually a disease of the elderly, it is increasingly being diagnosed in younger people. A recent report indicates that about 10% of patients are less than 50 years old [4]. In France, 50% of patients diagnosed with B and C stages of the disease and enrolled in national protocols were less than 60 years old.

Clinical stages [5,6], bone-marrow histology [7,8], blood lymphocyte counts [9,10], lymphocyte doubling time [11,12] and cytogenetics are reliable predictors of patient outcome. CLL in younger adults has no major distinctive features, prognostic factors being the same as those used with older patients. Median survival is less than three years for young patients with advanced CLL [4], and hence in such cases innovative dose-intensive therapies are often justified.

Choice of therapy

The choice of therapy for CLL in stages B and C remains difficult, with median survival times of five years and 22 months, respectively [13]. The disease can be treated with alkylating agents, with or without prednisolone [13]. The addition of anthracyclines may improve the outlook but the median survival time remains less than four and six years for stages C and B, respectively [13,14]. Considerable interest has recently been generated by the use of the new purine analogues: fludarabine, deoxycorfomycin and 2-chlorodeoxyadenosine (2-CDA) [15,16]. Despite high response rates, there is little evidence that fludarabine will result in durable responses and cures. Nevertheless, two randomized studies [17,18] have demonstrated that fludarabine achieves more complete responses than anthracyclines or chlorambucil-containing schedules. Fludarabine and cyclophosphamide used in a single regimen seemed extremely active with a response rate of close to 100% in patients not previously refractory to fludarabine [19]. One study showed that consolidation therapy with high-dose cyclophosphamide reduced minimal residual disease (MRD) in CLL patients treated with fludarabine as induction therapy [20].

The outcome of therapy for CLL patients who either received fludarabine as salvage therapy or who failed on, or relapsed after their first fludarabine regimen, has not been defined. A median survival of 48 and 72 weeks, and a response of 7% and 11% to subsequent therapies for refractory patients and those receiving fludarabine as salvage therapy justify the consideration of innovative dose-intensive therapies [21]. Recently, the outcome of 227 patients who failed on or relapsed after their first fludarabine regimen was studied [22]. The response rate was 21% complete remission (CR) and 21% partial remission (PR) for the relapsed patients, and 2% CR and 5% PR for the refractory patients.

The prognosis for patients failing therapy with fludarabine [21–22] depends upon the extent of prior therapy and other clinical variables, and the combination of fludarabine and cyclophosphamide appears to be emerging as the most effective salvage treatment for patients previously treated with fludarabine. The poor prognosis and median survival of 21 months and eight months for relapsed and refractory patients, respectively, allow consideration of intensive therapy followed by transplantation for these patients.

Allogeneic and autologous transplantation in CLL

Fludarabine may produce complete remissions [23,24] and allow autologous bone-marrow or peripheral blood progenitor cells (PBPC) harvest for transplantation. The feasibility of PBPC mobilization and transplantation after fludarabine therapy has been

demonstrated [25]. Positive and negative cell selection has become available to deplete small numbers of residual leukaemic cells present in the autologous marrow or PBPC. In addition to immunophenotyping, clonal rearrangement of the immunoglobulin heavy chain locus (IgH) and detection of CDRIII region by the polymerase chain reaction (PCR) provide useful markers for the detection of MRD after negative or positive cell selection in the graft and after intensive treatment. Finally, improved supportive care allows more effective use of dose-intensive therapies in older patients. CLL is generally sensitive to alkylating agents and radiation [26].

High-dose cyclophosphamide and total body irradiation (TBI) with bone-marrow transplantation is a well-established preparative regimen that can produce prolonged disease-free survival (DFS) in a variety of haematologic malignancies. Khouri *et al.* [27] reported results of 11 allotransplants and 11 autotransplants for CLL (7 received marrow purged with anti-CD19). All the autotransplanted patients had a lymphocyte infiltration in the marrow of less than 15%. The conditioning regimen was standard (cyclophosphamide 120mg/kg and TBI 10 Gy). Among allografted patients, 6 are alive in CR at more than three years post-transplant. Among autografted patients, 6 are alive, 3 in CR, 2 in relapse and 1 in PR. Five patients died, 3 from a Richter transformation post transplant (Figure 21.1).

MRD was determined in this series by co-expression of CD5 and either by CD19, CD20 on lymphocytes or monoclonal surface light-chain expression on CD5 cells. Immunoglobulin gene-rearrangement analysis was performed by Southern blot. Absence of MRD was observed in 5 autografted and allografted patients. Positive results were observed in 4 autotransplants and 4 allotransplants. Among 5 negative autografted patients, 1 relapsed, 2 developed Richter transformation, and 2 were in continuous CR. Among 6 negative allografted patients, 5 continued in

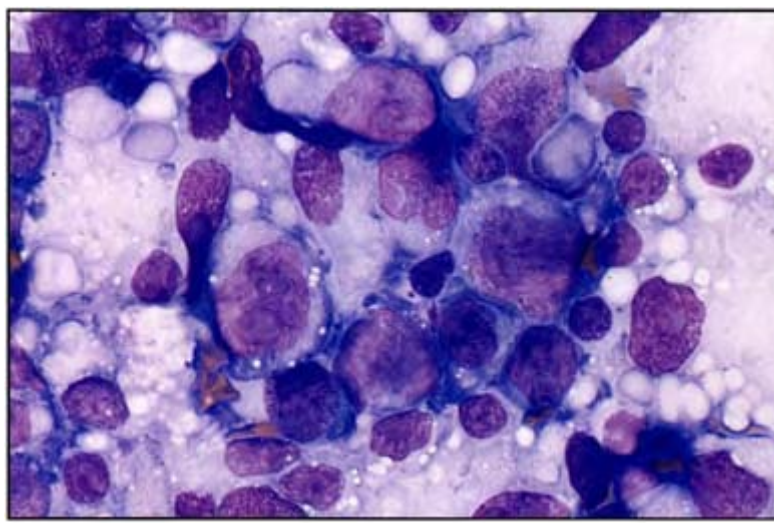

Figure 21.1 Richter transformation in chronic lymphocytic leukaemia.

CR. Among 8 positive patients, 6 relapsed and 2 are in continuous CR.

Rabinowe *et al.* [28] reported 8 allogeneic T-cell depleted transplants and 12 autologous transplants purged with anti-CD10 and anti-CD20. The conditioning consisted of cyclophosphamide at a dose of 120mg/kg and TBI of 1 Gy. Sixteen patients (10 autotransplants and 6 allotransplant) achieved CR post-transplant. The DFS was 82% at 1 month, and 70% at 30 months post-transplant. MRD was also studied using the same method as previously described and disappearance of MRD was observed between 3 and 12 months post-transplant in 11 patients.

The same group has recently published the results of 3 autotransplants and 13 allotransplants: 26 autotransplants and 8 allotransplants were alive in CR with a median follow-up of 12 months. All patients autotransplanted were in stages B or C pretransplant.

In a recent study, Provan *et al.* [29] amplified and sequenced the CDRIII region in patients with poor prognosis CLL referred for autologous and allogeneic haematopoietic stem-cell transplants. They showed that a significant number of patients remained disease-free and with no evidence of PCR-detectable MRD after transplant, suggesting that high-dose therapy may contribute to improved outcome in selected patients with CLL. Other studies have shown the feasibility and results of autotransplantation in CLL. Dreger *et al.* [30] found very interesting results after early autotransplantation in selected patients with CLL, and he observed early and durable molecular remissions post-transplant.

We have published results of allogeneic bone-marrow transplantation from human leukocyte antigen (HLA)-identical sibling donors in younger patients with CLL [31]. We recently updated pooled data on the outcome of allogeneic transplants from HLA-identical sibling donors from the International Bone Marrow Transplant (IBMT) and European Blood and Marrow Transplant (EBMT) Registries (group I= 73 patients) and autologous (group II=46 patients) transplants from the EBMT Registry.

Patients and methods

Allogeneic transplants

Seventy-three patients receiving HLA-identical sibling bone-marrow transplants for stage C disease were reported to the EBMT Registry and the IBMT Registry between 1984 and 1996. Median age was 43 years (range 21–58 years). There were 55 males and 18 females. Median interval from diagnosis to transplant was 38.5 months (range 1–130 months). Pretransplant therapy varied. Three patients received no treatment, 19 chlorambucil with or without prednisone and 8 cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP). Twenty-six received other drugs alone or combined, including cyclophosphamide, vincristine, doxorubicin, bleomycin, fludarabine and interferon. No data were available for 18 patients. Two patients received local radiation, and 3 total lymphoid radiation and chemotherapy. Ten patients had splenectomy. Of 52 evaluable patients, 7 (13%) were considered to have responsive disease at transplant including 2 complete remissions (4%) after fludarabine, 20 with stable disease (38%) and 25 with progressive disease (48%). Patients were selected for transplant according to

separate criteria at individual transplant centres. Donors were HLA-identical siblings; 36 were male and 34 female. The median age was 41 years (range 21–55).

Autologous transplants

Forty-six patients receiving autologous transplant for CLL were reported to the EBMT Registry between 1988 and 1996. There were 41 males and 5 females. Median age was 49 years (range 30–60 years), and median interval from diagnosis to transplant was 32.5 months (range 5–124 months). All patients had received pretransplant therapy: 44 had received polychemotherapy, 1 had received CHOP and 1 chlorambucil. Of 41 evaluable patients, 34 were considered to have responsive disease at transplant, including 27 complete remissions (66%), 6 with stable disease (15%) and 1 with progressive disease (2%).

Pretransplant conditioning

Allogeneic transplants

Of 71 evaluable patients, 60 received cyclophosphamide (median dose 120 mg/kg, range 90–150 mg/kg). Fifty-nine also received TBI: median dose 12 Gy (range 8–14 Gy); median fractions=5 (range 1–9 fractions). Twelve patients received additional melphalan (*n*=1) or etoposide (*n*=11). Two patients received cyclophosphamide and busulfan (16 mg/kg) without radiation. Eleven patients received other conditioning regimens including additional drugs (cytarabine, chlorambucil and daunorubicin).

Autologous transplants

Of 46 evaluable patients, 39 received TBI (12 Gy). Twenty-nine also received cyclophosphamide (120 mg/kg), and 2 patients received melphalan (140 mg/m^2). Eight patients received additional etoposide, and 6 received the BEAM protocol (BCNU, etoposide, cytosine, arabinoside and melphalan) without radiation. One patient received another conditioning regimen which included additional drugs.

Graft-versus-host disease prophylaxis

All allogeneic bone-marrow transplant recipients received graft-versus-host disease (GvHD) prophylaxis, 2 with methotrexate alone, 8 with cyclosporin alone, and 48 with methotrexate and cyclosporin. Eight patients received a T-cell-depleted graft, 7 of whom also received cyclosporin. One patient received a monoclonal anti-interleukin-2 receptor antibody and cyclosporin. Six patients received prednisone in addition to these regimens.

The graft

For allogeneic transplants, all patients received bone marrow from HLA-identical sibling donors. For autologous transplants, 18 patients received PBPC, 3 of which were $CD34^+$-cell selected. Twenty-five patients received bone marrow, 20 of which were purged with a monoclonal antibody cocktail. Three patients received purged PBPC in addition to bone marrow.

Results

Sixty-five (96%) of 68 evaluable allogeneic patients, and 44 (96%) of 46 evaluable autologous patients had stable engraftment. Three (4%) allogeneic and 2 autologous (4%) recipients experienced graft failure. Twenty-five of 63 patients at risk (40%) developed an acute GvHD≥grade II and 25 of 46 patients at risk (54%) developed chronic GvHD which was extensive in 12. Fifty-four (76%) in the allogeneic group and 51 (91%) in the autologous group achieved haematologic remission post-transplant.

In the allogeneic group, 32 patients are alive at a median of 37 months (range 1–117.5 months) post-transplant, and in the autologous group, 38 patients are alive at a median of 13 months (range 1–65 months) post-transplant. Five-year survival probability for allogeneic transplants is 41±12% with a maximum follow-up of 10 years and a plateau, and 32 ±46% for autologous transplant with a maximum follow-up of five years without a plateau (P=0.0006) (Figure 21.2).

The five-year leukaemia-free survival for allogeneic transplants is 34±12% and 28±42% for autologous transplants (P=0.01). In the allogeneic group, patients grafted with responsive disease have a five-year survival probability of 56±22%, and patients grafted with non-responsive disease a probability of 24±24% (P=0.01). Among the 70 patients alive post-transplant, 32 are allogeneic, and 38 autologous patients (29 allotransplant and 36 autotransplant are in CR; 3 allo and 2 autotransplant are in relapse).

Sixteen allogeneic and 28 autologous patients underwent an immune phenotype study post-transplant. Of these, 11 allogeneic and 26 autologous patients had a normal profile. Four allogeneic patients had molecular studies carried out pre- and post-transplant. These did not show gene rearrangement suggestive of persistent leukaemia. Risk of relapse after allogeneic transplant is 40±24% and 70±48% after autologous transplant (Figure 21.3). In the allogeneic group, 41 patients died. Five deaths were disease-related and 36 treatment-related. In the autologous group, 8 patients died. Of these, 3 deaths were disease-related and 5 treatment-related.

Conclusions

These results all demonstrate the feasibility and relevance of allogeneic or autologous transplantation for selected patients with CLL. Selection of patients and indications for transplantation should take into account patient age, existence of an HLA-identical sibling donor, CLL prognostic factors and disease status with conventional therapy.

For allogeneic transplantation, a high morbidity and transplant-related mortality still exist. However, some patients appear to be cured after a very long follow-up post-transplant with durable molecular remissions corresponding to a durable graft-versus

leukaemia (GvL) effect. To enhance the GvL effect and to decrease the toxicity of high-dose chemotherapy or TBI, some authors have shown engraftment and induction of a GvL effect with fludarabine-based non-ablative preparative regimens in patients with CLL

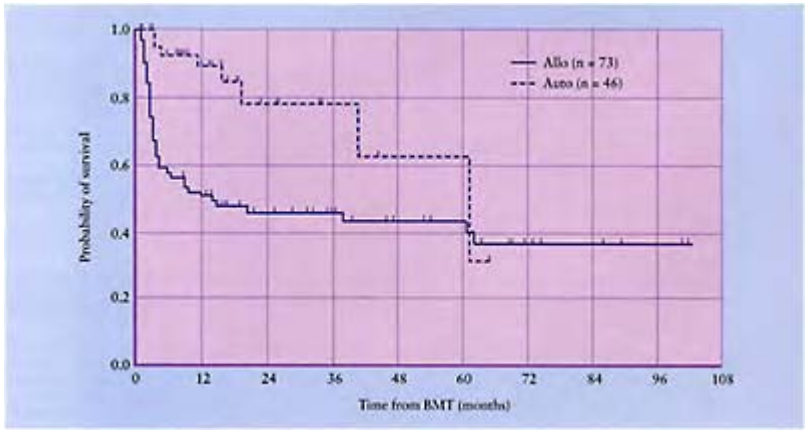

Figure 21.2 Probability of survival among (——) 73 patients after HLA-identical sibling bone-marrow transplantation for CLL and (------) 46 patients after autologous bone-marrow or PBPC transplantation for CLL.

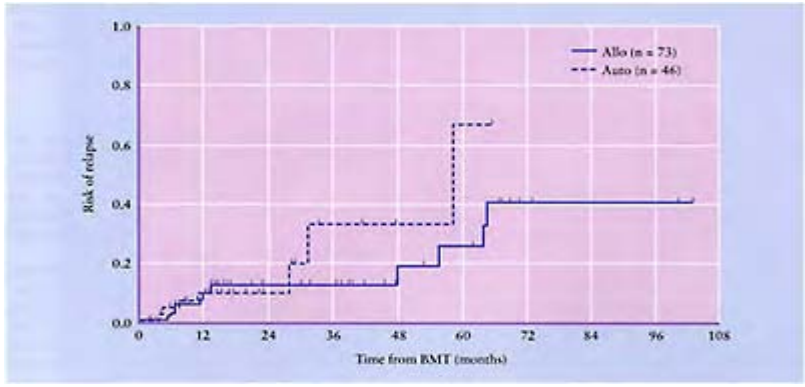

Figure 21.3 Risk of relapse after allogeneic (———) or autologous (------) bone-marrow transplantation and PBPC transplantation for CLL.

[32]. This preliminary experience suggests that conventional dose chemotherapy with a fludarabine-based regimen is sufficiently immunosuppressive to allow engraftment and subsequent donor lymphocyte infusion to enhance GvL. This less toxic approach may provide a feasible means for allogeneic transplantation of older or medically infirm patients with CLL.

With CLL, allogeneic transplantation can only be recommended in the context of approved clinical research protocols for patients with disease stages B or C, early after conventional second-line therapy, especially if they have sensitive disease. Autologous transplantation in CLL results in a very high percentage of complete remissions post-transplant which are durable on molecular assessment and are not associated with a high transplant-related mortality.

We also recommend that autologous transplantation for CLL is only carried out using approved clinical research protocols for patients with stages B or C disease. Autografting should be undertaken early in the course of the disease for patients in CR or in very good PR after conventional therapy, or as soon as progression is evident in other patient groups.

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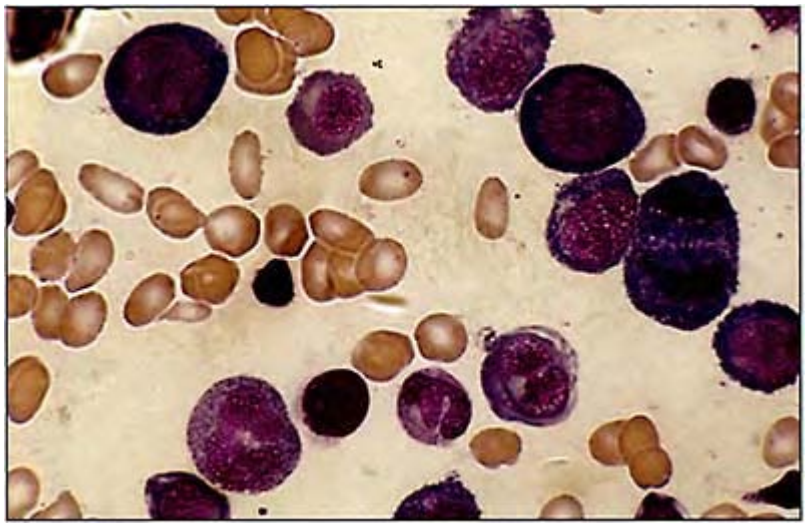

Chapter 22
Autoimmune disorders

Sally Killick

Introduction

Autoimmune diseases (AID) such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and systemic sclerosis follow a chronic relapsing course causing considerable morbidity and mortality. Immune-mediated haematological disorders, such as immune thrombocytopenic purpura (ITP) and autoimmune haemolytic anaemia, usually respond to conventional immunosuppression but can sometimes prove resistant to such therapy. These diseases may be modified only by treatment with immunosuppressive agents whose toxicity limits the dose which may be given. Immunoablative therapy has been proposed as a means of achieving profound immunosuppression and durable remissions in such patients whose disease becomes refractory to conventional treatment. However, since this approach would also result in myeloablation, a means of stem-cell rescue must be simultaneously provided. Because of the higher mortality associated with allogeneic sibling donor stem-cell transplant, autologous peripheral blood stem-cell transplantation is the currently favoured procedure

for stem-cell rescue following immunoablative therapy. However, the ideal immunosuppressive protocol and the means and degree of T-cell depletion required to prevent relapse of the AID by autoreactive T-cells is yet to be determined. Additionally, the specific disease indications for this approach to treatment have not been formally clarified. For these reasons, a liaison group between the European Group for Blood and Marrow Transplant (EBMT) and European League Against Rheumatism (EULAR) has been established. It has already announced a written consensus report with preliminary recommendations, and provided a means of data reporting to evaluate new techniques and monitor clinical outcome in order to determine whether the benefits of this approach outweigh the potential risks of this powerful immunoablative and myeloablative treatment. It is as yet uncertain whether such a procedure can result in cure of the AID. A realistic aim may, instead, be good long-term control of the disease allowing, for example, corticosteroid independence or dose reduction.

Background

The prevalence of AID in the Western world is estimated at 6–7% [1]. Severe AID are chronic, incurable and associated with profound mortality as well as morbidity. Current aggressive therapy at best controls inflammation and suppresses disease activity but does not prevent damage or disability.

Rheumatoid arthritis

Rheumatoid arthritis is the most common inflammatory arthropathy in the world [2], affecting 1–2% of the population worldwide, of whom 50% are unable to work ten years after contracting the disease [3]. Although for the majority of patients it is not a life-threatening condition, for those with severe disease it may be lethal. It has been shown that actuarial survival in patients with RA may be equivalent to that seen in patients with malignancy [4].

The aetiology of RA is unknown. Initiation of the disease occurs with presentation of an arthritogenic peptide, which is complexed to the human leukocyte antigen (HLA) class II molecule on an antigen presenting cell, to a CD4-positive T-lymphocyte carrying the appropriate T-cell receptor rearrangement. T-cell activation results in release of cytokines from lymphocytes, monocytes and synovial cells. These cytokines include interleukin-1, tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and granulocyte-macrophage colony-stimulating factor (GM-CSF) which induce an acute-phase response with stimulation of mesenchymal cells to produce enzymes leading to degradation of collagen and proteoglycans. This ultimately leads to joint destruction.

There is no known cure for RA. Current treatment modalities include non-steroidal anti-inflammatory drugs (NSAIDs), used as a first-line therapy, which reduce swelling and inflammation but which do not prevent joint destruction. Second-line therapy with disease modifying antirheumatic drugs are commenced if the disease remains active after a trial with NSAIDs. Glucocorticoids, associated with a high number of side-effects with chronic usage, are reserved for acute exacerbations of active disease, used either systemically or by local injections.

New developments in immunosuppressive treatment of RA continue to emerge, including cyclosporin and FK-506, monoclonal antibodies such as Campath-1H, anti-CD4 and anti-interleukin-2 (IL-2) receptor, cytokine inhibitors such as anti-TNF-α and anti-IL-6 monoclonal antibodies, in addition to peptides and antigens such as collagen II, myelin basic protein and T-cell vaccination [5].

Systemic lupus erythematosus

Often considered the archetypal systemic AID, SLE has the potential for multi-organ involvement with diverse clinical features. The overall prevalence in the USA is 1 in 2000, but higher in women and the black population [6]. Prognosis in the Western world has dramatically improved over the last four decades. Today in the 1990s, the five-year survival is greater than 90% compared with 50% in the 1950s [7]. This improvement has been due to advances in supportive care (antibiotics, antihypertensives, renal dialysis) and lupus-specific treatment. Despite these improved survival rates, the mortality rate in SLE is three times that of the general population [7]. Poor-risk features of the disease include major organ involvement, particularly the central nervous system and renal disease, and disease activity assessed by SLEDAI (SLE Disease Activity Index).

Treatment of SLE is individualized to the patient and based on disease manifestations. Mild arthritis and serositis are treated with NSAIDs followed by antimalarials. Immunosuppressive drugs and cytotoxics are usually reserved for major organ involvement but are not curative.

Other AID

Systemic sclerosis

The natural history of systemic sclerosis varies. However, only a minority of patients experience spontaneous remission with the majority progressing to multi-organ damage. The cumulative survival is 30% at 12 years with poor-risk features including the presence of antitopoisomerase autoantibodies and diffuse skin involvement [8].

Systemic necrotizing vasculitis

Systemic necrotizing vasculitis is a chronic relapsing disease also with potential for multi-organ involvement. Mortality has improved with the advent of immunosuppressive treatment, and currently more than 80% of patients with Wegener's granulomatosis and microscopic polyangiitis survive for longer than five years [9]. Despite improvements in long-term survival, disease-free remission is not achieved in the majority of patients due to disease relapse, cumulative tissue damage from irreversible scars and drug toxicity. The risks associated with long-term cyclophosphamide therapy are dose-related and include haemorrhagic cystitis, bladder carcinoma, lymphoma, infertility, bone-marrow suppression and myelodysplasia [9]. Potentially curable treatment of systemic vasculitis with immunotherapy is limited by dose related toxicity.

ITP and autoimmune haemolytic anaemia

ITP and autoimmune haemolytic anaemia are common haematological disorders that usually respond to conventional therapy with prednisolone, other immunosuppressive agents, or splenectomy in the acute or chronic phase. However, occasionally they may be resistant to all these therapies and such patients may develop fulminant, life-threatening haemorrhage or anaemia, respectively.

Rationale for considering immunoablative therapy in AID

Experimental data

Experimental animal models have shown two distinct categories of AID: hereditary and induced forms [10]. The transfer of bone marrow from hereditary AID rodent strains to normal mice leads to development of AID in the recipient. This led to the hypothesis that AID is a haemopoietic stem-cell disorder. However, in the induced form the susceptibility or resistance to AID is genetically determined, and in rodent strains this determinant has been shown to be transferred by bone marrow [11]. There is some evidence that human stem cells carry only the susceptibility for AID (comparable to the induced animal models), requiring external triggers for clinical disease [12]. This is supported by the fact that AID do not occur in the first months of life [13] and concordance in monozygotic twins is limited [14].

In inducible animal models of AID, the fully developed disease has been shown to respond with long-lasting remissions to allogeneic bone-marrow transplantation (BMT) from normal donors and autologous BMT where high-dose total body irradiation (TBI) seems superior to high-dose cyclophosphamide [15]. As there is overwhelming evidence that AID are T-cell dependent, *in vivo* and graft T-cell depletion in autologous settings are required for optimal success.

Clinical models

There is clear clinical evidence that allogeneic BMT can lead to cure of AID, as confirmed by long-term follow-up data from Seattle (K.Sullivan, personal communication). A review of 901 patients undergoing allogeneic BMT from 1969 to 1989 for haematological/oncological diseases revealed 14 cases of pre-existent AID. Follow-up of patients for a median of 12 years (range 5–20 years) showed no recurrence of AID post-BMT. All patients in the look-back received cyclophosphamide as conditioning, with or without TBI or busulphan.

Anecdotal cases of cure of AID have also been reported from other centres in patients receiving allogeneic BMT for leukaemia or aplastic anaemia [16–20], although one patient transplanted for gold-induced aplastic anaemia (AA) had a recurrence of RA at eight years post-BMT despite being cured of the AA [21]. Prolonged remissions of AID have also been reported after autologous stem-cell transplantation for solid tumours and

haematological malignancies [22–24]. Clinical data on immunoablative therapy and haemopoietic stem-cell rescue performed specifically for AID are anecdotal with limited follow-up, but results appear promising [25–30].

Advantages of using autologous stem-cell rescue and immunoablation compared with allogeneic stem cells

The procedure-related morbidity and mortality of allogeneic stem-cell transplantation (15–30%) exceeds that of autologous peripheral blood stem-cell transplantation (<2%), and for this reason it is not currently recommended as a clinical option for treatment of this patient group unless the transplant is being performed for a coincidental haematological disorder. Furthermore, the risks of allogeneic transplantation may be higher in autoimmune disease patients with pre-existing multisystem disease. Allotransplantation does have the advantage of not reinfusing autoreactive T-cells, and there may be a 'graft-versus-alloimmunity' effect, analogous to the graft-versus-leukaemia effect seen after allogeneic transplantation for leukaemia. Autologous stem cells are a safer haemopoietic product for transplantation in these patients.

The use of granulocyte colony-stimulating factor (G-CSF) mobilized blood stem/progenitor cells, improved transfusional support and antibiotics have resulted in a transplant-related mortality of ≤2% [31]. However, T-cell contamination is ten times higher with blood progenitor cells [32], necessitating the use of T-cell depletion to reduce the risk of reintroducing autoreactive T-cells.

Mobilization of blood progenitor cells in patients with autoimmune disease

Patients who have been heavily pretreated with potentially marrow-toxic agents may have poor haemopoietic reserve. In addition, the autoimmune disease itself might theoretically cause damage to the bone-marrow stem cells as well as the stroma. Conventionally, haemopoietic cells for autologous transplantation have been mobilized using cytotoxics and haemopoietic growth factors in combination [33]. Some concerns have been expressed that haemopoietic growth factors may exacerbate autoimmune disease [34]. The inflammatory lesions seen in AID contain mature haemopoietic cells such as lymphocytes and macrophages which are involved in tissue destruction and inflammation [3]. Proliferation of these cells may occur under the influence of growth factors. Furthermore, the mobilization and activation of neutrophils by G-CSF may potentially exacerbate AID such as systemic necrotizing vasculitis where neutrophils are actively involved in the pathogenesis of the disease. However, for patients with lymphoma or solid tumours, blood progenitor cells are routinely mobilized with a combination of cyclophosphamide and G-CSF, and the additional use of cyclophosphamide in patients with AID may be of benefit in preventing a flare-up of the

underlying disease with G-CSF. It has been recommended by the EBMT/EULAR group that G-CSF 10 μg/kg once daily is given following cyclophosphamide 1.5 or 4 g/m^2, and that the target dose of CD34$^+$ cells should be >2 ×10^6/kg or >2×10^4 CFU-GM/kg [35]. Cell doses will need to be higher if graft manipulation is planned, since some degree of cell loss during the manipulation procedure is inevitable.

Conditioning

The aim of conditioning is ablation of the diseased immune system. Recommendations remain unclear. Possible regimens recommended by the EBMT/EULAR group [35] include:

- Cyclophosphamide 50 mg/kg×4, which remains standard for patients with aplastic anaemia prior to allogeneic BMT. ATG or Campath may be added.
- Cyclophosphamide 60 mg/kg×2 followed by TBI.
- Cyclophosphamide 60 mg/kg×2 and busulphan 16 mg/kg for four days.
- Combination chemotherapy BEAM (BCNU 300 mg/m^2 day −7, VP-16 250 mg/m^2 twice daily, days −7 to −4, cytosine arabinoside (Ara-C) 200 mg/m^2 twice daily, days −7, −6, −4, melphalan 140 mg/m^2 day −3.

Data from animal and clinical models have demonstrated that conditioning with cyclophosphamide alone in AID can be associated with relapse [15,36]. Treatment of RA with Campath-lH, the humanized monoclonal antibody, which induces a profound and persistent peripheral blood lymphopenia, has shown that the degree of peripheral blood lymphopenia is not related to activity of the disease. Furthermore, synovial biopsies taken at the nadir of lymphopenia show T-lymphocytes still present. It can therefore be concluded that the peripheral blood changes do not reflect synovial changes [37]. Sanctuary sites such as the synovium containing residual auto-aggressive T-cells thus need to be penetrated by conditioning regimens in order to prevent early relapse and promote durable remissions. Cyclophosphamide used alone appears not to penetrate such sites, whereas the additional use of TBI or busulphan may achieve this aim. However, TBI when used with high-dose chemotherapy as conditioning regimens for transplantation is associated with an increased risk of secondary malignancies including solid tumours, acute leukaemias, lymphomas and lymphoproliferative disorders.

Multivariate analysis of risk factors in patients developing solid cancers after BMT demonstrated that higher doses of TBI were associated with a higher risk of solid cancers [38]. It is important however, to take into account the following observations/facts: previous mortality studies in RA indicate that patients with severe disease (based on joint count or activities of daily living scores) show five-year survival patterns in the range of those with three-vessel coronary artery disease or stage IV Hodgkin's disease [4]. Furthermore, patients with AID also have a higher risk of secondary malignancies, most commonly bladder tumours, often associated with long-term use of cyclophosphamide [9,39,40]. Therefore, when considering conditioning regimens for transplantation in AID, the long-term risks associated with TBI/busulphan must be weighed up against the morbidity and mortality of the underlying AID.

T-cell depletion

Autologous transplantation of AID with an unmanipulated graft has been associated with clinical relapse [41,42]. Euler *et al.* describe early relapse or persistence of autoimmune diseases in 5 patients with CREST syndrome, myasthenia gravis, Hashimoto's thyroiditis, SLE, atopic dermatitis and RA who underwent autologous BMT (n=1) or blood stem-cell (n=4) transplantation with unmanipulated grafts [41].

The cause of the relapse in AID may be due to the following:

- Survival of auto-aggressive lymphocyte clones in sanctuary sites which have survived conditioning. The additional use of TBI/busulphan which are more likely to penetrate sanctuary sites may reduce the risk of relapse.
- Reinfusion of autoreactive T-lymphocytes. This is likely to be reduced by T-cell depletion of the autograft, although the degree of lymphocyte depletion required to achieve this goal has not been established (see later).
- Recipient-inherent autoantigen challenge. Following immunoablative therapy, tolerance may be acquired to antigenic challenges by the newly acquired immune system, assuming memory T-cells have been ablated.
- Reinfusion of defective stem cells. However, stem cells are thought only to have a genetic susceptibility to AID requiring an external trigger which still remains after peripheral blood stem-cell (PBSC) transplant. A basic defect in stem cells to account for AID would be associated with early relapse following autologous PBSC transplant, while in fact longer remissions have been reported.

The aim of graft manipulation in AID is to eliminate autoreactive (memory) T-cells and produce *de novo* regeneration of T-cells from autologous haemopoietic stem cells that re-acquire unresponsiveness to self (tolerance). The preferred method of reducing T-cell numbers in the graft, whether by T-cell depletion strategies or positive selection of $CD34^+$ cells or a combination of these approaches has not been established. Preliminary analyses in normal donors have assessed T-cell depletion using:

- $CD34^+$ selection using the Isolex™ and Ceprate™ separation [43].
- High-speed fluorescence-activated cell sorting of $CD34^+$ cells using the Systemix system [44].
- The monoclonal antibody CAMPATH-1 [40,42].

Further work is needed to compare T-cell depletion by each of these methods. Reduction of T-cells to transplant $<1\times10^5$/kg is preferred, but it is not yet known whether the magnitude of T-cell depletion by current techniques is enough to prevent regrowth of pathogenic lymphocyte clones [35].

EBMT and EULAR Autoimmune Disease Stem-Cell Project

The First International Symposium on Haemopoietic Stem-Cell Therapy in Autoimmune Diseases took place in Basel between the 26 and 28 September 1996 to discuss patient

selection, outcome measurement, collection of marrow/blood stem cells and immunoablative regimens. Guidelines from this meeting have been published [35].

The major problem of stem-cell therapy of AID will be selecting suitable cases. In patients with concomitant haematological disorders, the treatment of choice is allogeneic transplantation. In AID, conversely, transplantation is still experimental and therefore individual cases need careful selection with informed consent. Patients should be selected in whom conventional treatment has failed, end-organ damage has not resulted and the disease itself is severe enough to increase risk of mortality. Accurate predictors of outcome for AID are needed to guide treatment. For instance, those RA patients at present not recommended by the EBMT/EULAR group for transplantation may in the future be selected by HLA types DR4 and DRW14 which are associated with a poor prognosis [45].

From this meeting, the Autoimmune Disease Stem-Cell Project has recently been initiated to collect data on clinical pilot studies, and hopefully to plan subsequent prospective comparative trials [35,46].

Future of stem cell transplantation for AID

The safety of transplantation for AID requires a number of questions to be answered:

- Patient tolerability to high-dose chemotherapy, due to pre-existing end-organ damage and/or haemopoietic reserve. The maximum dose of chemotherapy for efficacy and safety needs to be established.
- Selection of patients to assure maximum benefit with minimum transplant-related morbidity (see Table 22.1).
- Mobilization regimens of peripheral blood stem cells from patient population with close observation for inducing flare-up of the underlying disease due to administration of haemopoietic growth factors. Comparison of peripheral blood stem-cell harvest collections with historical autologous and allogeneic donor populations.
- The best method and degree of T-cell depletion has not yet been established. Although formal guidelines on this subject have not been issued, it is evident from early observations that early relapse will be associated with unmanipulated grafts.

The role of stem-cell transplantation in AID has not yet been determined. There is strong anecdotal evidence that patients with severe AID, in whom reversible disease is present, will benefit from immunoablative therapy with haemopoietic stem-cell support. It is possible that these patients will enjoy at least long-term remissions from their disease. The EBMT/EULAR group is already collating data on AID transplants in order to help advise clinicians on selection of appropriate cases, conditioning regimens, graft manipulation and transplant-related morbidity/mortality rates.

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Table 22.1 Autoimmune disorders currently under consideration for haemopoietic stem-cell transplantation (reproduced from Tyndall and Gratwohl, with permission) [35].

Rheumatological disorders
Systemic sclerosis (scleroderma)
Autoimmune pulmonary hypertension (after adequate trial of immunosuppression)
Necrotizing vasculitis (following induction with standard immunosuppression)
Rheumatoid arthritis (RA) with severe complications, e.g. necrotizing vasculitis, scleritis RA, poor prognosis, rapidly progressive and destructive, resistant to adequate treatment
Systemic lupus erythematosus—major organ threat, failed conventional therapy
Antiphospholipid antibody syndrome
Severe, uncontrollable cryoglobulinaemia
Paediatric rheumatology; systemic sclerosis with pulmonary fibrosis, severe dermatomyositis, severe necrotizing vasculitis. Not juvenile arthritis at this stage
Neurological disorders
Multiple sclerosis
Myasthenia gravis
Haematological disorders
Severe refractory immune thrombocytopenia
Autoimmune haemolytic anaemia
Immune neutropenia
Combinations thereof

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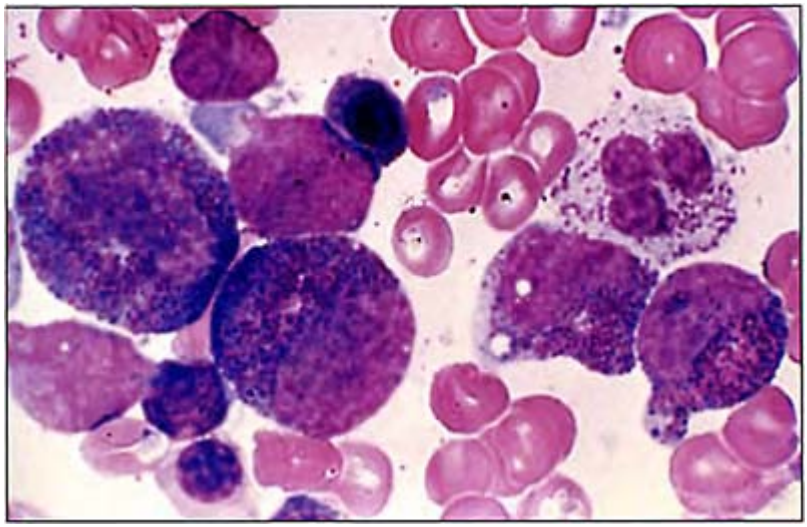
Part 2
Practical aspects of stem-cell transplantation



Chapter 23
Pretransplant evaluation of the patient and donor

Jayesh Mehta and Seema Singhal

Introduction

With the advent of peripheral blood stem cells for haematopoietic rescue, autograft-related mortality has dropped to less than 5–10% in most patient groups. At the other extreme, procedure-related mortality is still in excess of 50% in high-risk patients undergoing allografts from mismatched and unrelated donors [1,2]. Although only one death has been reported so far in a healthy donor as a consequence of donating marrow [3], it is a procedure associated with significant morbidity, including the risk of anaesthesia, blood loss and trauma to skin, blood vessels, nerves, muscle and bone [4,5] and is of no intrinsic benefit to the donor. Also, blood and marrow transplants are among the most expensive medical procedures, the ultimate burden of which falls upon society. A decision to embark upon a transplant procedure is therefore not a casual one. It is important to determine the appropriateness of the proposed procedure in a given individual and in the disease and stage being treated.

Considering a patient for transplantation

Before a patient is seen for the first pretransplant interview, a preliminary decision regarding appropriateness of transplantation has often been made, based upon available data. Guidelines and policies on referral and selection of patients for transplantation vary considerably between countries and centres [6–8]. A detailed discussion of indications for transplantation in specific diseases and choice of conditioning regimens is beyond the scope of this chapter, but is dealt with in individual sections. If transplantation is indicated from the point of view of the disease, the two major patient-related factors which determine selection are age and performance status. While advancing age is usually associated with higher transplant-related toxicity and poorer long-term survival [9–12], relapse is not necessarily adversely affected by older age [11,12], and improved supportive therapy has resulted in increasing numbers of successful autologous and allogeneic transplants in patients over the age of 50–60 years [13–15]. Poor performance status at the onset, especially if due to acute medical problems or to active infections, usually undermines treatment outcome by increasing transplant-related mortality. Some disease-specific complications need not necessarily be considered contraindications for transplantation: patients with renal failure, including those dependent on dialysis, have been successfully autografted after high-dose melphalan [16]. Allografting for myeloma patients with dialysis-dependent renal failure is also feasible, but little information is available on this in the literature. Few data are available on transplantation for other diseases in patients with renal failure, or significant dysfunction of other organs. Table 23.1 outlines a general approach to a patient who is being considered for a transplant procedure.

Type of transplant

Results of autologous and allogeneic transplantation vary considerably depending upon the disease and stage being treated, and more marked than differences in survival are the causes of failure [17]. In general, allogeneic transplantation is preferable to autologous transplantation in diseases where graft-versus-tumour reactions are active [18]. However, the higher procedure-related morbidity and mortality means that it is usually not a practical option for older patients. Allografting is the only suitable option in patients with marrow-failure syndromes, those with genetic diseases and in patients with advanced disease (usually haematological malignancies) involving the marrow.

In diseases such as most solid tumours, where increasing dose intensity is the major aim, autografting is the preferred option. Peripheral blood stem cells have replaced marrow as the source of stem cells almost completely. However, the safety of growth factor-mobilized blood-derived stem cells for autografting in acute myeloid leukaemia has been questioned [19,20]. This is the only clinical situation where, until more data are available, marrow may be preferable.

The first pretransplant consultation

The first consultation is the time to assess suitability for transplantation and when to transplant. Patients receive information concerning the transplant at this stage. Patients who have had at least some treatment at the transplant centre in the past, especially in the same unit, are usually much more comfortable and better informed about the proposed therapy and local conditions and protocols. In such patients,

Table 23.1 General approach to a patient who is being considered for a blood or marrow transplant

- Is transplantation the best therapy under the circumstances?
- Are there any obvious contraindications to transplantation (age, performance status, complicating medical problems, etc)?
- Is autograft or allograft preferable?
- If allograft preferable, is there a suitable HLA-compatible donor available?
- If no information is available on donors:
  Has typing on family members been arranged?
  Has a preliminary search for unrelated donors been started?
- If autograft preferable or no allogeneic donor available:
  Have cells already been harvested?
  When to harvest?
  Peripheral blood stem cells or marrow?
  How to mobilize peripheral blood stem cells?
  Purged/selected or unmanipulated?
- If allograft preferable and a donor is available
  Peripheral blood stem cells or marrow?
  What type of graft-versus-host disease prophylaxis?
- When to transplant?
- What conditioning regimen to use?

appropriateness of transplantation as the next course of therapy has usually already been determined, and the first formal clinic visit is for the ‘transplant talk’.

Patients who have specifically been referred for transplantation from elsewhere are often nervous, and retain limited amounts of information. It is important to be factual as well as reassuring and welcoming. It may be useful to give only a simple explanation of the transplant on the first visit, with more detailed discussions on subsequent visits. Although it is often desirable for patients to see transplant physicians more than once before the transplant, this is not always possible. The presence of a nurse transplant coordinator during the transplant talk is helpful. The nurse coordinator is then aware of the extent of the information given to the patient, and is able to answer some of the queries that arise after the transplant talk. Approximately 20–30% of patients have serious doubts and questions which require a second meeting with a physician. The nurse

transplant coordinator and ward nursing staff usually deal with questions pertaining to isolation, diet, visiting policy and so on.

The presence of partners or parents provides support and reinforces the information given. The discussion should be individualized for each patient's circumstances. This is particularly important for children and adolescents, and patients from a different cultural background. Parents of paediatric patients should be seen separately while the child is being introduced to the ward.

The 'transplant talk' usually starts with a brief explanation of the biology and natural history of the underlying disease, the role of marrow transplantation, and the role of alternative therapy. The next point is an explanation of the rationale behind a transplant procedure with a discussion about dose intensity and, in case of allografts, immunologic graft-versus-tumour effects. Short- and long-term adverse effects of the transplant could be discussed next. While discussing adverse effects, especially serious long-term problems such as development of secondary malignancies, it is important to ensure that the relatively small magnitude of the risk of these complications is kept in perspective. It is important to discuss risks, prognosis, and the probability of survival honestly and sensitively. This exhaustive scheme is not always appropriate, and it is essential to adapt the talk to the level of the patient's understanding and culture. All the information must be disseminated in a simple manner.

If the transplant is being performed as part of a clinical trial, information about this should be given at the first visit so that the patient's thinking is oriented accordingly. A detailed discussion may be deferred until the next visit. It may be best to leave any discussion about associated clinical studies, including infection prophylaxis or therapy, for which the patient may be eligible, until the second visit.

A number of patients reaching the stage of transplantation may already have had the issue of fertility addressed. They may have become sterile as a result of prior chemotherapy, or they may have semen or embryos cryopreserved. Although it is currently difficult to cryopreserve unfertilized ova satisfactorily, there is some evidence to suggest that wedges of ovarian tissue can be frozen for future fertilization.

Patients need to be made aware that the incidence of infertility following transplantation is high. However, it is by no means universal, and some chemotherapy-only regimens may spare fertility [21,22]. It is relatively easy for male fertility to be assessed and semen to be collected over a limited period of time. However, this is considerably more difficult in females and could take a few months. The tempo of the disease dictates whether females have the opportunity to undergo embryo cryopreservation or not. Although prolonged or permanent sexual dysfunction is not expected as a direct result of the treatment, the incidence of sexual dysfunction in the first few months after transplantation is high [23,24].

Patients must be made aware of the time required away from work or schooling. A large number of patients do not return to work for six to nine months after allografting. Recovery after autografting, especially with blood-derived cells, is much more rapid, with some patients back at work after a few weeks. Assistance of the clinical social worker may be needed to organize sickness benefits, time off work for spouses or parents, and child care during hospitalization and recovery.

Fitness for transplantation

In addition to the history and physical examination, the investigations outlined in Table 23.2 help in deciding about fitness for transplant, the conditioning regimen to be employed, and some of the supportive care measures, particularly infection prophylaxis. These also include investigations required prior to an autologous bone-marrow harvest under general anaesthesia. Ensuring freedom from any active infection is particularly important to prevent a serious exacerbation of an otherwise innocuous infection.

Table 23.2 Pretransplant investigations for the patient

Essential
Blood group and antibody screening
Bone-marrow examination
Coagulation studies
Complete blood count
Creatinine clearance
DNA restriction fragment-length polymorphism/ minisatellite studies
Liver-function tests
Toxoplasma titre
Urea and creatinine
Viral serology
Cytomegalovirus Epstein-Barr virus
Hepatitis B (HBsAg)
Hepatitis C
HIV
Herpes simplex virus
Varicella-zoster virus
Chest X-ray
Echocardiogram and/or MUGA scan
Electrocardiogram
Lung-function tests
Complete disease restaging
Dental examination
Under specific circumstances

Computed tomography scan of paranasal sinuses
Chest computed tomography
Lumbar puncture
Lymphocytotoxic cross-match
Pregnancy test

Dental assessment

Dental assessment at least one or two weeks prior to commencing the conditioning regimen is an important part of pretransplant assessment. Potential sources of dental sepsis and abscess formation should be dealt with in advance, to allow adequate time for healing should dental work be required. Antibiotic cover should be considered if a central venous catheter is *in situ.*

Nutritional assessment

A formal dietetic assessment will help identify undernourished patients who should be encouraged to gain optimal weight prior to transplantation, and also patients who are likely to be potentially at risk after the transplant when oral intake is compromised. The dietitian can advise the patient on the type of food that is permissible at various stages after the transplant, and give caloric supplements when oral intake is insufficient.

Selecting a donor

The exhaustive investigation scheme shown in Table 23.3 is relevant to determine fitness for the harvest after a donor has been provisionally selected. In order to select the donor, the minimum essential investigations which should be included are class I and II human leukocyte antigen (HLA) typing, blood grouping and cytomegalovirus (CMV) serology. Once a donor has been provisionally selected, the next step is a detailed history and physical evaluation, along with the investigations outlined in Table 23.3, to assess fitness to donate marrow or blood, and fitness for anaesthesia.

Donors who can potentially transmit bloodborne infections are generally not suitable. This is especially true for human immunodeficiency virus (HIV)-positive donors. Post-transplant morbidity due to liver complications is likely to be higher with hepatitis B surface antigen (HBsAg)-positive and hepatitis C virus (HCV)-positive donors as well. Pregnancy is a contraindication for a marrow harvest. Adequate data are not available to support the use of growth factors to mobilize stem cells in healthy pregnant women.

HLA typing is clearly the most important factor in selecting a donor. When more than one HLA-identical donor is available, the biological factors unrelated to tissue-typing (i.e. excluding assays such as CTLp [25]

Table 23.3 Pretransplant investigations for the donor

Essential
Blood group and antibody screening
Coagulation studies
Complete blood count
DNA restriction fragment-length polymorphism/ minisatellite studies
Liver-function tests
Pregnancy test
Urea and creatinine
VDRL
Viral serology
Cytomegalovirus
Epstein-Barr virus
Hepatitis B (HBsAg)
Hepatitis C
HIV
Herpes simplex virus
Varicella-zoster virus
Chest X-ray Electrocardiogram
Under specific circumstances
Cytogenetic studies (chromosome
Bone-marrow examination
Echocardiogram and/or MUGA scan
Haemoglobin electrophoresis
Lung-function tests
Lymphocytotoxic cross-match
Sickling
Toxoplasma titre

HTLp [26], minor histocompatibility antigens [27] or lymphocytotoxic cross-matching [28]) which are considered in donor selection are CMV serology, age, blood group, and sex and parity.

Cytomegalovirus serology

Despite the efficacy of antiviral agents such as ganciclovir and foscarnet [29], CMV infections still remain a significant source of morbidity after transplantation. In CMV-seronegative individuals, the donor marrow is a potential source of CMV. A CMV-seronegative donor is therefore preferable for a CMV-seronegative patient. Donor CMV serostatus is generally not important for CMV-seropositive patients, although there is some evidence to suggest that CMV-seropositive donors are preferable for CMV-seropositive patients receiving T-cell-depleted allografts [30].

Blood group

Although ABO incompatibility is not a barrier to successful allogeneic transplantation, ABO-matched donors are preferred because of potential immune complications of ABO-mismatched transplants [31] and increased transfusion requirements [32]. The number of donor lymphocytes transferred into the patient at the time of allogeneic blood-cell transplantation is as much as ten times the number infused at the time of marrow transplantation. This may result in clinically significant haemolysis in the second week following the transplant when donor B cells start producing haemagglutinins if there is minor ABO incompatibility [32] between the donor and the recipient [33].

On the other hand, there are two reports suggesting significantly decreased relapse of leukaemia after ABO-mismatched sibling allografts [34,35]. This intriguing observation requires further study, and if confirmed, may make ABO-incompatible donors more attractive.

Age

Young adult donors are likely to tolerate the procedure of marrow donation better than older patients. There is also evidence to suggest that the risk of treatment failure increases with advancing donor age [36,37]. Although very young infants have also been used as marrow donors safely [38,39], there are very complex ethical issues at stake [40,41].

Sex and parity

Donor-recipient sex mismatch [42,43] and donor parity [42,44] have been shown to be associated with increased risk of graft-versus-host disease and treatment failure. It is not clear that male donors are superior to nulliparous females. The order of preference for male patients therefore would be male, nulliparous female and parous female; that for females would be nulliparous female, male and parous female.

Source of cells

Peripheral blood-stem cells have replaced marrow as the preferred source of autologous stem cells for almost all diseases with the possible exception of acute myeloid leukaemia [19,45]. Preliminary data indicate that blood-derived allogeneic stem cells from HLA-identical sibling donors are at least equivalent to marrow (and probably superior) in terms of rapidity and consistency of engraftment [46,47]. The morbidity of the transplant may be lower because of the considerably higher number of cells infused [48], but there are no data to support this premise yet.

Although donation of blood stem cells is easier and more convenient, administration of myeloid growth factors to mobilize stem cells in a healthy person is an unusual therapeutic step. The available evidence suggests that this is safe, but long-term follow-up is not available. This is a potential area of concern which has prevented most volunteer donor registries from approving growth factor-mobilized blood stem cell harvests as the primary method of donation. However, basal-state apheresis for immunotherapy is approved for volunteer donors. In general, donors with complicating medical factors are far more likely to tolerate leukapheresis than a marrow harvest under general anaesthesia.

Venous access

In an individual (patient or normal donor) who is likely to undergo leukapheresis, it is important to assess peripheral venous access. It is preferable to avoid placing indwelling catheters in healthy donors if possible, especially if only one or two aphereses are likely to be needed. However, this has been done successfully with limited morbidity [47]. In order to avoid placing a central line in donors who do not have large bore peripheral venous access, consideration may be given to using an intermittent flow cell separator which requires a single intravenous access [49].

Indwelling vascular catheter

A tunnelled device such as a Hickman or a Broviac catheter is best under most circumstances as it can be left *in situ* for several months if required. However, recipients of peripheral blood stem-cell grafts recover so quickly and with often such limited transfusion support that an ordinary, non-cuffed central venous line may be sufficient. The former needs to be placed a few days in advance so that the insertion site can heal adequately. It is possible to manage an autograft with a port that is implanted subcutaneously, but this is suboptimal and inconvenient for allogeneic transplantation.

Psychological aspects of blood or marrow donation

Marrow donation entails undergoing a procedure with significant morbidity and a small risk of death without any benefit to the donor. Although relatives rarely refuse to donate marrow, there is often direct or indirect pressure on the donor, and it is very difficult for a

family member to refuse to donate marrow that could potentially save the life of a relative.

If a donor is fit to donate marrow under general anaesthesia and does not have concomitant medical complications which could increase the risk of a marrow harvest [50], the important thing is to make them feel at ease with the procedure. The donor's perception of marrow donation and the transplant procedure must be assessed.

Donors often have a distorted idea of the procedure, which can usually be remedied by counselling. Drawing an analogy with blood donation and highlighting the difference from organ donation often help the anxiety considerably.

Donors, especially those who show any degree of hesitation, must be made aware of the lethal and irreversible nature of the conditioning regimen so that full commitment to marrow donation is ensured prior to beginning the conditioning. If there is a serious question about the reliability of a donor, it is probably best to harvest and cryopreserve allogeneic cells prior to commencing the preparative regimen.

For the primary transplant procedure, there is no psychological pressure on unrelated volunteer donors. However, an element of emotional blackmail could enter the picture in cases of graft failure or relapse, when additional cells from the same donor may be of help. To prevent this, direct contact between an unrelated marrow donor and the marrow recipient/treating transplant team is prohibited by the two largest registries (National Marrow Donor Program and the Anthony Nolan Research Center).

Arrangement for autologous blood donation

Barring unforeseen circumstances, it is unacceptable to transfuse allogeneic blood to healthy donors during a bone-marrow harvest. However, if adequate arrangements are not made to reinfuse autologous cells, a substantial proportion of donors may require allogeneic blood. Many centres arrange to have one or two autologous units of blood collected over the two to four weeks preceding the marrow harvest for intra- or post-operative transfusions. It is important to prescribe folate and iron supplementation in advance if this is done. Administration of erythropoietin to normal donors (and patients) has also been utilized to minimize transfusion requirements [51]. Separation of red cells from the harvested marrow by centrifugation irrespective of donor-recipient ABO matching and reinfusion of these into the marrow donor is a relatively simple way of minimizing donor blood loss [52]. For patients or donors who are donating only peripheral blood stem cells, blood loss is not significant [52].

Consent

The last part of the pretransplant evaluation, after all investigations have been completed, consists of obtaining consent. The consent form could be a generic, institution-specific document which may then be accompanied by an information sheet which is specific to the proposed procedure. Alternatively, since the patient (or donor) and the physician (usually with a witness) sign only the consent form, it may be desirable to combine the

information sheet with a generic consent form to produce detailed, procedure-specific consent forms.

If generic consent forms are used, it is important to specify the type of anaesthesia for bone-marrow harvests (e.g. bone-marrow harvest under general anaesthesia). For blood stem-cell harvests, consent for administration of growth factors should specifically be included in the case of normal donors, along with consent for line insertion if this is needed (e.g. up to three aphereses after G-CSF administration, and insertion of Quinton catheter under local anaesthesia). The transplant consent form should ideally specify the exact nature of the transplant, the conditioning regimen, the source of cells, and the graft-versus-host disease prophylaxis (e.g. T-cell depleted allogeneic bone-marrow transplant from a mismatched, unrelated donor after total body irradiation [14]).

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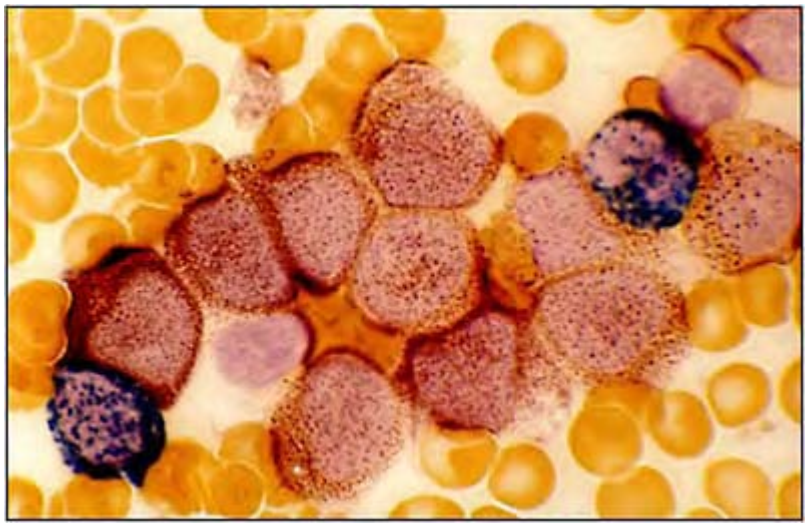

Chapter 24
HLA matching and compatibility testing

M.Tevfik Dorak and Christopher H.Poynton

Introduction

This chapter will review recent advances in our knowledge of the human leukocyte antigen (HLA) system and its clinical significance in bone-marrow transplantation (BMT). The use of unrelated donor bone-marrow transplants has shown how unpredictable the results can be, and has led to further efforts to try to clarify how we may select more suitable donors. Mismatched family donors with only one HLA locus mismatch result in a generally favourable outcome compared to unrelated donor transplants, and such transplants provide an excellent model for the study of the significance of specific mismatches. Firstly, we will introduce the ‘contemporary’ major histocompatibility complex (MHC) and the subsequent section will discuss latest typing techniques and their relevance to BMT.

The genetic control of allogeneic graft rejection resides on human chromosome 6 in a complex of closely linked genes. This is called the major histocompatibility complex, and was discovered in mice during transplantation experiments, where its first recognized function was self-non-self discrimination [1]. There are more than 20 different genetic systems involved in transplantation which include not only the MHC but also multiple minor histocompatibility loci [2]. However, the MHC is the major determinant of transplant outcome. With better understanding of its genetic content and the products of those genes, it has become much more than a simple histocompatibility complex. The numbers of the histocompatibility loci in the MHC and their alleles are continually increasing, together with the development of new methods to type them.

An introduction to the HLA system

The human MHC encodes molecules called HLA which are expressed on the cell surface only when associated with peptide antigens. Conventionally, HLA antigens are divided into two groups: class I (HLA-A, B, Cw) and class II (HLA-DR, DQ, DP). However, new genes within the HLA complex with structural similarity to the known HLA genes have been recently identified and designated as HLA-E, F, G, H, J, K and L (Figure 24.1, Table 24.1). Note that the 'w' (for workshop) has been dropped after the locus names except for HLA-Cw and HLA-B supertypes, since all alleles of the HLA loci have now been sequenced and officially recognized. It remains in use for HLA-C simply to avoid confusion with the complement loci (C4, C2). The class II gene products are composed of two chains, α and β. For DR, DQ and DP, those genes

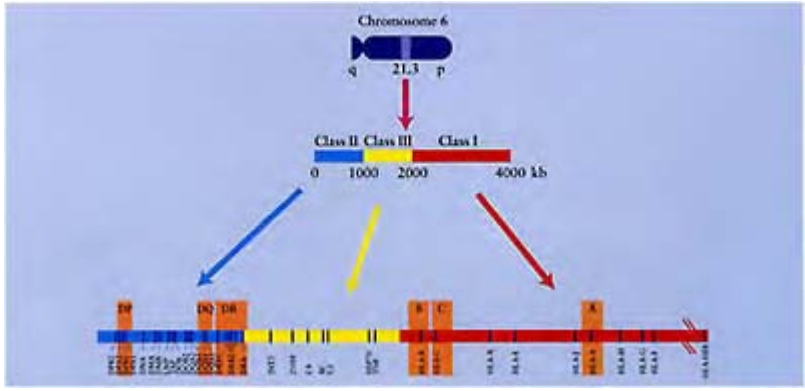

Figure 24.1 A simplified genetic map of the current major histocompatibility complex.

Table 24.1 Officially recognized HLA loci within the human MHC

HLA-A	Encoding for α-chain of HLA-A antigens A1, A2, A3, A9(23/24), A10(A25/26/34/66), A11, A19(29/30/31/32/33/74), A28(68/69), A36, A43, A80 (number of DNA-defined alleles is 83)*.
HLA-B	Encoding for α-chain of HLA-B antigens B5(51/52), B7, B8, B12(44/45), B13, B14(64/65), B15(62/63/75/76/77), B16(38/39), B17(57/58), 618, B21(49/50), B22(54/55/56), B27, B2708, B35, B37, 640(60/61), 641, 642, 646, B47, 648, B53, B59, B67, B70(71/72), B73, B78, 681 (number of DNA-defined alleles is 187).
HLA-C	Encoding for α-chain of HLA-Cw antigens Cw1, Cw2, Cw3(9/10), Cw4, Cw5, Cw6, Cw7, Cw8 (number of DNA-defined alleles is 42).
HLA-E	Encoding for α-chain of HLA-E antigen which has five DNA-defined alleles.
HLA-F	Encoding for α-chain of HLA-F antigen with undetermined polymorphism.
HLA-G	Encoding for α-chain of HLA-G antigen which has seven DNA-defined alleles.
HLA-DRA	Encoding for α-chain of HLA-DR antigens. Two DNA-defined alleles have been recognized.
HLA-DRB1	Encoding for DRβ1-chain determining specificities HLA-DR1, DR103, DR2(15/16), DR3(17/18), DR4, DR5(11/12), DR6(13/14), DR7, DR8, DR9, DR10, DR1403, DR1404 (number of DNA-defined alleles is 184).
HLA-DRB3	Encoding for DRβ3-chain determining the supertypic specificity HLA-DR52 antigen which has 11 DNA-defined alleles.
HLA-DRB4	Encoding for DRβ4-chain determining the supertypic specificity HLA-DR53 antigen which has nine DNA-defined alleles.
HLA-DRB5	Encoding for DRβ5-chain determining the supertypic specificity HLA-DR51 antigen which has 12 DNA-defined alleles.
HLA-DQA1	Encoding for α-chain of HLA-DQ antigens. Eighteen DNA-defined alleles have been recognized.
HLA-DQB1	Encoding for more polymorphic α-chain of HLA-DQ antigens DQ1(5/6), DQ2, DQ3(7/8/9), DQ4 (number of DNA-defined alleles is 31).
HLA-DOB	Encoding for the expressed, non-polymorphic HLA-DO α-chain.
HLA-DMA	Encoding for α-chain of HLA-DM. Four alleles are known.
HLA-DMB	Encoding for β-chain of HLA-DM. Five alleles are known.
HLA-DNA	Encoding for the expressed, non-polymorphic HLA-DN α-chain.
HLA-DPA1	Encoding for α-chain of HLA-DP antigens. Ten DNA-defined alleles are known.

HLA-DPB1	Encoding for β-chain of lymphocyte-defined HLA-DP antigens DPw1, DPw2, DPw3, DPw4, DPw5, DPw6= (number of DNA-defined alleles is 77).

Pseudogenes and genes not known to be expressed: HLA-H, HLA-J, HLA-K, HLA-L, HLA-DRB2, -DRB6, -DRB7, -DRB8, -DRB9, HLA-DQA2, -DQB2, -DQB3, HLA-DPA2, -DPB2.
*The allele numbers are from the latest Nomenclature Committee report [4].

encoding for α-chains are marked as A, and those encoding for β-chains are marked as B, e.g. DRA and DRB. When there is more than one gene as for DRB, they are numbered consecutively (DRB1, DRB2, and so on). Their further subdivision into alleles are numbered after the name of the locus and a *, like HLA-DRB1*0401 or HLA-B*0702. The protein products are marked as a for the α-chain, and β for the β-chain (e.g. DRα and DRβ).

The main function of the HLA molecule is to present peptide antigen, as it is only in this context that an appropriate immune response against foreign antigens can be initiated. The cooperation of macrophages, B-cells and T-cells is required for a fully functional immune response which depends upon strict MHC identity. Thus, the concept of MHC restriction was established [3]. Since they are very immunogenic themselves, HLA antigens may act as inducers of an immune response, as is the case in histo-incompatible transplantation.

The HLA system is the most polymorphic genetic system expressed in the human genome. This suggests that HLA polymorphism is advantageous in the population, and one reason for this is that different allelic products have the capacity to bind and to present different peptides. Consequently, the more alleles a population has, the better it is able to cope with a large number of pathogens. Maintenance of this polymorphism is undoubtedly aided by the selective advantage given to heterozygotes, possibly through the immune functions of HLA in a subsequent stage of evolution. Each locus in the HLA complex has a variable degree of polymorphism reflected by the number of alleles. Currently, the most polymorphic loci are HLA-B and HLA-DR, with more than a hundred officially recognized alleles each (currently more than 180 alleles are known), followed by HLA-A [4]. Out of 184 HLA-DRB1 alleles recognized by the 1996 nomenclature committee, 26 belong to the same serological specificity HLA-DR4.

In the HLA-B and DR loci, there are evolutionarily related alleles that fall into the same ‘supertypic’ group. Each HLA-B allele belongs to either Bw4 or Bw6 supertypic group. In recent years, the mystery of HLA-B supertypes has been solved. The antigens HLA-Bw4 and HLA-Bw6 reside on a unique epitope on each HLA-B molecule and are distinctly different from the epitopes that determine the HLA-B specificity. Each HLA-B molecule expresses either Bw4 or Bw6 supertype in addition to the (private) HLA-B specificity. Typing for HLA-B supertypes either by serology or DNA analysis may help to double-check HLA-B type or to sort out discrepancies. A list of the distributions of common HLA-B alleles in supertypic groups is presented in Table 24.2.

Likewise, HLA-DR alleles are also associated with supertypes. However, the HLA-DR supertypes are encoded individually by separate genes and are distinct molecules. Another difference is that not all DR alleles are associated with a supertype. Table 24.2 shows a list of DR alleles in each supertypic group.

Serological cross-reaction between certain alleles of each HLA locus is well known. This often creates problems in serotyping. The cross-reaction is due to some shared epitopes (public antigens) among several distinct specificities (private antigens). Antigens which are cross-reactive with each other are commonly referred to as cross-reactive groups (CREGs). With the advent of molecular typing, cross-reactivity (as defined serologically) is no longer a technical problem. Nevertheless, CREGs may still have some significance in donor selection (see below). There are six major CREGs: Bw4, Bw6, B5, B7, B8 and B12. A more detailed list of most common CREGs is presented in Table 24.3.

The group of alleles encoded by several HLA loci on a single chromosome is called a haplotype. Considering the number of alleles at each locus, millions of different combinations of these alleles (haplotypes) are to be expected. However, because certain alleles are commonly associated with one another due to linkage disequilibrium (LD), only a relatively small number of haplotypes can be observed. LD is a tendency for some alleles of different loci to segregate together as blocks. The inheritance unit of the HLA complex is a haplotype. It is fortunate if a patient who needs an unrelated donor carries the haplotypes which show a high LD, as this will mean the donor will be fully matched with the patient [5]. The haplotypes that show a high LD among their alleles are generally ancestral haplotypes (see below) [6]. A selection of such haplotypes is presented in Table 24.4.

HLA loci are linked which show a recombination fraction of only 1–3% across the HLA complex, i.e. in 100 meioses, the haplotype will be broken and

Table 24.2 Supertypic groups of HLA-B and HLA-DR alleles

HLA-Bw4	HLA-Bw6	HLA-DR51	HLA-DR52	HLA-DR53
B5 (51/52)	B7	DR1 (rare)	DR3 (17/18)	DR4
B12 (44)	B8	DR2 (15/16)	DR5 (11/12)	DR7
B13	B12 (45)		DR6 (13/14)	DR9
B15 (63/77)	B14 (64/65)		DR1403	
B16 (38)	B15 (62/75/76)		DR1404	
B17 (57/58)	B16 (39)			
B21 (49)	B18			
B27	B21 (50)			
B37	B22 (54/55/56)			
B47	B2708			
B53	B35			
B59	B40 (60/61)			
	141			

also:	B42
A9 (23/24)	B46
A10 (25)	B48
A19 (32)	B67
	B70 (71/72)
	B73
	B78
	B81

Table 24.3 Cross-reactive groups of HLA-A and HLA-B alleles

HLA-A					HLA-B									
A1	A9	A10	A19	A2	B5	B12	B14	B8	B15	B16	B7	B7	B7	B13
A3	A23	A25	A29	A28	B18	B21	B64	B59	B17	B38	B27	B22	B40	B47
A11	A24	A26	A30	A68	B35	B44	B65		B46	B39	B42	B54	B41	
A36		A34	A31	A69	B51	B45			B57	B67	B73	B55	B48	
		A43	A32		B52	B49			B58			B56	B60	
		A66	A33		B53	B50			B62				B61	
			A74		B70				B63				B81	
					B71				B70					
					B72				B71					
					B78				B72					
									B75					
									B76					
									B77					

*Table 24.4 Common HLA-B:DR haplotypes in the British population**

Haplotype	Frequency (%)**
HLA-(A1) B8DR3	11.9
HLA-(A2) B44DR4	5.5
HLA-(A3) B7DR2	5.9
HLA-(A29) B44DR7	4,2

HLA-(A1) B57DR7	3.0
HLA-(A2) B62DR4	1.2

*For a comprehensive list of all ancestral haplotypes see [5,6], **The frequencies in the British population are from [7]. The commonest HLA-A allele of each haplotype is shown in parentheses.

reconstituted one to three times. However small, this may have important implications in the selection of an HLA-identical sibling for transplantation. When it occurs, a new composite HLA haplotype is created, representing the parts of the two haplotypes of the parent in which recombination has occurred. Thus, the child will have a recombinant haplotype which is slightly different from all four haplotypes in the family. There are two well-recognized recombinational 'hotspots' in the HLA complex: one between HLA-A and HLA-B/Cw, and the other between HLA-DR/DQ and HLA-DP. The frequency of genetic recombination between HLA-A and HLA-B is approximately 1%. Separation of the HLA-DR/DQ segment from the DP region is the most common form of recombination (1–2%). As a result of this, HLA-DP incompatibility occurs in around 80% or more of HLA-ABDRDQ-matched unrelated pairs [8–10].

HLA genes segregate co-dominantly in progeny with one haplotype from each parent. Although there are reports that individual haplotypes may show preferential transmission, in general HLA haplotypes are transmitted in a Mendelian fashion. If both parents are heterozygous for HLA haplotypes, there are four possible genotypes for their offspring. Thus, any two siblings have a 25% overall chance of inheriting the same set of haplotypes (HLA identity). The likelihood of being totally different is also 25%, the rest being one haplotype identical. In the normal population, the HLA-identical sibling frequency is 25%. For young leukaemic patients, however, it is significantly higher than that, at around 35% [11–14]. This can be explained by a presumption that leukaemia-associated HLA haplotypes are transmitted preferentially from parents to offsprings. Using this idea as the starting point, a consistent HLA association between the HLA-DR53 supertype and all major types of leukaemia has been shown [14–16].

The fact that HLA-identical sibling frequency is higher than 25% in leukaemic families should not be confused with the overall chance of having an HLA-identical sibling, which correlates with family size (equal to $[1-(0.75)^n]$, where n is the number of siblings). This probability may go up to 55% in areas where families are traditionally large [17].

Genetic structure of the MHC

The chromosomal location of the human MHC is 6p21.3, and altogether it spans about 4 Mb. The MHC is divided into three regions [18]. The first HLA loci discovered lie in the class I region (telomeric), and a different group of HLA loci found later lie in the class II region (centomeric). The class III region of the MHC is between the other two regions and does not contain an HLA locus. This region is currently the most populated part of the HLA complex and accommodates the genes for tumour necrosis factor (TNF), the heat-shock protein *HSP70*, complement proteins and many other genes, many with yet unknown functions. In the class II region, with the exception of *RING3*, all genes

expressed are associated with the immune response. The HLA class I genes are interspersed with a variety of genes with many different functions.

A list of officially recognized HLA loci is given in Table 24.1. Apart from the classical class I and II loci, no other locus in the HLA region has so far been shown to be relevant in transplantation. Among the new HLA loci, some are not polymorphic, or non-expressed pseudogenes, and some have tissue-specific expression patterns. Thus, most are not expected to play a role in the immune reactions against an allogeneic transplant.

There are interesting new genes among those recently mapped to the MHC. To mention a few, the class II gene *RING3* is a developmentally expressed gene with a high expression level in leukaemia [19]. The *TAP* (transporters associated with antigen processing), *LMP* (low-molecular-mass polypeptide proteasome or large multifunctional proteinase) and *HLA-DM* genes have crucial roles in peptide presentation and cell surface expression of HLA antigens. Briefly, *LMP* gene products are the subunits of proteasome which degrade endogenous proteins to peptide fragment; *TAP* gene products are involved in the translocation of these peptides into the endoplasmic reticulum; and *HLA-DM* is probably required for peptide loading into the HLA molecule. Further insight may now be gained into the unresolved issue of HLA associations with malignancies with the mapping of a proto-oncogene in the class III region of the mouse and human MHC [20]. The proto-oncogene *INT3* is the integration site of the mouse mammary tumour virus and has transforming ability when mutated. There are also some transcription factors within the 'new' MHC such as *OTF3* [21], and a potential member of the NFk B family [22].

The genomic organization of the DR region is different in different haplotypes, since the number of genes and pseudogenes expressed may vary among the major supertypic groups. The HLA-DR1 group which also contains DR10, and the DR8 group, are not associated with a supertype and only express one DR molecule (Table 24.2). The DR52 group (DR3, 5, 6), DR53 group (DR4, 7, 9) and DR51 group (DR2) haplotypes express two DR molecules, one representing the supertype-encoded by *DRB3*, *DRB4* and *DRB5* genes, respectively, and the other representing the private specificity.

It is still under debate whether the variable number of *DRB* genes on different haplotypes may be the reason for the size difference among the class II regions of the supertypic haplotypes. In the area between the *DQA1* and *DRB1* genes, the DR53 supertypic group haplotypes carry a 120kb-long extra piece of DNA in comparison to the DR3 (DR52) haplotype [23]. It is tempting to speculate that this extra bit of DNA might well contain a gene that would be exclusive to the DR53 supertypic haplotypes. Such a gene could be responsible for DR53-associated phenomena such as preferential transmission, and disease associations such as leukaemia and rheumatoid arthritis.

Structure of HLA molecules

All HLA molecules are membrane integrated glycoproteins. Class I molecules consist of a polymorphic heavy (α) chain encoded in the MHC, and a non-covalently associated invariant light chain β_2 microglobulin (β_2M) encoded on chromosome 15. The heavy chain comprises three distinct portions: hydrophilic cytoplasmic tail (30–40 amino acids), transmembrane domain (around 30 amino acids), and extracellular portion, each encoded by a separate exon. The extracellular part of the heavy chain is composed of α_1, α_2 and α_3 domains, each containing around 90 amino acids. The polymorphic residues are not

randomly distributed throughout the molecule but located mainly in the outer domains α_1 and α_2. These two membrane distal polymorphic domains, encoded by exon 2 and 3 of the gene, form one structural domain (α_1/α_2), supported by membrane proximal non-polymorphic domains α3 and β_2M. The groove in the α_1/α_2 superdomain is the peptide-binding groove (antigen-binding site) with the majority of variable residues located in this cleft. The groove consists of two nearly antiparallel α helices above a floor of eight antiparallel α-strands. The groove is in the most membrane distal portion of the molecule, which is an ideal orientation for interaction with the antigen receptor of patrolling T-cells.

Class II molecules are also α/β heterodimers with extracellular, transmembrane and cytoplasmic portions. Both chains of class II molecules span the membrane. The membrane distal domains of the chains (α_2/β_2) form the peptide-binding groove, whereas, the membrane proximal domains (α_1/β_1) which have a strong sequence homology with immunoglobulin constant region domains such as α1 and β_2M of class I, are non-polymorphic. The polymorphism of class II molecules stems from the β-chains, mainly in the exon 2-encoded part. The α-chain is non-polymorphic. The peptide-binding groove of class II is very similar to that of class I except that both α and β chains contribute to it. The peptide-binding unit of class II maps to the amino terminal region of the outer domain of the β-chain, whereas the T-cell recognition site maps to a segment of the same domain spanning the third hypervariable region (HVR3). HVR3 is the most polymorphic and immunodominant part of the β-chain. To disguise themselves as self to their target cell, many viruses mimic the HVR3 sequences of HLA class II antigens [24]. This is one of the viral immuno-evasive mechanisms involving HLA [25].

Peptide presentation by HLA molecules

The following is a brief introduction to this rapidly developing field in immunology. Interested readers are referred to more detailed recent reviews [26–28].

The recognition of antigens by B-cell receptors (surface immunoglobulin) and their secreted counterparts, antibody molecules, is a direct phenomenon. However, for T-cell recognition of an antigen to occur, the antigen must be processed and presented to the T-cell receptor (TCR). HLA molecules are assembled inside the cell, incorporate antigenic peptide in the groove and are then expressed in order to present this assembly to T-cells for immune recognition (Table 24.5, Figures 24.2–24.5). Peptide presentation shows different patterns for each class of HLA antigens. Class II molecules associate with peptides derived from endocytosed externally derived antigen, during biosynthesis and assembly. Class I has evolved to make a sample of intracellular contents available for surveillance by the immune system.

The following simplified example summarizes the differences in peptide presentation by two classes of HLA molecules: if a cell is exposed to viral proteins exogenously, they are taken up by the cell (endocytosis), degraded by acidic proteasomes (cathepsins) down to 12–25 residues, stored at acidified cytosolic vesicles (endosomes) where they meet HLA class II molecules [29]. After association with them, the HLA:peptide complex is transported to the cell membrane, ready for recognition by $CD4^+$ T-cells. To get the same antigen presented by HLA class I molecules to $CD8^+$ T-cells, the virus would have to infect the cell and its endogenously-synthesized proteins should be cut down to 9±1-mer peptides. Some subunits of proteasomes involved in this process are encoded by the *LMP*

genes within the MHC class II region. Short peptides produced by the proteasome are transported into the endoplasmic reticulum by the product of another set of MHC genes, *TAP1* and *TAP2*, where they bind and stabilize HLA class I molecules before surface expression. In general, HLA molecules do not recycle to present different peptides. There are exceptions in that class I may handle exogenous antigen and class II may present endogenous peptides that have not come from endosomes.

Peptides are essential for proper folding, assembly and stabilization of both classes of HLA molecules. After the assembly of the molecule and irreversibly bound peptide in the peptide-binding groove, these peptide-HLA complexes are transported to the cell surface where they can be scrutinized by T-cells. In fact, class I molecules do not leave the endoplasmic reticulum unless they bind peptides. In the absence of peptide, class I heavy chains can interact with β_2M,

Table 24.5 Characteristics of class I and class II molecule assembly and peptide presentation

Parameter	Class I	Class II
Expression	All nucleated cells	Antigen-presenting cells
Recognition	CD8+ T-cells	CD4+ T-cells
Length of peptides bound	9±1 mer	12–25 mer
Associated molecules (chaperons)	Calnexin	Invariant chain (Ii)
Source of antigens	Endogenous	Exogenous
Protease used	Proteasome complex (incl. LMP2/LMP7)	Cathepsin
Cellular compartment for incorporation of peptides	Endoplasmic reticulum	Endosome/Lysosome (MIIC)

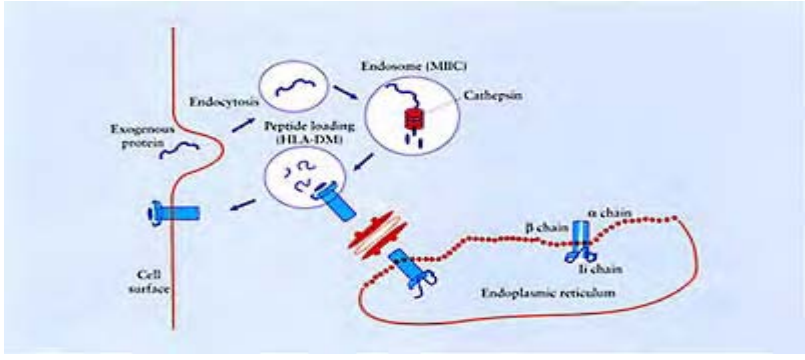

Figure 24.2 HLA class II molecule assembly and exogenous peptide presentation.

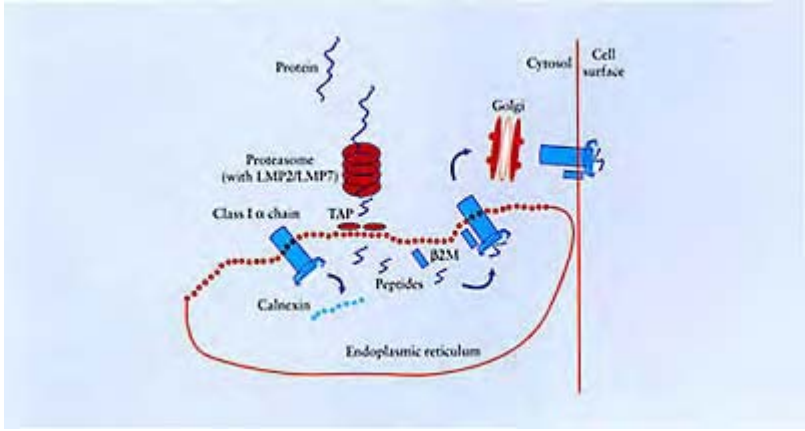

Figure 24.3 HLA class I molecule assembly and endogenous peptide presentation.

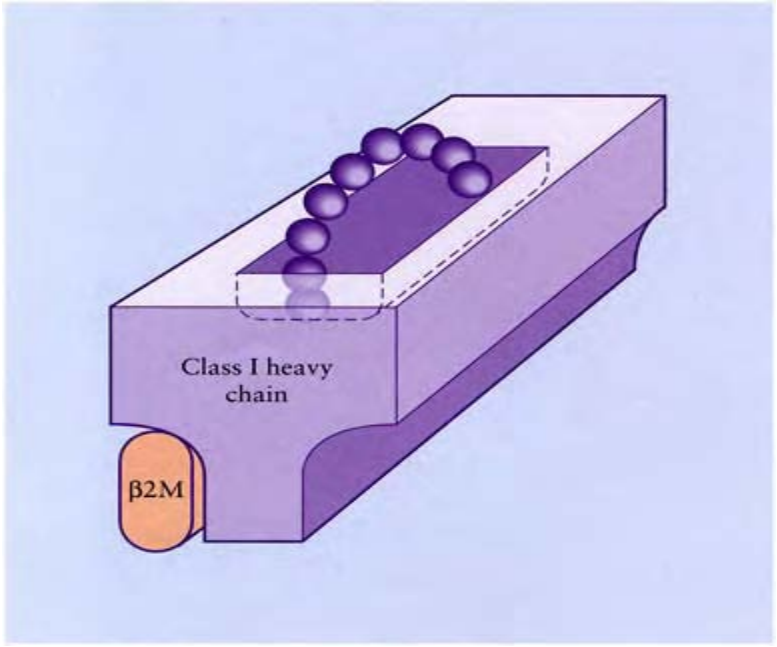

Figure 24.4 HLA class I peptide binding. HLA class I molecules can accommodate only short peptides, being a more closed structure than

class II molecules. Class I molecules preferentially bind nonamers, with the termini anchored and a bulge in the middle.

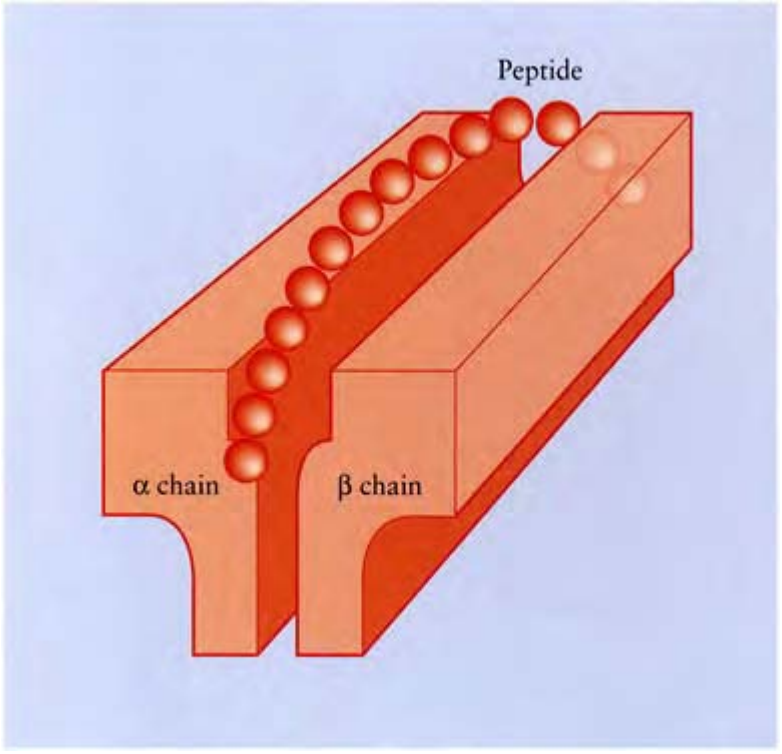

Figure 24.5 HLA class II peptide binding. HLA class II molecule resembles a more open grooved structure, with the peptide (12–25 amino acids in length) anchored at one or two sites. For peptides longer than 15 residues, it is likely that the ends extend beyond the margins of the groove.

and some are transported to the cell surface, but being unstable, they are rapidly lost. The main determinant of antigen presentation is the rate of HLA class I exocytosis, not the level of class I surface expression [30]. As peptides presented by class I (to $CD8^+$

cytotoxic T-cells) are usually derived from cytosolic and nuclear proteins, the immune system can respond to internal changes in infected/transformed cells.

Peptide presentation to HLA class II restricted $CD4^+$ T-cells generally requires newly synthesized class II molecules, associated invariant chain (Ii; CD74) and HLA-DM. HLA class II molecules are directed to the specialized endosomal/lysosomal compartment (MIIC= MHC class II compartment) of antigen-presenting cells by Ii, to bind processed peptides. Ii prevents binding of (endogenous) peptides to class II before they reach MIIC [31]. Ii is dissociated in the acidic pH of endosomes to allow the peptides to bind to class II prior to cell surface expression of mature class II heterodimer. Stable surface expression of Ii prevents peptide presentation by HLA-DR. Another HLA class II molecules, HLA-DM, is involved in releasing Ii and loading antigens on to class II molecules in MIIC. Unlike the conventional HLA class II molecules, HLA-DM molecules are not highly polymorphic, reside in the MIIC, have only an intracellular role, and do not require peptide binding to form a stable heterodimer [32].

Peptides bound to HLA class I molecules exhibit a typical length of 9±1 amino acids and carry 'anchor' residues crucial for binding in position 2 (P2) and the C-terminal position (PC). However, the presence of anchor residues in a given peptide is in itself not sufficient to determine binding to HLA molecules [33]. Several examples of known immunogenic and naturally processed peptides exist that do not carry a perfect binding motif. The MHC binding affinity of each peptide determines its immunogenicity, only high or intermediate affinity peptides being immunogenic. Due to the presence of allele-specific motifs in the peptide binding site of HLA alleles, each peptide can be presented only by one or several alleles of a locus. This is the basis of MHC restriction of antigen presentation. Being heterozygous at an HLA locus would increase the chances of a peptide being presented.

Minor histocompatibility antigens (mHag)

In BMT from HLA genotypically identical siblings, graft-versus-host disease (GvHD), the major immunological complication of BMT, can still occur. In such patients, donor T-lymphocytes, T-helper cells (T_H) or cytotoxic T-lymphocytes (CTL), directed against minor histocompatibility antigens (mHag) of the recipient, presented by HLA class I or class II antigens, can be shown [34,35]. Two decades after the first report suggesting the possible involvement of mHag in human transplantation, the nature of mHag and the immune response against them is better characterized [36]. Minor histocompatibility antigens are naturally processed peptide fragments originating from intracellular proteins that associate with MHC products. The first human mHag identified at the molecular level are HA-2 and H-Y antigens [35,37]. The HA-2 peptide most probably originates from a member of the myosin family. The H-Y antigen presented by HLA-B7 is an 11-residue peptide derived from an evolutionarily conserved protein encoded by the Y chromosome called SMCY.

T-cells recognize mHag in an HLA-restricted manner, and the ability of T-cells to mount a specific response can be described as peptide alloreactivity. This response occurs between HLA-identical individuals when a mHag is present (or expressed) in one individual, but absent in the other. The best known mHag is the male-specific H-Y antigen [38,39]. The majority of mHag are determined by autosomal, non-HLA-linked

genes and are most frequently presented by HLA-A1, A2 and B7 [36]. An interesting phenomenon is that expression of most autosomal mHag seems restricted to the haemopoietic cell lineage, including epidermal Langerhans cells [40]. This may have implications for the graft-versus-leukaemia effect [41].

The link between mHag and GvHD is hard to pin down. Prospective typing for the mHag HA1 may be useful to predict GvHD [42]. It has been speculated that a gene distal to the HLA complex may be associated with increased risk for acute GvHD in HLA-identical sibling transplants [43]. This idea was based on the finding that those pairs which were not identical at the *F13A* locus which is 25 cM away from HLA-A, had a higher frequency of GvHD. An unknown gene in this interval could be responsible for an immunological reaction against the recipient. Recently, a β_2M-associated, expressed HLA class I-like gene has been found 5 cM telomeric to HLA-A (shown as HLA-HFE in Figure 24.1) [44]. This gene is mutated in a very high percentage of patients with hereditary haemochromatosis and may also have a role in transplantation immunology.

Donor selection for BMT

From the above discussion, it will be clear that mismatching at major HLA or minor histocompatibility antigens increases the risk for the main immunological events after BMT-GvHD and graft failure. The risk of graft failure/rejection and GvHD increases proportionally with the degree of HLA incompatibility. Even if engraftment occurs, other problems associated with HLA incompatibility continue—infections (delayed immunological reconstitution), lymphoproliferative neoplasms and decreased overall long-term survival [45]. In HLA-identical sibling transplants, acute and long-term immunological complications are seen less frequently than in unrelated transplants. Because HLA serologically matched siblings have inherited the same parental haplotypes, the same degree of matching cannot be achieved in unrelated transplants. However, it is sometimes possible to find recipient-donor pairs who share whole haplotypes. As opposed to random allelic resemblance at HLA loci, identity for (ancestral) MHC haplotypes between unrelated pairs yields mixed lymphocyte culture (MLC) results comparable with those of HLA-identical siblings [46,47]. In the clinical situation, patients matched to their unrelated donors for the haplotypes instead of only alleles have better survival rates both in renal [7] and marrow transplantation [48].

The first choice for a patient in need of BMT is an HLA-identical sibling. If an HLA-identical sibling is unavailable, an alternative donor should be considered. The options are the patients themselves (autologous BMT), a family donor who is HLA-haplo-identical with one-antigen mismatched on the other haplotype, an unrelated volunteer donor matching as closely as possible, or an HLA disparate cord blood stem-cell donor. In the absence of a matched sibling, it may still be possible to find a good family match, especially in families where parents are related, as a remote relative may be found who is HLA-identical to the patient. This search may also yield a potential donor within the family who is partially matched with the patient. Usually, depending on the nature of the mismatched antigen involved, an intrafamilial one-antigen mismatched donor may be as good as an HLA-identical sibling. This issue is discussed in detail later. A one-antigen mismatched relative is available for about 5% of patients, but an HLA-matched unrelated

donor for about 70% of patients lacking an HLA-identical related donor [49]. Therefore, if a suitable family donor is not available, a computer search for an unrelated donor should be commenced. No clear preference among alternate donor options has been established.

Current practice in the selection of unrelated donors involves initial serological typing, followed by DNA typing of class II genes (and more recently class I genes). No matter what the resolution of the typing system is, identity for HLA genes/antigens in unrelated pairs does not mean identity for the MHC region. In first degree relatives, if the donor and recipient are matched for HLA-A and HLA-B antigens, there is an excellent chance that they will also be matched for HLA class II antigens and other loci within the MHC. However, in random pairs, matching for one or two antigens is not predictive for a full MHC matching.

One reason for higher frequency of immunological complications following unrelated BMT is that unrelated subjects have more mismatches for minor histocompatibility antigens [50]. In terms of HLA matching, the reason for the immunological problems after unrelated transplants is the lack of haplotypic identity which can only be established through extensive family studies, which is not practical. In pairs fully matched for HLA alleles at all loci examined, haplotypic identity and random allelic identity can be distinguished by analysis of non-HLA loci within the MHC [48,51]. As expected, haplotypic matching improves survival following BMT [48]. However, such additional tests can only be performed in the presence of multiple-matched unrelated donors to choose the haplotypically matched one. On the other hand, when strict criteria for identifying HLA-matched donors are used, the survival of patients transplanted from unrelated donors does not differ from that of patients transplanted from HLA-identical family members [52,53]. This observation suggests that lower survival rates in unrelated BMT are probably due to HLA mismatches remaining undetected. This assumption has strong support coming from retrospective studies using molecular HLA-typing techniques [9,54,55]. In kidney transplantation too, a retrospective molecular study revealed HLA mismatches in serologically-matched pairs and survival in the DNA-matched group was notably higher [56]. It was concluded that many transplants that were previously thought to be HLA matched were, in fact, mismatched, and those undetected mismatches were responsible for the lower success rates in HLA-matched cadaver transplants than in HLA-matched sibling grafts.

Sensitization against donor antigens

Sensitization of the recipient against donor antigens through blood transfusion or pregnancy is one of the major risk factors for rejection [57]. The presence of donor-specific antibodies can be tested by using the patient's serum for antibodies and donor lymphocytes. This test, referred to as the lymphocyte cross-match (a complement-dependent cytotoxicity (CDC) assay) which is particularly important in solid-organ transplantation as there are more mismatched HLA antigens involved, is still important in allogeneic BMT. The presence of a positive pretransplant antidonor cross-match is associated with an increased risk of primary graft failure in allogeneic BMT, although graft failure can happen despite a negative CDC test, due to its low sensitivity. A more sensitive test uses flow cytometry for detection of alloimmunization. Simultaneous two-

colour flow can discriminate anti-class I antibodies directed against all lymphocytes from anti-class II antibodies which are directed against only B-lymphocytes. The flow cytometry test has a ten-fold greater sensitivity than the CDC cross-match assay [58].

Functional histocompatibility tests

Recipient-specific alloreactivity of donor T-cells can be studied before BMT to predict immunological complications. The commonest test performed for this purpose is the MLC assay which is discussed in detail later. Several additional techniques have been described to improve the outcome of unrelated BMT. These are designed to detect minor or major histocompatibility differences at the functional level between individuals. The functional histocompatibility tests are shown in Table 24.6.

Cytotoxic T-cell precursor (CTLp)

Although still far from being routine tests, some of the functional tests have been shown to be predictive for

Table 24.6 Functional tests for optimizing unrelated donor selection

Mixed epidermal cell reaction [59]
Skin explant model [60]
Mixed lymphocyte culture (MLC) and modified versions [61]
Cytotoxic lymphocyte precursor frequency assay (CTLp) [62]
IL-2-secreting helper T-cell precursor frequency assay (HTLp) [63,64]

GvHD, and can therefore be used as additional histocompatibility tests [59–64]. These methods are designed to test the repertoire of circulating T-cells that are reactive against the recipient's HLA. Cytotoxic ($CD8^+$) T-cells tend to recognize HLA class I better, and helper ($CD4^+$) T-cells, class II better. Cytotoxic T-cell precursor (CTLp) frequency analysis has been most promising in unrelated BMT performed with T-cell depletion [62,64,65]. Those patients with CTLp frequencies higher than 1:100000 tended to have more severe GvHD than those patients with lower CTLp frequencies. CTLp-positivity rigorously correlates with the presence of class I mismatches at the DNA level. The CTLp analysis is performed by limiting dilution assays, whereby limiting numbers of responder (donor) cells are cultured with a constant number of stimulator (recipient) cells and assayed for reactivity against additional stimulator cells. The frequency of responding cells is determined by a maximum likelihood estimation using a computer program. The CTLp frequency analysis may be used as a test to assess class I incompatibility and it is not sensitive to class II mismatches. There is now preliminary evidence that high CTLp frequencies can be predicted by DNA typing of class I genes that may have appeared to be serologically identical [54].

Helper T-cell precursor (HTLp)

A more recent functional histocompatibility test relies on the same principle but assays the frequency of interleukin-2 (IL-2)-secreting helper T-cell precursors (HTLp) [63,64]. Two studies have shown that high frequencies of host-specific HTLp in the peripheral blood of donors correlate well with the development of moderate-to-severe GvHD in recipients after HLA-identical sibling [63,66] and unrelated BMT [64]. This assay has been found to be as informative as the CTLp assay in predicting acute GvHD in unrelated BMT. Frequency analysis of recipient-specific HTLp is more rapid, less labour intensive and more sensitive to HLA class II and mHag disparities than CTLp, so it may be another functional histocompatibility test for donor selection and GvHD prediction. It remains to be seen whether the results of the HTLp assay can be predicted by fine-resolution HLA class II typing. In conclusion, the role of these functional histocompatibility tests is still a little unclear and current molecular HLA typing methods may be able to give the same answer.

HLA matching

HLA typing can be done in two different ways: detecting HLA antigens on the cell surface (serology) or analyzing the genes encoding for HLA antigens (DNA typing). With increased knowledge of the HLA genes and alleles, the concept of HLA matching has evolved to a degree which could not have been predicted a decade ago. What would have been considered a good match in the recent past may be a disastrous incompatibility today. For example, a single amino acid difference between HLA-B44 subtypes has been shown to induce acute GvHD [67] or rejection [68]. In the class II region, mismatching for subtypes of HLA-DR4 is associated with an increased risk of acute GvHD in related or unrelated BMT [49]. On the other hand, there are HLA-DRB1 and HLA-DPB1 disparities retrospectively demonstrated in unrelated BMT with no influence on the immunological outcome [67,69]. These observations suggest that, although HLA matching is crucial for the success of BMT, in analogy to kidney transplantation [70], there may be permissible mismatches which would not have any detrimental effect. Our knowledge on this issue in BMT is currently insufficient, but is increasing rapidly.

A useful way to view the mismatches is to divide them into three categories [71]:

1. Major, for example an HLA-A2 in the recipient versus an HLA-A3 in the donor.
2. Minor, for example an HLA-A2 versus an HLA-A28 (CREG mismatching).
3. Micro, for example an HLA-A2 subtypic mismatching determined by isoelectric focusing and/or polymerase chain reaction (PCR).

For HLA class II alleles, micromismatching identitifies HLA-Dw determinants, but differences must be identified by fine resolution PCR typing; a minor mismatch is that which is distinguishable by HLA-Dw typing but not by serology; and a major mismatch is serological incompatibility (Table 24.7).

In haematological malignancies, survival in one-antigen mismatched unrelated transplants is equivalent to HLA-identical sibling transplants because of a decreased risk of relapse [49,72,73]. If the mismatch concerns a CREG (i.e. minor) antigen mismatch, this donor would seem to be a reasonable alternative to a fully-matched donor for BMT

[10,71,72] as well as for platelet transfusion [74]. It has thus been recent practice to accept (in the absence of a better choice) unrelated donors with minor mismatches, at least in younger patients with haematological malignancies [10,71,72].

When there are several potential donors, all one-antigen mismatched at different loci, the question arises as to whether there is any difference in the relative relevance of HLA loci in BMT. Different studies have demonstrated the equivalent importance of class I and class II matching in BMT, regardless of the locus [71,72]. In kidney transplantation, it is generally believed that matching at HLA-B and HLA-DR is of prime importance and the role of HLA-A is somewhat less important [75]. The basis for this may be that in healthy individuals, CTLp frequencies against HLA-A antigens are generally lower than CTLp frequencies against HLA-B [76]. Also, at the functional level, qualitative differences exist between CTL precursors against HLA-A and HLA-B, the affinity for the latter being higher [77]. These observations will have a bearing on the selection of one-antigen mismatched donors for BMT if confirmed by long-term survival studies. It should be remembered that when the mismatched HLA-A antigen is in a CREG (minor mismatch), the GvHD risk is lower than that of a non-CREG HLA-A mismatch (major mismatch), but for the DR locus, all mismatches have the same influence on the development of GvHD [71].

Among the issues on HLA-matching in unrelated BMT that remain unanswered is the functional role of HLA-Cw (class I) and HLA-DP (class II) in clinical outcome. Although several studies have attributed a

Table 24.7 Classification of mismatches

HLA-A/HLA-B	HLA-DR/DRB/Dw
Genotypic identity	
Determined by SSOP/SSP/SBT	
B*0702 versus B*0702	DRB1*0401 versus DRB1*0401
Phenotypic identity	
Determined by serology, IEF or MLC (for Dw)	
B7 versus B7	DR4 (Dw4) versus DR4 (Dw4)
Micromismatch	
Determined by high-resolution SSOP/SSP/SBT or IEF (for class I)	
B*0702 versus B*0705	DRB1*0401 versus DRB1*0404
Minor mismatch	
Determined by serology (class I) or MLC (Dw) or low-resolution SSOP/SSP (DRB)	
B7 versus B27 (CREG)	DR4 (Dw4) versus DR4 (Dw10)
Major mismatch	
Determined by serology or low-resolution SSOP/SSP (DRB)	
B7 versus B8 (non-CREG)	DR3 versus DR4

role to HLA-DP mismatching in the induction of acute GvHD, in the light of recent molecular studies in otherwise 'well-matched' unrelated pairs, it is believed that HLA-DP mismatching should not be an exclusion criterion for selection of a donor [8,78]. Previous studies may perhaps have suffered from HLA-ABDR mismatches remaining undetected because of the technology then available, as retrospective studies strongly suggest [9,54].

Until the advent of molecular typing of HLA-Cw genes, it was not possible to study the relevance of HLA-Cw disparity in transplantation immunology. In view of the fact that HLA-Cw molecules can be recognized by cytotoxic T-lymphocytes *in vivo* [79], they are expected to have a role in the immunological outcome of BMT. This issue requires particular attention, since in one study, 12.5% of 56 HLA-ABDRDQ-matched unrelated pairs had HLA-Cw mismatching detected by molecular methods [80]. It has further been proposed that recipient natural-killer cells might mediate rejection of marrow grafts by destroying donor haemopoietic cells incompatible for HLA-Cw antigen [81], but clinical data to test this hypothesis are not yet available. Evidence for a direct role for HLA-Cw antigens comes from the demonstration of a significant correlation between molecular HLA-Cw incompatibility and the frequencies of CTLp following BMT from HLA-ABDRDQ-matched unrelated donors [54,55]. In the light of these observations, molecular compatibility for HLA-Cw genes in unrelated BMT may have to be included in donor selection, although this adds further to the difficulties of finding a suitable match.

One of the possible strategies previously proposed to increase the number of potential donors was using mismatched donors where the mismatched donor antigen is a non-inherited maternal antigen (NIMA) of the patient. This idea was based on the finding that in about half of pregnancies, life-long B-cell tolerance can be induced against the maternal antigens that were encountered but not inherited by the foetus [82]. If true, this would immediately open up many more possibilities for selecting donors. Although there were initial reports on the biological influence of NIMAs in kidney transplantation, firm support to this concept is still lacking [83]. Furthermore, recent reports have suggested no influence of NIMAs on the alloreactive T-cell repertoire in cord blood or in adults [76,84]. At present, it would be too optimistic to expect any role for NIMAs in donor selection for BMT.

Functional assays could potentially help in selecting one-antigen mismatched unrelated donors on the basis of 'intelligent mismatching', i.e. low CTLp frequencies against the mismatched antigen might be indicative of a good outcome [65]. The more recently introduced HTLp frequency assay may also provide additional information for suitability of the donor for a particular recipient [64]. A summary of relevant information on HLA-matching and donor selection is provided in Table 24.8.

Current HLA typing techniques

Serology

Until recently, HLA typing of donor and recipient for BMT was exclusively carried out by complement-

Table 24.8 Guidelines for HLA-matching and donor selection in BMT

- HLA matching benefits all transplants.
- Graft rejection and GvHD risk correlate with the degree of HLA mismatching.
- Best option as a donor is an HLA-identical sibling.
- Alternative options are:
  Haplo-identical one-antigen mismatched family donor; HLA-matched unrelated donor, and one-antigen mismatched unrelated donor.
- Mismatching at HLA-A, HLA-B, HLA-DR is equally bad, but whenever possible an HLA-A CREG mismatching is preferable as a one-antigen mismatch, whereas mismatching at HLA-DR should be avoided.
- Mismatching at HLA-DP may be ignored.
- Mismatching at HLA-C may be more important than HLA-DP mismatching.
- MLC has no predictive value for acute GvHD and is replaced by PCR typing.
- CTLp and HTLp assays are equally informative for predicting GvHD and survival in unrelated BMT.
- Fine-resolution PCR typing decreases the chances of finding a well-matched donor but nevertheless increases survival rate.

dependent microcytotoxicity, and functional compatibility by the MLC assay [85]. The microcytotoxicity assay (serology) requires viable cells expressing HLA antigens, and specific antisera. The antisera for HLA typing are generally obtained from multiparous women, individuals heavily transfused and solid-organ recipients who have rejected their transplant. The available serological reagents cannot detect most of the currently recognized allelic specificities, for example by serology only 18 alleles and a few subtypes of them can be recognized at the HLA-DRB locus. Common problems associated with serology are misassignment and missing an antigen, causing wrong phenotype detection, or identification of false homozygosity of a heterozygous subject, or vice versa. These are due to cross-reactions at the protein level and the poor quality of antisera used. The use of highly specific and pure monoclonal antibodies can overcome some of these problems, but still cannot cope with the ever growing number of alleles. Serological typing is difficult to perform when lymphocytes are scarce, if there are contaminating malignant cells, or when separation of B-cells is difficult due to a high granulocyte count. The significance of these practical difficulties is seen when retyping of retrospective samples by DNA analysis is carried out, showing that around 25% of the HLA-DRB alleles may be incorrectly assigned serologically [86,87].

Cellular assays

The MLC assay detects alleleic differences in the HLA molecules of the donor and recipient T-cells. With this method, peripheral blood mononuclear cells from one

individual (the responder) are cultured together with mononuclear cells from another individual (the stimulator) that have been irradiated or otherwise treated to prevent proliferation. The degree of proliferation (as measured by ^{3}H-thymidine incorporation) by the responder cells is thought to represent the extent to which the responder cells can recognize any histoincompatible antigens expressed by the stimulator cells. In addition to HLA-DR/Dw, incompatibilities for HLA-DQ, HLA-DP and class I antigens have been shown to contribute to the proliferative response [88], For this assay, live lymphocytes are required, and the culture system is very sensitive to environmental factors. A satisfactory result can be obtained in only two-thirds of transplant pairs [88,89]. It takes a minimum of four days, interpretation is difficult, and moreover, its value for predicting GvHD has been questioned. Most studies have reported failure of the MLC to predict severe acute GvHD and mortality following HLA-identical sibling [90,91] and unrelated BMT [69,88,89,92,93]. The MLC is reaching the end of its useful life.

The current PCR-based typing methods are as informative as the MLC and largely replace it in practice [92,94–96], whereas restriction fragment-length polymorphism (RFLP) matching for HLA-DR and HLA-DQ fails to predict a negative MLC [97]. This is further evidence for the relevance of HLA-DR/DQ subtypes in allorecognition. DNA typing has also replaced the cumbersome technique of cellular Dw typing originally described by Dupont *et al.* [98]. The question still remains of whether the MLC can provide information concerning acceptable mismatches, that is, polymorphisms which are not recognized as being different by the effector T-cells.

The failure of the MLC to predict GvHD has tempted researchers to develop modified methods based on the conventional MLC. By addition of T-cell growth-promoting agents (IL-2, IL-4) and agents that increase HLA antigen expression on cells (γ-interferon and tumour necrosis factor), amplification of otherwise undetectable proliferative T-cell responses has been achieved [61]. This modified MLC correlates better with the development of acute GvHD following HLA-identical sibling BMT.

Isoelectric focusing (IEF)

IEF is a technique used to determine the polymorphism of HLA antigens that can only be used in addition to serology. HLA class I polymorphism can be detected by electrophoretic analysis, whereby immunoprecipitated class I proteins are separated on IEF gels and charge variants are identified. Since the isoelectric points of many class I alleles are similar it is not possible to assign the HLA type of a cell without its serological type. IEF is most useful for defining HLA-A and HLA-B types when there is confusion over the serological typing of the cell. This technique requires live cells. Analysis of HLA class I molecules by IEF has shown structural heterogeneity for many serological specificities and helped to characterize subtypes of some serotypes [99]. This additional information on the polymorphism of class I antigens has been shown to be enough to improve the results of unrelated BMT [65]. However, sequencing of class I alleles has revealed further polymorphism that cannot be detected by IEF, and current DNA-based typing techniques are replacing IEF for high-resolution class I typing in practice [100].

DNA-based techniques

DNA-based HLA typing techniques have evolved rapidly in the last few years. For a detailed review of this topic, readers are referred to recently published textbooks [85,101]. The first DNA-based technique for HLA typing was restriction fragment-length polymorphism (RFLP) analysis [102] which has been widely used for all class II loci. Most of the sites in genomic DNA where the restriction sites for the enzymes lie are located in the introns of the HLA genes, and the RFLP obtained generally reflects genetic linkage disequilibrium between the location of the restriction sites and the allelic variation in the coding regions [103].

RFLP analysis is a somewhat time-consuming technique. It involves digestion of good quality DNA, size separation on an agarose gel by electrophoresis, transfer of digested and size fractionated DNA on to solid phase, hybridization with a labelled HLA probe and autoradiography. Technical problems mainly concerning digestion and transfer may interfere with the accuracy of the results obtained. It is superior to serology for class II typing, but fails to distinguish much of the HLA class II sequence polymorphism. In some instances, typing DR alleles by RFLP analysis relies on DQ associations, which is undesirable [104]. It was a valuable tool at the beginning of the molecular era for HLA typing and is still useful for research purposes, especially for homozygosity studies. It has, however, lost its attraction in routine tissue-typing laboratories owing to the development of quicker, cheaper and more practical polymerase chain reaction (PCR)-based techniques.

Almost all DNA-based tissue-typing techniques routinely used today utilize PCR to obtain a large amount of DNA [85,105] (Table 24.9). A post-amplification typing process is then required to discriminate the HLA alleles, i.e. amplified fragment-length polymorphism (AFLP), sequence-specific oligonucleotide (SSOP)-dot-blot or -reverse-blot techniques. The PCR-sequence-specific primers (SSP) technique differs from the other PCR-based tissue-

Table 24.9 Main features of common PCR-based HLA typing methods

Method	PCR	Post-PCR	Detection
AFLP	One reaction using locus-specific primers	Multiple restriction enzyme digestions	Direct visualization in agarose gel for interpretation of band patterns obtained by each enzyme
SSOP	One reaction using locus-specific primers	Immobilization of DNA and hybridization with allele-specific probes	Usually calorimetric detection of positive or negative signal
SSP	Multiple reactions using allele-specific primers	None	Direct visualization in agarose gel for presence or absence of allele-specific bands
SBT	One or more reactions depending on the locus and the use of RNA or	Direct sequencing	Automated

DNA

typing techniques in that the discrimination of alleles takes place during the PCR-amplification step. All these techniques required the full sequencing of each allele in order to make the primers, as they require amplification of the most variable part of the coding region (e.g. exons 2 and 3 of class I and exon 2 for class II genes). DNA-based techniques give a greater resolution than serology for both class I and class II loci and do not suffer from cross-reactivity. They identify most of the serological blanks. PCR-based techniques also require a very small amount of cells for DNA extraction, and the DNA can be kept for retyping or further typing if necessary. No viable cells are required; frozen samples, blood or marrow smears, or paraffin-embedded tissue can be used as a source of DNA. Important points in PCR-based HLA typing are the sensitivity, specificity and reproducibility of the particular method, reliability in heterozygous combinations, suitability for large numbers of samples, requirements of time and labour, simplicity of interpretation and cost. Development of non-organic nucleic acid extraction methods and non-radioactive visualization methods has eliminated most of the safety concerns of DNA analysis. The techniques for identification of alleles that are sufficiently simple and informative in a clinical setting are PCR-SSOP and PCR-SSP. These two methods are currently used in most tissue-typing laboratories and are also the method of choice in commercial typing kits.

PCR fingerprinting Rather than typing for HLA alleles, this method permits the rapid matching of HLA-DR and Dw specificities between individuals [105]. It relies on the formation of homo- and heteroduplexes during PCR reaction which would create haplotype-specific patterns when run in non-denaturing polyacrylamide gels. In practice, haplotype-specific gel banding patterns (PCR fingerprints) are unique to each homozygous or heterozygous haplotype combination. Matching patterns between two individuals indicate DR-Dw identity, whereas mismatching patterns suggest disparity. The technique is sensitive enough to detect DR4 subtype mismatches [106]. The simplicity and speed of PCR fingerprinting allow same-day elimination from donor searches of HLA class I-matched but DR-Dw-mismatched candidate donors. Identity between a donor and recipient can be examined by mixing their DNAs before the final stage of the PCR (DNA cross-matching). In the event of identity, individual samples and the mixed sample should yield identical fingerprints [106].

PCR-AFLP (PCR-amplified fragment-length polymorphism) In this method, PCR-amplified products are digested separately with several endonucleases. The digested products are run in an agarose gel and visualized directly on the gel. The assignment is based on the patterns obtained with multiple enzymes. It is a laborious technique and suffers from the fact that expensive enzymes are required. With the advent of new generation enzymes allowing amplification of longer fragments, this technique is likely to replace the conventional RFLP analysis.

PCR-SSOP (PCR-sequence-specific oligonucleotide probing) (Figure 24.6) Using group-specific primers, the polymorphic parts of the genes are amplified, blotted on to a membrane and separately hybridized with multiple non-radioactively labelled oligonucleotide probes specific for the hypervariable region of each allele or a group of alleles. One nucleotide mismatch will prevent the annealing of the probe. To monitor the amount of DNA amplified and loaded on to the membrane, locus-specific consensus probes are

used which are expected to recognize all PCR products. The assignment is based on the reaction pattern obtained with several sequence-specific oligonucleotide probes. SSOPs can be labelled using either radioisotopes, digoxigenin or biotin. The labels are detected by autoradiography or enzyme-linked secondary probes using chemiluminescence or chromogenic substances. The PCR-SSOP typing can be summarized as one amplification and multiple hybridizations of membrane-bound PCR products. Cross-reactivity of some SSO probes in the assignment of alleles in certain heterozygous combinations may cause difficulty. It is a reliable technique, routinely used in many laboratories. The hybridization steps make it slightly more laborious and longer than PCR-SSP. However, it only requires one PCR reaction per locus to type and is more cost-effective than PCR-SSP. High-resolution class II typing by PCR-SSOP is impractical because of the number of probes involved and their cross-reactivity. It is, however, a very robust and reliable technique for low-resolution typing of a large

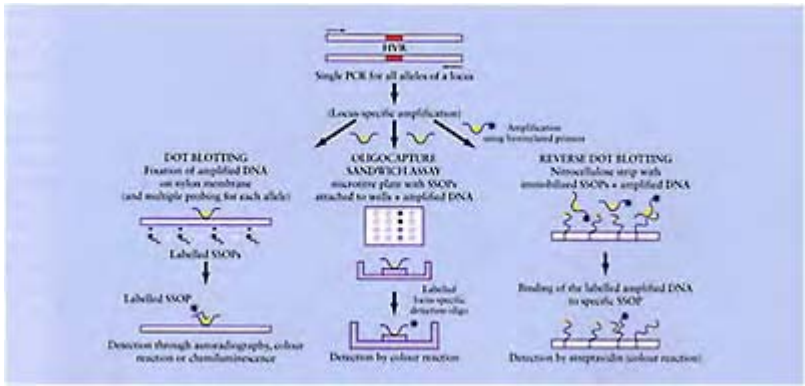

Figure 24.6 The outline of the PCR-SSOP and various detection techniques.

number of samples [104]. The reproducibility of large-scale SSOP typing for HLA class II genes has been shown to be greater than 99% for HLA-DRB1 and 98% for DQB1 [107,108].

PCR-SSOP and reverse-hybridization dot-blotting As the allelic diversity at a locus increases (hence, the number of SSOPs), so does the complexity of the PCR-SSOP typing technique. Each sample must be immobilized on multiple membranes, and each membrane must be hybridized to a different probe. To overcome this complexity, a reverse dot-blot typing procedure has been developed, in which the probes are immobilized on a membrane in a spatial array, and the PCR product is hybridized to the probes (all on the same membrane) in one reaction. In this method, only one hybridization reaction per sample is required for analysis by multiple probes. For later detection by chromogenic or luminescent substrates, the primers for PCR are biotinylated. In commercially available kits, the SSOPs are immobilized on a single strip to facilitate calorimetric detection, data interpretation and storage. This method is

currently used for class I and class II typings. Automation of this technique is available as a complete membrane strip processor. The commercially available system is programmed to handle hybridization, stringent wash and colour development. The membrane strips, amplified DNA and denaturation solution are placed in the trough. In less than three hours, the strips are completely processed, with no human intervention.

A further development of the reverse-hybridization technique is the microtitre version of it. In this method, hybridization takes place in wells and not in tubes. The wells are coated with specific capture oligonucleotide probes to anneal to the PCR product if they match. After stringent washing, only those DNAs attached to the specific SSOPs remain in the wells. The presence of attached DNA to a specific SSOP is examined through binding of labelled locus-specific oligo to the amplified DNA in the well. This labelled DNA is detected through calorimetric reaction. Reading is performed visually, or by using a simple microtitre plate reader. Identification is obtained in 90 minutes after PCR amplification. Today, most commercial kits use this straightforward method, which is suitable for automation.

PCR-SSP (PCR-sequence-specific primers) In this method, typing specificity is part of the amplification step. The primers are designed to anneal to the allele-specific sequences so that positive amplification will mean the presence of a particular allele (Figure 24.7). The PCR-SSP typing system is a derivative of the amplification refractory mutation systems (ARMS) method [109]. The SSP technique requires setting up multiple PCR reactions using a different set of primers in each one. The absence or presence of amplification is determined by direct visualization on agarose gels. All PCR-amplification reactions are sensitive to various inhibiting components as well as to cycling temperature variation. Since the PCR-SSP technique is based upon the presence or absence of a PCR product, it is important to know that amplification has functioned as expected in the PCR reaction. To avoid misassignments due to amplification failures, two approaches are usually employed: an internal control primer pair is included in the reaction mixture to amplify a non-HLA gene. The internal control primers are chosen to be more sensitive to non-optimal PCR conditions and used to monitor PCR efficiency. If there is no amplification of the control fragment, this is a PCR failure. Absence of an HLA allele of an expected size in the presence of strongly amplified internal control fragment indicates a genuine absence of that allele in the sample. In PCR-SSP sets, primer pairs for group-specific alleles or supertypes are also included as internal controls. The amplification of group-specific alleles or supertypes must correspond to the amplification of private alleles in the sample. In summary, PCR-SSP involves multiple amplifications and a single detection step (agarose gel).

One important point about PCR-SSP typing is that the concentration of the DNA to be used should be quantified accurately to avoid non-specific amplifications. It is a very rapid and simple method, ideally suited for high-resolution typing of a small number of samples simultaneously. The PCR reaction takes less than two hours. The cost of typing one individual is low, and is independent of the number of samples analyzed simultaneously. Depending on the time available, it could be less convenient when large numbers of samples are to be typed in parallel. As a breakthrough in tissue typing, a complete HLA-A, HLA-B, HLA-C, DRB, DQB typing can now be achieved within three hours by PCR-SSP method using 144 primer sets [110]. Since the individual components in the typing procedures and their reproducibility have previously been reported and

shown to be excellent, this typing system may readily replace conventional HLA typing by serology, especially in large centres involved in organ exchanges.

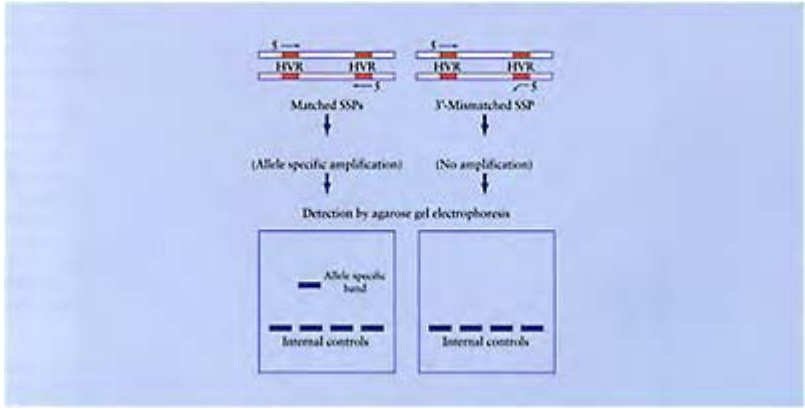

Figure 24.7 The outline of the PCR-SSP technique.

SBT (sequence-based typing) This is the definitive HLA class I and class II typing method [111,112] which is now being automated [113–115]. With the ever-increasing numbers of HLA alleles at each locus, it is possible that there will be so many HLA alleles that SSOP or SSP typings would require huge panels of oligonucleotide probes or primers, respectively. It may be more practical and informative to use DNA sequencing to determine the HLA allele whether known or yet unknown. It is, however, unlikely to provide results as fast as other PCR-based tests when there are time constraints. However, because of the high numbers of probes or primers involved in common PCR-based typing techniques, direct sequencing methods applied to PCR-amplified genomic DNA are more likely to be the methods of the future.

Complete SBT can be accomplished for both class I (HLA-A, HLA-B, HLA-Cw) and class II (HLA-DRB, HLA-DQB1, HLA-DQA1, HLA-DPB1, HLA-DPA1) genes. It can be completed in a single working day, running multiple reactions simultaneously. Each of the individual reactions involves five steps:

1. Nucleic acid extraction (RNA or DNA).
2. Synthesis of locus-specific cDNA from RNA (total genomic DNA can also be used).
3. PCR.
4. Direct fluorescent sequencing of the PCR product.
5. Analysis of the sequencing patterns to assign alleles.

In case of heterozygosity, as the sequencing ladders from both alleles overlap, the assignment of alleles requires extreme caution. The reaction conditions and oligonucleotide combinations designed for HLA SBT have been optimized in order to ensure characterization of all possible heterozygous combinations at each locus. Thanks to powerful allele assignment, software packages are available and heterozygous allele

combinations can be detected through direct sequencing without cloning the PCR products.

Alternatively, a low-resolution PCR-SSP typing can be performed initially, followed by direct automated sequencing of the positive samples for high-resolution typing. This combination provides a high degree of confidence in the final typing result which is particularly important in the case of heterozygosity.

Class I DNA typing Polymorphic products of HLA class I genes are traditionally assigned by serology, with additional heterogeneity detectable using IEF. Further improvements in the results of unrelated BMT can be predicted with the use of higher resolution typing for HLA class I genes. A single-centre study consisting of 115 consecutive chronic myeloid leukaemia patients undergoing unrelated BMT showed that class I identity determined by IEF had the major impact on survival [65]. In this study, all pairs were serologically matched, and this was supplemented by DNA analysis of HLA-DR and HLA-DQ. Given the fact that IEF does not detect all sequenced HLA-A and HLA-B alleles, DNA typing is expected to be superior to it and is already replacing it [100,116]. PCR-SSP [110] and PCR-SSOP [100,116,117] techniques have been used successfully for HLA class I typing. It is expected that DNA typing will soon replace serology and IEF for class I typing.

Serology has been used for almost 30 years for class I typing as a low-resolution typing system. This is because of the unavailability of antisera to identify all HLA class I alleles. In contrast to the situation with serology, class I alleles are more complicated to identify by DNA-based methods than class II alleles because the polymorphism is found in two exons. Technically, it has been more demanding to design typing systems for class I loci. However, with the full sequencing of class I alleles, both SSP and SSOP have been successfully used for DNA typing, and commercial kits are available for HLA-A, HLA-B and HLA-Cw typing.

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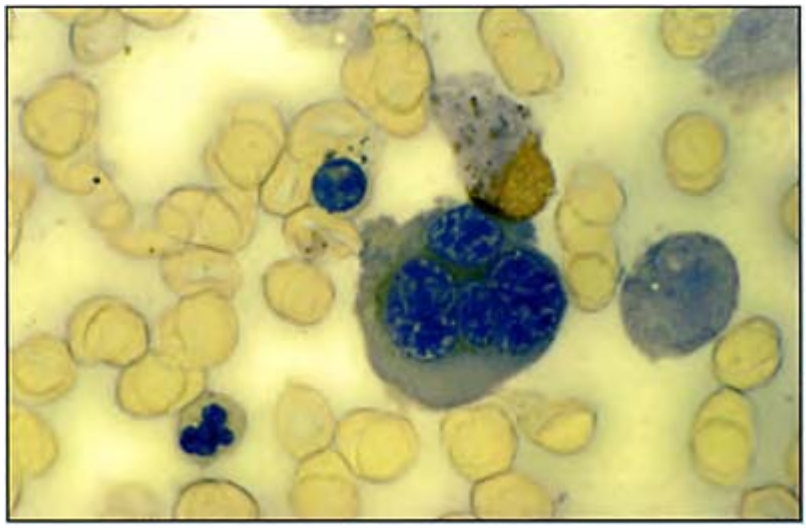

Chapter 25
Searching the Registry for an unrelated donor

Susan Cleaver

Introduction

In 1995, the Anthony Nolan Bone Marrow Trust (ANBMT) passed its twenty-first year in operation as a volunteer donor registry and had provided bone marrow for more than 1400 patients with haematological malignancies, immune deficiency diseases and other disorders of bone-marrow function.

The rationale for registers of volunteer donors is to offer the chance of a cure to an estimated 70% of patients requiring allogeneic bone-marrow transplants (BMT) who do not have a closely human leukocyte antigen (HLA)-matched family member to act as donor [1]. By 1996 a total of approximately 8500 unrelated BMT had been carried out worldwide, with 37 registries of volunteer unrelated donors in operation around the world.

The ANBMT was set up in 1974 as a registered charity, named after a child born in 1971 with the rare immune deficiency disease, Wiskott-Aldrich syndrome. Although Anthony Nolan died in 1979 without receiving a transplant, the work has continued in his name, with many other patients providing impetus over the years for continued donor recruitment. By the middle of 1996, 272000 donors were registered with the Trust which has grown to employ over a hundred staff, operating from purpose-built laboratories on the Royal Free Hospital site in North London, with administrative operations close by. A Medical Advisory Panel oversees donor care and management and a Scientific Advisory Committee coordinates the research activities of the Anthony Nolan Research Institute.

The search

Search statistics

Requests for searches of the register are received for patients being treated at accredited transplant centres in many parts of the world, predominantly Europe, North America and Australia, as well as the UK. New patient requests number approximately 3500 annually, of which 500 originate from UK transplant centres. This annual figure for new requests has remained relatively constant since the early 1990s, despite the emergence of registers of donors in many other countries. The profile of the tissue types of patients referred from abroad has changed, however, as matches for the more common tissue types are being satisfied locally.

Registering a search

Within the UK, an arrangement exists between the ANBMT, the British Bone Marrow and Platelet Donor Panel (BBMPDP), and the Blood Transfusion Service Register, to exchange requests for searches which originate from UK transplant centres. In this way, transplant physicians or their representatives need only transmit one request, which will result in a separate donor listing and report summary from each panel. To facilitate the collection of all necessary information to initiate a search, a standard search request form has been designed, which is used throughout the UK.

The information which is absolutely essential to initiate a search includes the patient's tissue type and the unique reference number of the patient assigned by the transplant centre or national registry making the request. Family HLA typings should be performed to confirm patient haplotypes and to eliminate the possibility of a matched or one-antigen mismatched family donor where such a donor would be preferred to a matched unrelated donor. HLA typing should employ molecular-based techniques at the outset, to a resolution commensurate with the resolution of the match required, or to that stipulated by the registry being accessed. Other factors which facilitate searching and which may be considered desirable or essential by some parties, include the patient name, date of birth, diagnosis, status of disease, racial/ethnic group and intended transplant centre.

As the UK national registry, or 'hub', it is necessary for the ANBMT to ensure that only those searches which meet internationally recognized guidelines [2] in terms of such

factors as patient age, diagnosis and stage of disease, are referred internationally and that such requests originate from EBMT accredited [3] UK transplant centres. Hence we may require more information from UK transplant centres when a new search is initiated than when processing new requests from international hubs who will have performed the same monitoring in their own country. It is also our duty to advise UK transplant centres of any foreign registry procedures or regulations which will impinge on an international search. Such variable factors include cost of services, HLA-typing requirements stipulating loci to be tested and resolution required, and time to work-up a selected donor.

Transmission of the search

Search requests are transmitted by a variety of modes, including the postal system (within the UK), telefax, electronic mail, or through systems stemming from such projects as the European Donor Secretariat (EDS) and its intended successor, the European Marrow Donor Information System (EMDIS)[4]. Since the early 1990s the EDS has been facilitating the exchange of searches between countries, via modem link to France Greffe de Moelle (F-GM), the French registry, located in Paris. As a progression from this, the ANBMT became partners with Zentrales Knochenmark-spenderregister, ZKRD (the German national register) and F-GM in a Healthcare Telematics Project supported by the Directorate General XIII of the European Union (3rd Framework Programme Advanced Informatics in Medicine A2006. The purpose of this project, which commenced in 1992, was to integrate the databases of 12 European bone-marrow donor registries, in a seamless fashion, allowing a smooth flow of data between what are extremely heterogeneous systems and wide-area national networks. The scope of the project was ambitious, extending from the initial search, through blood-sample requests to transplant work-up. EMDIS is now functional and has considerably enhanced the exchange of searches between those registries which are connected.

A separate arrangement with the National Marrow Donor Program (NMDP), USA, provides an automated searching facility to process incoming searches from the USA to the ANBMT. Search requests are received via electronic mail, as text file attachments, which are imported to a database and processed by a central personal computer. Resulting reports are E-mailed back to the NMDP immediately after searching. The process involves no user intervention whatsoever and allows fast searching of the register around the clock.

Format of results

Initiating a search for a patient results in the generation of a report listing donors who are potentially HLA matching with the patient. Generally, such reports are divided into those donors with HLA-A, -B and -DR data and those with only HLA-A and -B data. The matching programme developed at the ANBMT includes the DRB1 molecular typing data in the sorting algorithm. Where ambiguities exist, resulting in intermediate resolution DRB1 results, the allele codes developed by the NMDP are utilized to enter data on to the computer. Priority is given in the donor sorting to the degree of HLA match, and within that degree of HLA match to younger donors, listing in ascending order of age, with males before females, males being clinically preferred for some

patients. This gender listing can be reversed if requested. Additional information provided on matching donors includes age, sex, cytomegalovirus (CMV) status, blood group, ethnic group and weight. If it is known that a donor cannot presently participate in tests for the patient for whom the search has been run, this is indicated as 'temporarily unavailable' (TU) with a release date, or 'in test' (IT), i.e. for another patient. The search programme is quick, producing listings of donors within a matter of minutes.

Bone-marrow donors worldwide

Early on in the search for a donor, reference is usually made to 'Bone Marrow Donors Worldwide' (BMDW) [5,6]. During 1988, the Immunology Working Party of the EBMT took the initiative to collect together the data on all the tissue types of all the donors on registers around the world. This is collated at the Europdonor Foundation, the Dutch national registry in Leiden, and numerous updates have been produced over the years. Currently it is possible to receive or download BMDW on the Internet, and updates are produced every two months. On-line matching facilities are planned to be introduced on the Internet during 1996 and 1997, to include mismatching and prognostic matching. Links exist to BMT-related homepages, HLA-related homepages, EBMT News and donor registry homepages (http://bmdw.leidenuniv.nl). National hubs support BMDW financially and are given rights over distribution of and access to BMDW on a national basis. The main function of BMDW is to inform the physician treating the prospective BMT recipient as to whether a given HLA phenotype is very rare or not. To this end, it has been the policy of the ANBMT to provide a relevant print-out of BMDW, to supplement the ANBMT search report, with every search requested through a UK transplant centre. Hence the requesting physician has the global picture regarding possible matches at the outset, and may decide that a formal international search could be beneficial to the patient early on in the proceedings.

Outcome of searches

On the initial search of the ANBMT register it is very rare for a patient referred from a UK transplant centre not to have HLA-A, -B matches, regardless of the ethnic or racial group concerned. Such matching donors were identified for 99% of patients referred in 1995. The percentage of new searches referred from UK transplant centres with HLA-A, -B, and -DR matching donors has increased steadily over the years, but does vary considerably according to whether the patients are UK resident Caucasians, or a racial or ethnic minority, who may or may not be UK resident (Table 25.1).

The options available for those patients without HLA-A, -B, and -DR matches on the initial search include DR typing of available HLA-A, -B matches, extending the search internationally, and/or a national or international one-antigen mismatch search. Very often it is advisable to pursue more than one option simultaneously. HLA-DR typing of ANBMT donors can be undertaken upon request and all typings are performed in-house for reasons of quality control and standardization of results. As an extension to our services, if several HLA-A, -B, -DR matching donors are listed without DNA typing, confirmatory typing of donors can be undertaken at the ANBMT. This confirmatory

typing service was introduced in 1993 and has been used by a large number of transplant centres. The purpose of the service is to provide the transplant physician with more specific details on donors' typings, thereby reducing the overall number of donor samples being shipped to transplant centres. As it is the policy of the ANBMT to interview all donors at the sample shipment stage, to ensure the volunteer is fully briefed on the processes and implications of being a donor, such confirmatory typing also reduces inconvenience to donors by eliminating those who are mismatched from the search.

Accessing international donors

Each year, over a hundred UK searches are extended internationally at the request of transplant physicians, and by the end of 1995, a total of 49 patients being treated at UK transplant centres had received marrow from donors registered on international panels (Table 25.2).

International searches are transmitted and coordinated through the national registry or 'hub' of a country [7], in the UK through the ANBMT, to the national hubs of other countries. As some countries have in excess of a hundred donor centres and likewise, numerous transplant centres, the national hubs play an essential coordinating role. Resources are concentrated, and standardized practices and procedures can be ensured for the country as a whole in the interaction with other countries. International financial exchanges can more easily be regularized. At the end of July 1996, the tissue types of 3550279 donors were recorded through BMDW, from 34 bone-marrow donor registers and seven cord blood banks in 28 countries. The search information provided through BMDW gives an indication only of the likelihood of identifying a match for a patient and in which registries a match might be found. Once a formal international search has been requested by the transplant physician, staff at the ANBMT transmit the

Table 25.1 Percentage of patients searches referred from UK transplant centres finding 6/6 antigen-matching donor(s) on the preliminary search of the Anyhony Nolan Register by enthnic group

	1991		1993		1995	
Patients	Total searches	With 6/6. match(es)	Total searches	With 6/6 match(es)	Total searches	With 6/6 match(es)
Caucasian UK resident	169	57%	295	67%	388	69%
Racial or ethnic minority	60	22%	69	26%	82	32%

Table 25.2 Number of UK recipients of bone marrow international registries, per year, by donor country

Country	1992	1993	1994	1995	Total
Australia				2	2
Austria				1	1
Belgium			1		1
Canada		1	1	2	4
France	2	3		1	6
Germany		1		2	3
Holland		1	2		3
Italy	1		1		2
Switzerland			1		1
Singapore				1	1
USA	5	6	7	7	25
Total	8	12	13	16	49

request to each registry in the required format and collate results into a comprehensive report, returning to the transplant physician the overall worldwide results. Requests for donor blood samples and donor work-up are then coordinated by the ANBMT.

The largest register, with two million donors, is that of the NMDP, which was founded in the USA in 1986, through a contract with the federal government. The Canadian Unrelated Bone Marrow Donor Register (UBMDR) of the Canadian Red Cross Society was founded in 1989 and has recruited 152 000 donors. In Europe, the French Register, France Greffe de Moelle, was established in 1985 and the German Registry of Bone Marrow Donors (Zentrales Knochenmarkspenderregister, ZKRD) in 1991. The Dutch Register (Europdonor Foundation) and the ANBMT Register date back to the early 1970s. Numbers of bone-marrow donors in Europe in 1996 total in excess of 1.4 million. Outside of Europe and North America, registers exist in Australia, Hong Kong [8], Singapore, Taiwan, Japan [9] and South Africa, all of which can be accessed by the ANBMT for UK patient searches.

Successful searches: transplant statistics using ANBMT donors

By mid-1996, the ANBMT had provided bone marrow for 1600 patients, with small numbers of transplants in the early years increasing to over two hundred per annum since

1992 (Figure 25.1). It has always been the policy of the Trust to provide marrow for patients wherever they may reside in the world, provided they are being treated at a recognized transplant centre. The establishment of registers of volunteer donors in other countries since the mid-1980s has brought about a change in the geographical distribution of the patients receiving marrow from ANBMT donors (Fig 25.2). Increased transplant activity in the UK using unrelated donors has been seen over the period. During 1994, 73 ANBMT donors provided marrow to patients at 18 UK transplant centres. In 1995 the corresponding figures were 110 donors providing marrow to patients at 24 UK transplant centres.

For many years, chronic myeloid leukaemia (CML) was the predominant disease for which the ANBMT provided unrelated donors. This changed in 1995,

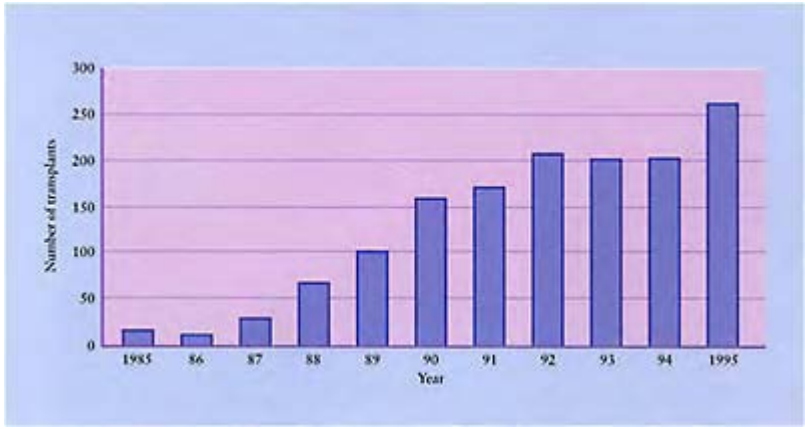

Figure 25.1 Number of transplants using ANBMT donors.

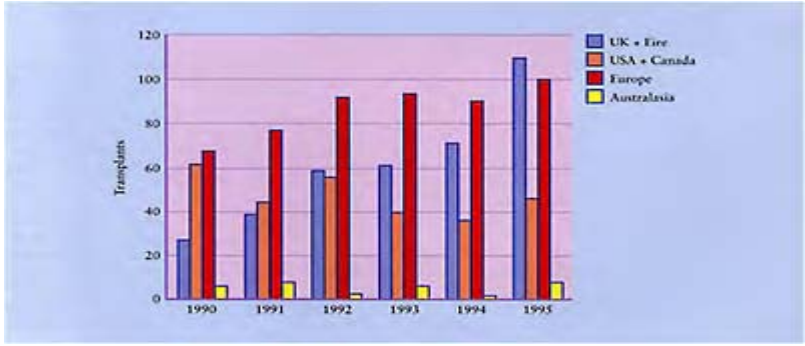

Figure 25.2 World distribution of transplants performed using ANBMT donors.

when for the first time more donors were provided for acute lymphoblastic leukaemia (ALL) than for CML. The median length of time from a preliminary search request to deciding on the optimum donor for patients with ALL is three months, with the majority proceeding to transplant within four weeks of donor selection. CML is a disease where there is usually more time available to identify the optimum donor and so, generally speaking, testing of donors progresses without urgency. Transplants may be planned some time after optimum donor identification with median time from search request to transplant being around eight months.

Strategies to improve the outcome of donor searches

The success of donor searches depends upon fully tissue-typed, matching donors being available in a timely fashion. These factors are influenced by donor recruitment strategies, efficient register maintenance procedures and tissue-typing policies.

Recruitment strategies

Current recruitment strategies at the Trust are designed to increase the ethnic diversity of the panel, lower the mean age of the donor pool and increase the proportion of male volunteers. Whichever group is being targetted, however, it is essential that donors are fully informed at the outset of the health requirements and of the processes involved should they be selected to donate [2] (Table 25.3), so that the attrition rate is minimized.

In the middle of 1996, the size of the Anthony Nolan Register stood at 272000. The estimated figure for natural wastage from the register is 10% per year, due to such factors as donor illness, change in personal circumstances, passing the upper age limit, emigrating, or moving home. To maintain the size of the register, continued recruitment is necessary, with a target of 500 new donors per week.

Recruitment of new donors currently proceeds either by personal, postal application, or through clinics especially organized for the purpose. Between 1974 and 1988 most of the recruitment was via the latter route, clinics being set up with the assistance of the National Association of Round Tables of Great Britain and Ireland (RTBI) who have additionally

Table 25.3 Donor information should cover the following topics

• Emphasis on anonymity for donor and patient
• Requirement for further blood samples before donation
• Requirement for virological testing, expecially human immunodeficiency virus (HIV) and hepatitis B surface antigen (HBsAg)
• Risks of anaesthesia and harvest procedure
• Loss of time from normal activities
• Location of harvest procedure, i.e. proximity to donor’s home

- Requirement for collection of autologous blood unit
- Possibility of need for allogeneic unit and associated risks
- Donor's right to withdraw and consequences for the patient if this right is exercised after the transplant protocol has started
- Patient's need for BMT and chance of success expressed in general terms
- Possibility of second donation for the same patient (the fact that the donor is under no obligation to donate on a second occasion needs to be stressed)
- Details of compensation for loss of income and details of insurance cover affected

raised generous funds for the Trust. In 1988 the postal application route was introduced, but clinics have continued to provide an important source of donors. Over the past two years approximately 450 clinics have been organized on behalf of the Trust and run by volunteers, nearly half of these clinics being specifically for ethnic minority recruitment. Experience has shown that where small numbers of donors are recruited conscientiously, clinics can produce committed, long-term volunteers. It is important, however, to work within the realistic capacity of the tissue-typing laboratory and sudden surges of demand should be avoided.

The advantages of donor recruitment clinics are that specific donor groups can be targeted, volunteers can be identified willing to offer future assistance, and phlebotomy is diverted away from valuable NHS resources. Clinics provide access to monetary and other forms of support, with possible openings for publicity in the media. The disadvantages, however, include the tendency to place emphasis on donor quantity rather than quality, poor dissemination of information by the organizers, failure of donors to disclose relevant medical information and inappropriate peer pressure to join the register. The fact that clinics are often driven by emotive patient appeals compounds the difficulties of ensuring that people join the register for any patient and not just the one for whom a donor is sought at that time.

In order to address these potential disadvatages, a leaflet has been produced by the Trust setting out how to organize a clinic and what is expected of the organizers. Those responsible for running the clinic are asked to enter into a contract with the ANBMT which emphasizes the importance of the task they are undertaking. Organizers are permitted to recruit up to fifty donors on their first occasion and up to a hundred donors are permitted for experienced organizers.

In recent years the preferred route of donor recruitment has been via the postal route, where donors have more time to digest the information provided. They are asked to complete and return a comprehensive medical questionnaire and to read the leaflets which explain what is involved in becoming a donor. A blood-sampling kit is then sent to the donor who can take this along to his/her general practitioner or local hospital to have the blood sample taken and posted back. The Trust is greatly indebted to the numerous general practitioners, practice nurses and hospital phlebotomists who willingly give their valuable time to assist the work of the Trust.

Although donors remain on the register until their 56th birthday, the upper age limit for recruitment is 40 years. This strategy is in part to conserve scarce financial resources,

but also importantly to lower the mean age of the register. Whenever there is a choice of donor, other factors being equal, the youngest donor is preferred.

The Anthony Nolan Panel as a whole is only 39% male, whilst, due to our prospective tissue-typing policy and external requests for HLA-DR typing, the HLA-A, -B, and -DR typed portion of the panel is 46% male. A survey undertaken in 1993 on behalf of the World Marrow Donor Association (WMDA) of 15 national registries, showed that there is a tendency for more women to join registers than men [7]. Where a choice of donor exists, however, a male donor may be preferred, particularly where the patient is male. The percentage of males actually donating marrow from the Anthony Nolan Panel in 1995 was 57%. It is important, therefore, to redress the gender balance of the register by recruiting more male donors, particularly as at any given time a proportion of the females on the register is unavailable due to pregnancy and childbirth. Recruitment strategies include targeting predominantly male organizations and raising awareness generally of the need for more male volunteers, particularly through our newsletter to existing donors.

Despite considerable efforts by some registries, the majority of donors on existing panels are Caucasian. In a multiracial society such as exists in the USA, the need for minority recruitment has been recognized. In 1992, funding was provided by the National Heart, Lung and Blood Institute to increase the proportion of ethnic minority donors on the NMDP, USA Panel. By mid-1996, the NMDP had recruited nearly 480000 minority donors and provided transplants for more than 450 minority patients.

In the UK, the ANBMT has been actively recruiting ethnic minority donors and in 1995 produced a new donor recruitment leaflet designed to appeal to all ethnic groups. This replaced a previous one showing the Caucasian recipient of a successful transplant. Although the numbers joining the register are relatively small, recruitment has taken place from the Asian, Jewish, Greek Cypriot and Afro-Caribbean communities with much welcome assistance from voluntary organizations representing these ethnic groups.

Maintenance of the register

Having recruited the desired volunteers to the register, it is essential to maintain accurate records so that donors can be contacted quickly when required. Initially, once the tissue types of new donors are entered on to the computer, individual registration numbers are sent to donors acknowledging their entry to the database and including ‘change of address’ notification cards for their future use. Thereafter a twice-yearly newsletter is sent to all registered donors, to inform them of the activities of the Trust and to serve as a reminder of their registration. Staff are employed at the Trust specifically to trace donors who have moved from their registered address. Techniques include tracing through previous neighbours, and places of work, and through access to the electoral roll. In more recent years, two addresses have been recorded from new donors, the second being of a relative or close friend who is likely to know of their whereabouts at all times.

Typing strategies

The clinical variable most influencing search outcome has been found to be the patient’s disease status [10]. Patients with advanced disease are more likely to suffer search failure due to deterioration or death. Whilst the registry has no control over when a patient is

referred, strategies can be developed to minimize the delay in identifying a donor once referral has occurred so that fewer patients reach an advanced disease stage while waiting for a donor. At the ANBMT, the introduction of molecular-based HLA-typing technology, particularly for HLA-DR typing, has significantly increased the number of donors who can be tested each week, thus improving the chances of matching donors being identified on the initial search.

Historically, due to financial constraints and limitations of the techniques available, typing of donors on the register proceeded in two stages, class I (HLA-A, -B) typing on donor enrolment followed by class II (HLA-DR, -DQ) typing when a potential match had been found. However, such was the demand for class II typing of donors in the late 1980s and early 1990s that delays occurred in processing requests and consequently identifying matches. In the latter half of the 1990s, the situation is very different. Techniques have changed in the laboratory, and while external requests for DR-typing continue to decrease, increasing numbers of patients who attain transplant with a donor from the ANBMT have their matching donor identified on the initial search of the register (Table 25.4).

The typing stategy employed at the ANBMT utilizes both serological and molecular techniques for class I and a range of molecular techniques for class II, routinely testing on a large scale for DRB1 and DQB1 [11]. Typing is performed to intermediate or high resolution using the polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes (PCR-SSOP) and sequence-specific primers (PCR-SSP). DNA-based typing, certainly for class II alleles, has been shown to be highly reliable and reproducible in the large-scale typing situations common to registries [12].

By mid-1996, 126000 donors on the ANBMT register had been HLA-A, -B and -DR typed. DR typing of donors proceeds at the rate of 750 per week, meaning that a substantial proportion of this typing is prospective typing of the register. The policy of typing in two stages continues, i.e. new donors are not automatically DR typed, but numerically more DR typings are processed than donors joining the register. All new donors have a blood sample frozen for future testing. Selection for DR typing is based on a number of criteria which include class I type, donor sex and age. Preference is given to typing young males, those donors with apparently homozygous or unsplit class I antigens and to donors who are potentially matching on selected currently active patient searches. To speed the typing process further, many of the donor samples are retrieved from the frozen blood repository which currently holds 70000 samples. This prospective typing strategy has been effective in providing patients with donors for transplant where donor typing had not been requested by the transplant centre. To illustrate

Table 25.4 Percentage of transplant recipients whose donor was identified on the intial search of the Anthony Nolan Register, by year

1988	1989	1990	1991	1992	1993	1994	1995
35	51	75	77	81	87	82	85

the point, during 1995, donors were identified through prospective DR typing for 15 of the 266 patients who received transplants through the Trust. The strategy also, of course, increases the pool of fully typed donors for the potential benefit of future patients.

Clinical services

The clinical services branch of the laboratory continues to increase and expand its range of services and now provides histocompatibility testing for 12 leading bone-marrow transplant centres in the UK and Europe. During 1995, approaching 40% of the UK transplants using ANBMT donors involved tests, including cellular assays, performed by the clinical services department. Additionally, final confirmatory typing was performed on the patient and potential donor in half of the cases where an international donor was selected during 1995. The Donor Identification Advisory Service (DIAS) scheme, introduced in 1993 to provide a service for those transplant centres without state-of-the-art tissue-typing facilities, continues to be well used. The scheme involves both patient and prospective donor(s) being tissue typed in the ANBMT laboratories, cellular assays being performed and a recommendation being made to the transplant centre about the most suitable donor. The service streamlines the search process and considerably reduces the time taken to identify the preferred donor.

Successful searches for all patients?

As different allelic forms of the HLA antigens and varying haplotype frequencies are found in different races and ethnic groups, it is important that the ethnic composition of the donor pool corresponds to that of the patients referred. Experience shows that unrelated donor searches for Caucasian patients lead to a transplant in approximately 30% of cases with current size and composition. This figure decreases when patients from other races or minority ethnic groups are considered.

In the past, a major difficulty encountered in expanding recruitment to the register from non-Caucasian racial groups was the acquisition of appropriate HLA-typing sera. Much of that acquired through antenatal screening programmes was of Caucasian origin, raised against Caucasian HLA determinants. However, with the advent of DNA-based typing techniques the situation has changed, in many ways for the better. In contrast to serological reagents, those used in DNA-based HLA typing are synthetic and can therefore be manufactured in quantity to a high standard. As new alleles are sequenced from different ethnic groups, reagents to identify these alleles can, if necessary, be added to the typing repertoire.

In practice, the large number of alleles that has been identified at DNA level has made the matter of donor-recipient matching more complex. Given that most transplant centres prefer to match for HLA-A, -B, -DRB1, -DRB3, -DRB4, -DRB5 and -DQB, and some for Cw and DP additionally, the chances of identifying a match at the genotypic level are considerably reduced compared to a match at the generic level. This has led clinicians to question the resolution of typing necessary, at which loci, and whether acceptable

mismatches exist. All of these issues are topics of current research at the Anthony Nolan Research Institute [13].

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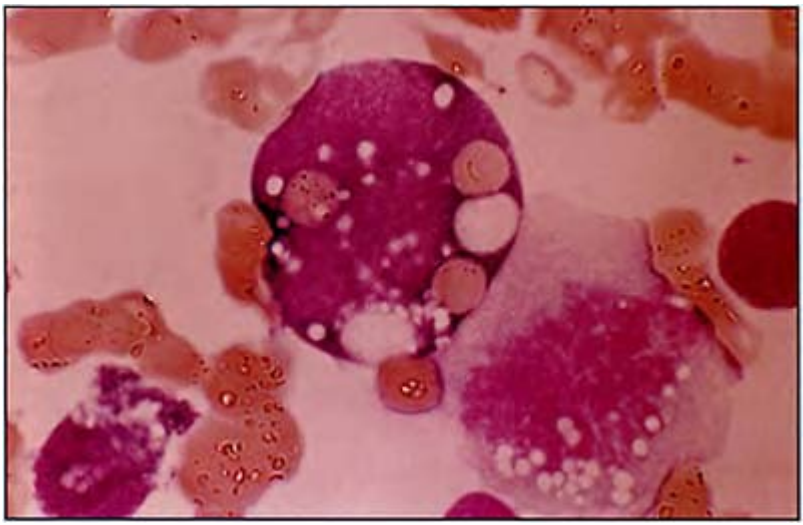

Chapter 26
Unrelated doner bone-marrow transplantation

Jacqueline Cornish, Nicholas Goulden and Michael Potter

Introduction

Allogeneic bone-marrow transplantation (BMT) has become a widely accepted form of treatment in selected haematological malignancies, bone-marrow failure syndromes and congenital disorders of the lymphohaemopoietic system and metabolism [1,2]. The donor of choice is currently a human leukocyte antigen (HLA)-identical unaffected sibling. However, with the decline in family size, only about 30% of patients have an HLA-matched sibling in North America and Western Europe [3]. Transplants have been reported using partially matched family members with single antigen mismatches which increases the donor pool by about 10% [4–6]. Haplo-identical family marrow transplants have also been performed with recent success [7]. Alternatively, many groups have explored the use of volunteer unrelated donors so that more patients can be offered the potentially curative therapy of an allogeneic transplant [4,8–10].

The first successful unrelated donor (UD) transplants were performed in the 1970s. A patient with severe combined immunodeficiency disease (SCID) achieved sustained engraftment from an unrelated donor in 1972 [11] and Foroozonfar *et al.* reported the case of a child with chronic granulomatous disease who received an unrelated donor transplant in 1977 [12]. In 1979, the first case of a child with acute lymphoblastic leukaemia (ALL) successfully engrafted from an unrelated donor was reported by Hansen *et al.* from Seattle [13] and the publication of additional case reports between 1973 and 1983 clearly established the feasibility of unrelated donor BMT [9,10,14]. Progress in this field was slow, however, and hampered initially by the lack of large donor registries. Advances in the last decade in the availability and success of UD bone-marrow transplants have been due to three main factors: firstly, increased understanding of the major histocompatibility complex (MHC) in man; secondly, improved techniques for the prevention of graft-versus-host disease (GvHD); and thirdly, the establishment of international bone-marrow donor panels [15,16]. The largest of these is the National Marrow Donor Program (NMDP) in the United States, currently with a panel size of >2.3 million, and this became operational in December 1987.

Many European countries have expanding registries under the growing impetus for the recruitment of larger numbers of HLA-typed unrelated volunteers, and in the United Kingdom most searches are channelled through the Anthony Nolan Research Centre and the British Bone Marrow Registry, a panel comprised of regular blood donors. There are currently around 4 million prospective donors on panels worldwide with over 30 countries contributing to a coordinated search.

The unrelated donor bone-marrow transplant activity in Europe has expanded, so that in 1973 a total of eight transplant teams performed five UD transplants, whereas in 1996, 373 teams performed over 14000 transplants, of which 950 utilized an unrelated donor, 7% of the total transplant activity in Europe and 22% of the allogeneic group (Table 26.1). The indication for UD transplant was chronic myeloid leukaemia in 36% of cases and acute lymphoblastic leukaemia in 27%, predominantly beyond first complete remission. There are major differences between participating countries in Europe. The absolute numbers of teams and transplants per country differ widely and range from 0.2 to 33.3 teams per 10 million inhabitants (median 5.8) with 0 to 47.7 unrelated transplants per 10 million inhabitants (median 8.7), as shown in Figure 26.1.

The use of unrelated donor bone-marrow transplant in paediatric practice has grown more

Table 26.1 Bone-marrow transplants (Europe) as registreted to the EBMT group (*Gratwohl* et al. *on behalf of the EBMT group*)

Year	Teams	BMT	UD
1973	8	16	5
1983	84	1202	7
1992	203	6065	296
1993	245	7510	442

1994	306	11336	567
1995	343	12101	764
1996	373	14424	950

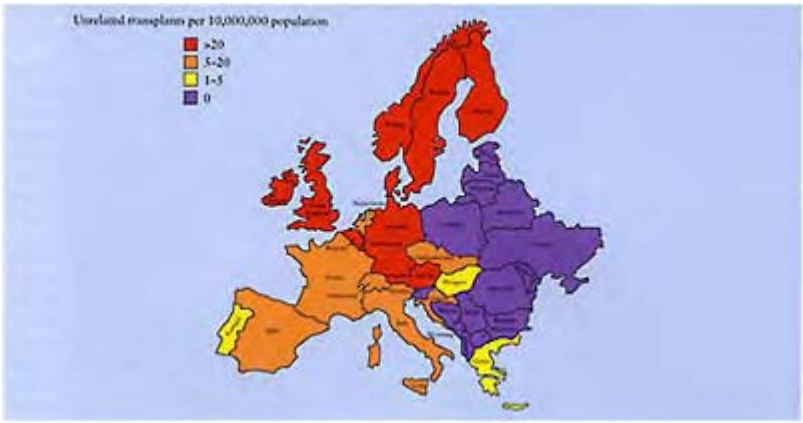

Figure 26.1 Unrelated donor BMT distribution throughout Europe as registered to the EBMT group. A Gratwohl et al. Bone Marrow Transplant *1997; **19**:407–419.*

rapidly than in the adult population. The International Bone Marrow Transplant Registry (IBMTR) reported an increase from 7% in 1989 to 30% of the total transplants performed in children aged 17 and under in 1995 (Table 26.2). The United Kingdom Paediatric Bone Marrow Transplant Registry, which started in April 1993 and collects data on all transplant procedures performed in the UK in children up to 17 years, shows a similar proportion of unrelated donor transplants (Table 26.3). The indications for UD transplant in paediatric practice differ from the overall indications as reported by the EBMT group in that 60% are performed for acute lymphoblastic leukaemia, and 60% of these are in second complete remission. The proportionately greater use of unrelated donor transplantation in children may reflect an increased tolerance to the procedure and possibly the willingness of clinicians to use mismatched donors.

Table 26.2 IBMTR children ≤17 year (USA, 1989–1995) (M Horowitz, personal communication)

Group	1989	1990	1991	1992	1993	1994	1995
Autologous	430	430	470	490	520	510	550
Identical siblings	330	350	350	400	450	470	540

Twin	2	8	5	6	12	12	10
Other related	130	130	180	160	250	210	260
Unrelated	70	160	200	240	320	410	580
Total	**962**	**1078**	**1205**	**1296**	**1552**	**1612**	**1940**

Table 26.3 UKBMT children ≤17 years (UK, 1993–1996) (JM Cornish on behalf of the UKCCSG Paediatric BMT group, 1997)

Group	1993	1994	1995	1996
Autologous	98	79	93	75
Identical siblings	98	103	90	97
Twin	2	1	1	0
Other related	27	14	18	27
Unrelated	63	89	91	89
Total	288	286	293	288

The probability of finding an HLA-A,B,DR match from the NMDP at the initial search has risen from 15%, when total donor numbers were small, to current figures of 70% for Caucasians and 25% for African-Americans [17]. Unfortunately, underrepresentation of the more rare haplotypes in the donor population remains a major problem for most panels. However, the use of a single-antigen mismatched donor will increase the chance of finding a donor to 90% in patients of European descent. Transplant-related toxicity in a younger age group is likely to be less than that reported in the mismatch situation in older recipients.

Donor selection

The problems which can be anticipated with the use of phenotypically identical unrelated donors include increased risks of primary non-engraftment, graft rejection, GvHD and infection [3,8,18–21]. This may lead to an increased transplant-related mortality (TRM) rate compared to the matched related setting. However, this may be partially or even totally offset by a lower rate of disease relapse using unrelated donors. This presumably results from an increased graft-versus-leukaemia (GvL) effect mediated by alloreactive donor lymphocytes. The current recommendations are that transplants from fully matched unrelated donors are optimal; however, a minor disparity at one HLA locus has not led to a decreased overall survival even with a significantly greater degree of GvHD in the mismatched group when using an unmanipulated graft [22]. Some degree of HLA disparity may be acceptable in recipients under the age of 35 years if a fully matched unrelated donor is unavailable.

Units employing the use of T-cell depletion combined with pretransplant immunosuppression of the recipient report a greatly reduced risk of GvHD when HLA-mismatched donors are used. These are usually single class I or class II antigen mismatches, and survival is similar to the matched groups [23–25]. The adverse effect of increased donor age and greater risk of GvHD is also minimized by the use of T-cell-depleted donor marrow.

Tissue typing no longer relies on serological methods alone, and the use of high-resolution molecular techniques is now standard procedure in many centres. Donor selection may be aided by the use of functional assays but their place is not yet clearly defined. The cytotoxic T-lymphocyte precursor (CTLp) assay measures the frequency of recipient antidonor cytotoxic cells, but data regarding its correlation with HLA-disparity and prediction of graft rejection have been conflicting [26,27].

It has also recently been demonstrated that there is an association of HLA-C disparity with graft failure after marrow transplantation using unmanipulated unrelated donor grafts [28]. As molecular typing becomes more precise, it is clear that the only perfectly matched donor is an identical twin, and there is probably no such concept as a perfectly matched unrelated donor. The recent World Health Organization nomenclature for factors of the ‘HLA System 1996 has demonstrated many more alleles than previously recognized [29]. This will emphasize the need to identify degrees of tolerable mismatch in unrelated donor transplantation and the subsequent development of better treatment protocols to minimize their clinical impact.

Current indications for unrelated donor transplantation in children

Childhood acute lymphoblastic leukaemia (ALL)

The outcome of childhood ALL has improved dramatically over the past twenty years so that now chemotherapy induces remission in almost all children and a cure rate of 70–80% can be expected on nationally directed protocols [30,31]. The main cause of treatment failure is relapse of disease. For those patients who relapse on, or shortly after, stopping treatment, subsequent cures are rarely achieved with further chemotherapy [32,33] or with autologous transplantation [34]. This is where allogeneic BMT has a major role [35]. In the absence of fully matched family donors for the majority of these children, the use of the volunteer unrelated donor panels has been explored, although it is only in recent years that sufficient numbers of these transplants have been performed to permit comparison with alternative treatments. Data from prospective randomized trials comparing the outcome of UD BMT with other forms of treatment in childhood leukaemia do not currently exist. However, given that improved survival for patients receiving matched sibling donor (MSD) transplant over chemotherapy or autologous BMT have been reported, comparison can be made between the outcome of UD BMT with MSD BMT. Reports from the adult literature suggest reduced survival following UD BMT due to increased toxicity, but this may not be so apparent in the paediatric setting,

and advantage can be made of the potential for an enhanced graft-versus-leukaemia effect (GvL) [36].

High-risk ALL in first complete remission (CR1)

Allogeneic BMT in first remission is usually restricted to those patients with high-risk disease who have an increased probability of relapse [37]. The relevant risk factors are summarized in Table 26.4. Patients in these categories have an overall leukaemia-free survival (LFS) of 18–28% with regimens containing intensive chemotherapy. Sibling allogeneic transplantation is appropriate in these children within six months of diagnosis and results in an overall LFS of 56–89% [38,39]. Within this group, the outlook for infant ALL associated with 11q23/MLL rearrangement or Philadelphia-positive childhood ALL is particularly poor in the absence of allogeneic BMT [40]. Several studies have suggested that BMT can result in long-term disease-free survival, and it would seem reasonable to proceed to an UD BMT in early CR1 in the absence of a matched sibling donor.

The decision about whether to undertake UD BMT in other children with high-risk ALL in CR1 is primarily based on the experience of the individual centre but should take account of the fact that sustained second remissions are often difficult to maintain, and that a number of patients may die of disease prior to BMT if an unrelated donor search is not initiated early [41]. Recommendations for UD BMT in childhood ALL in CR1 are summarized in Table 26.4.

Childhood ALL beyond first remission

Bone marrow with or without extramedullary relapse of ALL is the most frequent indication for allogeneic BMT in childhood leukaemia. Several groups have reported improved survival for patients receiving an MSD BMT following relapse compared to those who had no matched related donor and received conventional chemotherapy alone [42–44]. The probability of LFS following BMT has ranged from 40 to 58%, compared to 11–17% for chemotherapy although there may be less benefit for transplantation in children with long first remissions [45].

Within Europe, MSD BMT is currently regarded as a routine procedure for bone-marrow relapse of ALL by the accreditation subcommittee of the EBMT group [1]. This is endorsed by the Medical Research Council (MRC) Working Party on Childhood Leukaemia and the Paediatric BMT Working Party of the United Kingdom Children's Cancer Study Group (UKCCSG) whose table of indications for transplant procedures in children is shown in Table 26.5.

Table 26.4 High-risk ALL patients who are candidates for BMT in CR1 (P Veys on behalf of the UKCCSG paediatric BMT group, 1997)

Philadelphia-positive t(9,22)*
Near haploid*

Infant ALL with MLL gene rearrangement**
Failure to remit by day 28 of therapy**
High hazard score**
True biphenotypic leukaemia defined by morphology, markers, cytogenetics**
*Candidates for MSD BMT or UD BMT if available, **Candidates for MSD BMT. Certain may be considered for UD BMT within Clinical Research Protocols category on an individual basis.

Table 26.5 Proposed indications for transplant procedures in children 1997 (developed through the UKCCSG BMT group, updated 11 April 1997) (P Veys on behalf of the UKCCSG Paediatric BMT group)

Disease	Disease status	Allogeneic matched related	Allogeneic unrelated	Autologous blood or marrow
AML	CR1	R[a]	NR	NR
	CR2	R	CRP[b]	R[b]
	Relapse/refractory	D	D	NR
ALL	High-risk CR1	R[c]	R[c]	NR
	CR2[d]	R	R	CRP
CML	Chronic phase	R	R	CRP
	Advanced phase	R	R	CRP
	Blast crisis	D	NR	NR
NHL	As ALL			
Hodgkin's disease	CR1	NR	NR	CRP
	CR2	CRP	NR	CRP
	CR3	CRP	D	R
	Refractory	NR	NR	D
Myelodysplasia	JCML, CMML[e]	R	R	NR
	RA[f], RARS, IMo7, RAEBT, sAML[g]	R	CRP	NR
Immunodeficiency and inborn errors		R[h]	R	
Thalassaemia		R	NR	

Sickle-cell disease		R	NR	
Aplastic anaemia and inherited monocytopenia		R	CRP	
Solid tumours				
Neuroblastoma	Stage IV	NR	NR	R
PNET/Medulloblastoma	High risk/relapse	NR	NR	CRP
Ewing's sarcoma	High risk	NR	NR	CRP
Germ-cell tumour		NR	NR	CRP
Rhabdomyosarcoma		NR	NR	CRP
Wilms' tumour	Relapsed/refractory	NR	NR	CRP
Auto-immune disease		NR	NR	D

R=in routine use for selected patients; CRP—to be undertaken in approved Clinical Research Protocols, D=developmental or pilot studies can be approved in specialist units; NR=not generally recommended. [a]=Not t(15,17; t(8,21); inv(16); Down's syndrome. b=26.7, c=26.4, d=26.6. [e]=if pace of disease dictates. [f]=If cytogenetics abnormal or blood product dependent. g=RAEBT, sAML eligible for AML 12, except Down's syndrome where BMT is not necessary. [h]=Haplo-identical related donors may also be considered for these patients.

Over recent years, children with early relapse of ALL and without a MSD have undergone UD BMT. Overall survival has been significantly better than that reported for adults [24], and a LFS of between 40 and 53% compares similarly with MSD BMT [24,25,36,46,47]. A group of children who received uniform treatment from diagnosis and relapse (with Medical Research Council (MRC) chemotherapy protocols) and then went on to have UD BMT in second complete remission (CR2) was recently reported by Oakhill *et al.* [25]. In this series, a LFS of 53% was obtained overall with no significant difference between recipients of matched and mismatched UD transplants (Figure 26.2). There is uniform agreement that the subgroup of patients who relapse early on treatment have a particularly poor prognosis [32,48,49] and are likely to benefit the most from UD BMT, which appears to show superior survival over MSD BMT or chemotherapy alone.

T-cell depletion has reduced the incidence of transplant-related complications (particularly GvHD) and a 100-day mortality of 10–20% is reported in the best series. This has permitted the use of both matched and partially mismatched unrelated donors, so extending the applicability of UD BMT to the majority of children requiring allogeneic transplantation [23–25]. Also, unlike in chronic myeloid leukaemia (CML), T-cell depletion in the unrelated setting for ALL has not been associated with an increased relapse rate compared to MSD BMT. This suggests that despite the reduction in GvHD, a GvL effect has been preserved, perhaps mediated by increased genetic disparity.

The most common method of T-cell depletion in paediatric practice in the United Kingdom and some other European centres is the use of Campath monoclonal antibodies. This antibody also depletes B-cells which may explain the remarkably low incidence of Epstein-Barr virus (EBV)-related post-transplant lymphoproliferative disease compared

to other T-cell-depletion approaches. Considerable success has also been achieved using the monoclonal antibody T10B9, as shown by Casper's group [3, 24].

The management of the small number of children with isolated extramedullary relapse of ALL is complex and controversial. Central nervous system (CNS) or testicular relapse of ALL while receiving primary chemotherapy schedules is considered predictive of impending bone-marrow relapse [50], carries a poor prognosis, and patients should be

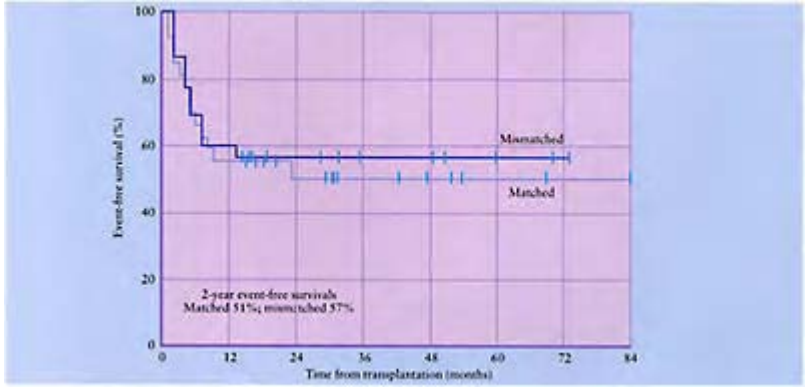

Figure 26.2 Actuarial event-free survival at 2 years (53%) of unrelated donor BMT for ALL CR2; *44 patients. After A Oakhill* et al. Br J Haematol 1996; ***94:****574–578.*

considered for MSD or UD BMT. Extramedullary relapse off therapy has a better prognosis than bone-marrow relapse, and isolated testicular relapse can be cured by chemotherapy and radiotherapy alone in 75% of cases, while isolated CNS relapse can respond to either MSD BMT or chemotherapy [51].

In summary, recent data would suggest that UD BMT is a rational substitute for MSD BMT in children with relapsed ALL [23,24,46,47]. The low transplanted-related mortality seen by some groups following UD BMT may suggest that this modality of treatment be offered in all cases when allogeneic BMT is indicated but no MSD is available. However, this needs to be considered in the context of recent improvements in chemotherapy regimens, particularly in children with a long first remission. Arguably, a more logical approach would be to advocate UD BMT in early relapsing disease whilst prospectively comparing UD BMT to chemotherapy in less aggressive disease. This may depend on centre experience of UD BMT. The most significant indicators for relapsing disease depend on the site of relapse and duration of first remission. Recommendations for the use of UD BMT in children with relapsed ALL based on these criteria are given in Table 26.6.

Table 26.6 Management of relapsed ALL according to duration of CR1 and site of replapse (P Veys on behalf of the UKCCSG Paediatric BMT group)*

Relapse site	Duration of CR1** <2.5 years	>2.5 years to <4 years	>4 years
Bone marrow with or without other site	*UD BMT**** *MSD BMT****	*MSD BMT* UD BMT or R2 chemotherapy	*MSD BMT* R2 chemotherapy
CNS Previous CNS radiotherapy	*MSD BMT* UD BMT or R2 chemotherapy+ radiotherapy	*MSD BMT* R2 chemotherapy+ radiotherapy	R2 chemotherapy+ radiotherapy
No previous CNS radiotherapy high hazard score	*MSD BMT* UD BMT or R2 chemotherapy+ radiotherapy	R2 chemotherapy+ radiotherapy	R2 chemotherapy+ radiotherapy
not high hazard score	R2 chemotherapy 4- radiotherapy	R2 chemotherapy+ radiotherapy	R2 chemotherapy+ radiotherapy
Testis	*MSD BMT* UD BMT	Chemotherapy+ radiotherapy	Chemotherapy 4- radiotherapy

Preferred option in italics.
*R2 chemotherapy=MRC UKALL R2 chemotherapy.
**Radiotherapy is to site of relapse. During BMT, testicular or CNS boosts may be given at discretion of clinician.
***Standard practice has been to use a MSD if available, however the results are poor; UD BMT may have a superior GvL effect and is being used in preference in certain cases.

Acute myeloid leukaemia (AML) in childhood

AML in CR1

Unrelated donor BMT is not currently recommended in AML CR1 and there remains considerable controversy over the role of MSD in CR1. A multicentre study comparing BMT with chemotherapy from the Children's Cancer Group reported a significant survival advantage for patients eligible for transplantation versus those without a sibling donor at three years (52% versus 41%), five years (50% versus 36%) and eight years (47% versus 34%) [52]. Publication is awaited from the MRC AML X trial which has accrued over 350 children, but preliminary results suggest that the disease-free survival (DFS) following intensified chemotherapy has improved to 60% at five years, and at present there is no significant overall survival difference between the BMT and

chemotherapy arms. This question is also being asked in the current MRC AML XII study, although children with favourable karyotypes, t(8,21) t(15,17), and inv(16) will not receive BMT in CR1.

Relapse of disease remains the main cause of treatment failure in children with AML. The relapse rate is lower following allogeneic BMT than chemotherapy, but for a clear advantage in favour of transplantation, demonstration of a reduction in the rate of transplant-related mortality will be required.

AML in CR2

For patients with AML in CR2 who lack a sibling donor, the main therapy of choice lies between UD BMT and an autograft. Currently, there are no published prospective randomized studies although most of the evidence suggests that these two options have similar efficacy. Results of non-T-cell-depleted UD BMT in children have been reported in a series of 13 patients from Seattle predominantly in CR2, with a three-year DFS of 46% [47], and by Davies *et al.* from Minneapolis who demonstrated a DFS of 33% at two years in AML CR2 or early relapse [53]. The use of T-cell depletion may reduce transplant-related mortality, and a two-year DFS of 70% has been recently described in a mixed young adult and paediatric population [54] despite the association with severe viral infections due to delayed immune reconstitution.

The role of UD BMT in the management of AML seems likely to change as it becomes a safer procedure. The intention to perform randomized studies within the EBMT group, and a case control study from the IBMTR may aid in the choice of therapy. Recommendations for the management of relapsed AML in childhood are illustrated in Table 26.7.

Table 26.7 Mangement of relapsed AML in CR2 according to previous treatment (P Veys on behalf of the UKCCSG Paediatrc BMT group)

No BMT	AutoBMT	MSD BMT
MSD BMT Consider UD BMT if relapse <1 years from diagnosis AutoBMT if relapse >1 year from diagnosis (with CR1 marrow)	Consider UD BMT if relapse >6 months since AutoBMT	Donor T-cell infusion Second MSD BMT if >12 months since first BMT
MSD=matched sibling (or family) donor (including 5 of 6 HLA matches in certain cases); UD=unrelated donor; Auto=autologous; CR=complete remission.		

Aplastic anaemia

Aplastic anaemia is usually an acquired disorder of haematopoiesis, although a proportion of cases are familial, associated with genetic disorders such as Fanconi's anaemia or Schwachman-Diamond syndrome. Marrow failure in the acquired form can

be a consequence of damage to stem cells (occasionally radiation- or drug-induced), damage to the stromal cells required for their production, or secondary to immune-mediated suppression of haematopoiesis as reviewed by Young and Barrett [55].

The treatment of choice in severe aplastic anaemia (SAA) is an early HLA-matched related BMT for the minority of patients in whom such a donor can be found. EBMT Registry data suggest a five-year overall survival of 72% for such transplants, although single-centre data indicate that improvements on this figure can be made, with a 92% survival at three years reported by the Seattle team [56,57]. For the majority of patients lacking a matched family donor, immunosuppression is the most appropriate form of initial therapy. Combinations of antilymphocyte globulin (ALG) or antithymocyte globulin (ATG), cyclosporin A, corticosteroids and growth factors produce initial responses in 70–80% of patients. However, two main factors suggest that alternative/unrelated donor BMT has a role in severe aplastic anaemia. First, approximately 20% of patients fail to respond to initial immunosuppression. Although a proportion may respond to a second course of ALG/ ATG given three to four months following the first course, BMT may also be considered at this time point (to reduce further exposure to infective complications and alloimmunization). There is evidence to suggest that it is patients with very severe aplastic anaemia (neutrophil count $<0.2\times10^9$/litre) who have the lowest initial response rate to immunosuppression, and an' early/rapid unrelated donor search should therefore be performed. Second, a number of responders eventually relapse or develop a second clonal bone-marrow diseases, e.g. paroxsysmal nocturnal haemoglobinuria (PNH), myelodysplastic syndromes or AML which may require allogeneic BMT for potential cure.

Results of unrelated donor BMT for severe aplastic anaemia are inferior to those reported from HLA-matched related donors. This is predominantly because of:

- increased graft failure—especially in patients alloimmunized by a large cumulative total of blood and platelet transfusions;
- increased rates of acute and chronic GvHD; and
- increased infectious complications—particularly in patients with a preceding history of prolonged aplasia.

Reports from the EBMT and NMDP Registries suggest a survival rate of only 20–30% [58,59]. The use of T-cell depletion reduces the risk of GvHD but potentially increases the graft failure rate. One successful approach by the Milwaukee group [60] has been to T-cell deplete using the narrow-spectrum T10B9 antibody. The rate of graft failure was reduced by intensive pre-BMT conditioning using total body irradiation, cyclophosphamide, cytosine arabinoside with or without ATG. In this study of 28 high-risk (largely heavily transfused and HLA-mismatched) but young (0.75–24.3 years; median 8.5 years) patients, the survival at almost three years was 54% with low rates of GvHD grades II–IV (28%) and graft failure (3 cases—all of whom had not received ATG as part of the intensive conditioning regimen) (Figure 26.3).

Davies *et al.* have also reported a similar survival with smaller numbers, showing that the best results using unrelated donors have been in children, possibly because they tolerate the highly toxic chemotherapy used for conditioning and are less prone to serious GvHD [61]. Without doubt, early referral to a transplant centre is recommended for young patients with severe aplastic anaemia so that a search may be initiated during

primary treatment with immunosuppression. This will avoid long periods of neutropenia pretransplant leading to profound infectious complications and an increased blood product requirement.

Fanconi's anaemia (FA)

Almost all patients with FA eventually develop haematological abnormalities, typically aplastic anaemia (often after an initial thrombocytopenic phase) but also myelodysplastic syndromes and AML. The characteristic genetic abnormality is increased susceptibility to DNA-crosslinking agents and probable defective processing of DNA damage. Currently, the only curative therapy for the haematological manifestations of FA is allogeneic BMT. Using HLA-matched sibling donors and low-dose cyclophosphamide/limited field (thoracoabdominal) irradiation as conditioning, the survival rate in 151 patients has been reported as 66% at two years [62].

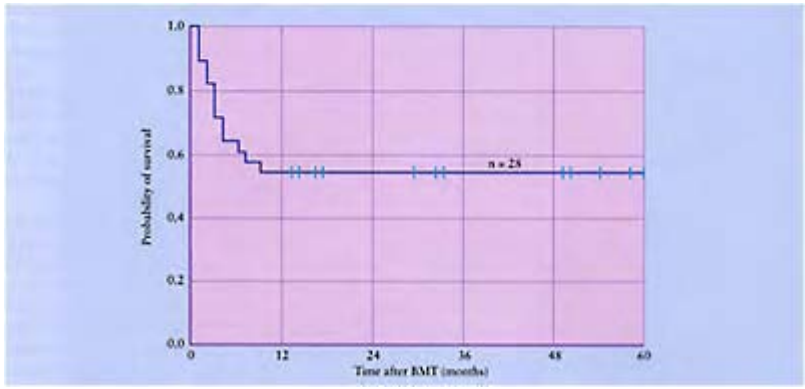

Figure 26.3 Survival after unrelated donor BMT for severe aplastic anaemia. After D *Margolis* et al. Br J Haematol *1996; **94:**65–72.*

However, there is an increased incidence of secondary malignancies (particularly involving the head and neck) in long-term survivors, reflecting non-haemopoietic tissue mutagenesis. In the absence of a family matched donor the outlook is poor—one recent analysis of 48 patients undergoing alternative donor BMT (matched unrelated and haplo-identical related) showed a 29% survival rate at two years [63]. This relates to high rates of graft failure (a consequence of transfusion-induced alloimmunization and the necessity for reduced intensity conditioning in FA), GvHD and infection. Alternative modalities of therapy include cord blood transplantation (related/unrelated), androgen or growth factor treatment, and for the future a gene therapy approach (following the recent cloning of the FA complementarity group C gene).

Myelodysplastic syndromes in childhood

Myelodysplastic syndromes (MDS) represent a heterogeneous group of disorders resulting from clonal expansion of the pluripotent stem cell, usually characterized by ineffective haematopoiesis causing life-threatening cytopenias. Death often occurs as a result of infection or transformation into acute leukaemia [64]. MDS is rare in childhood, and the majority of cases fall into the French-American-British Co-operative Group (FAB) classification of poor-risk categories (CMML; RAEB; RAEB-t) [65]. They account for 2% of all childhood leukaemias and present in 90% of patients under four years of age.

The most common variant of MDS (about 50%) of cases in childhood is juvenile chronic myeloid leukaemia (JCML), a morphologically identical but more aggressive variant of CMML, but lacking a monocytosis. JCML is a myeloproliferate disorder characterized by hepatosplenomegaly, leukocytosis, thrombocytopenia and increased foetal haemoglobin (HbF). Patients are usually extremely unwell when they present, with malaise, wasting, bleeding, fever, pallor and lymphadenopathy. A skin rash representing disease infiltration may be present. Bone-marrow examination shows myeloid and erythroid hyperplasia, and while the Philadelphia (Ph) chromosome is always absent, 40% of patients have a clonal chromosomal abnormality. The disorder is characterized by a rapidly progressive clinical course [66]. Response to chemotherapy is poor, producing at best only suppression not eradication of the malignant clone. Allogeneic BMT appears to be the only reported curative strategy with some patients having remained in remission for as long as eleven years post-transplant [65,67]. CMML may run a more indolent course but transformation to an acute leukaemia also occurs and BMT should be considered.

A report from the European Working Group on Myelodysplastic Syndrome in Childhood (EWOG-MDS) reviewed 43 patients affected by JCML/CMML who were given an allogeneic MSD or UD BMT. The probability of transplant-related mortality was 9% in the MSD group, versus 46% in the UD BMT group [64]. The overall probability of relapse was 58% for the group as a whole, with a five-year event-free survival of 31%. The event-free survival for children receiving MSD BMT was 38%, patients receiving busulphan having a better event-free survival compared with those given total body irradiation (TBI) (62% versus 11%).

Although there are relatively few published reports specifically addressing the question of BMT in children with CMML/JCML, it has become apparent that a significant malignant cell kill can be achieved by employing conditioning regimens containing three alkylating drugs that have a non-cell-cycle-specific action such as busulphan, cyclophosphamide and melphalan. These preparative regimes are at least as effective, and possibly more effective than TBI containing conditioning regimes previously reported by the Seattle group [68]. There is also a reduced risk of radiation-induced severe growth retardation, endocrine disorders, cataract formation and neuropsychological sequelae. A preliminary EBMT analysis of paediatric patients given allogeneic BMT for MDS variants other than CMML has also confirmed that a busulphan-containing regimen is at least as effective as radiotherapy in inducing long-term remission (E Locatelli, unpublished data).

Since myelodysplastic syndromes in childhood are rare disorders, it is almost impossible to perform a randomized study. A European study has opened and enrolled

patients from April 1997 and this is a prospective non-randomized multicentre study with the aim of standardizing the transplant approach. This protocol has been adopted by the EBMT Paediatric Working Party and the EWOG-MDS group and hopes also to accrue data from North American centres. In this protocol summarized in Figure 26.4, all children (MSD/UD) will receive a standard conditioning regimen with busulphan, cyclophosphamide and melphalan. Patients having an UD BMT will in addition receive a short course of methotrexate and either Campath-1G or ALG *in vivo* to prevent the occurrence of severe GvHD. The decision with respect to splenectomy in

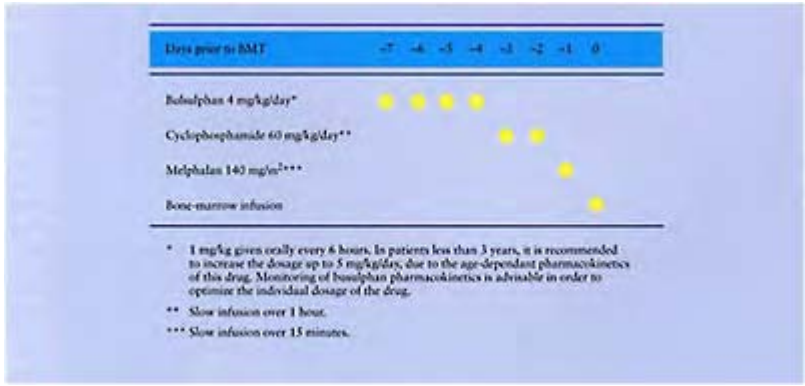

Figure 26.4 Conditioning regimen for children with MDS. After F.Locatelli on behalf of the Paediatric BMT Working Party of the EBMT.

cases of massive splenomegaly or hypersplenism is for the consideration of individual transplant centres. However, the potential for an engraftment advantage may support this intervention [24,69].

The rationale for this study derives from studies published by Locatelli *et al.* [64] and is supported by preliminary data derived from the UK Paediatric MDS Register (J.Chessells, unpublished data). The outcome in 39 cases reported to this registry was analyzed and showed a statistically significant survival advantage for MSD over other donors and for chemotherapy-only conditioning therapy versus TBI-containing regimens. There was a higher incidence of death from infection in the alternative donor group.

The additional problems to be addressed, therefore, in the use of unrelated donor BMT for paediatric MDS are those of increased graft rejection, GvHD and leukaemia relapse. The use of T-cell depletion in reported series may have reduced the potential for the GvL effect, although Casper *et al.* have described 9 children receiving UD BMT with TBI in the conditioning regimen and the use of T-cell depletion, 4 of whom survive [24]. Davies *et al.* have published data on 10 patients transplanted using an unrelated donor for MDS including TBI in the preparative regimen, of whom 6 survive [61]. These series are small but encouraging. In the absence of matched sibling donors, undoubtedly the best results

are achieved following early referral for UD BMT in order to avoid prolonged periods of neutropenia prior to transplantation, or acceleration to more advanced disease.

Current indications for unrelated donor transplantation in adults

Chronic myeloid leukaemia (CML)

Background: the role of allogeneic BMT

CML is a stem-cell disorder, and this provides the rationale for allogeneic transplantation as a potentially curative modality of therapy. However, even in non-transplanted patients there has been an increase in survival in CML over the last decade. This relates in part to earlier diagnosis, although the introduction of α-interferon has also improved survival - particularly for the relatively small subgroup of patients who demonstrate a complete or major cytogenetic response. Autologous transplantation using cells collected from peripheral blood or bone marrow at diagnosis may prolong subsequent chronic phase. A recent development in this field has been the collection of Ph-negative blood progenitor cells following intensive chemotherapy and the use of growth factors such as granulocyte colony-stimulating factor (G-CSF). The use of these cells following high-dose therapy may allow reconstitution of Ph-negative haemopoiesis. However, there are potential problems with this methodology particularly related to poor mobilization of progenitor cells, slow engraftment/prolonged cytopenias and the frequent collection of a proportion of Ph^+ cells. Also, even in patients who have a complete cytogenetic response to interferon or autologous transplantation the use of more sensitive polymerase chain reaction (PCR)-based techniques will frequently suggest low level residual leukaemia. Therefore, it still remains to be seen whether any patients with CML can be cured in the absence of an allogeneic transplant procedure.

Allogeneic BMT with a sibling donor remains the treatment of choice in CML. The long-term DFS is of the order of 60% in patients in first chronic phase (CP1) [70]. The results are best in patients under the age of 50 years within one year from diagnosis - the Seattle team recently reported a five-year survival of 77% in such patients [71]. Unfortunately, only approximately 15% of newly diagnosed patients have both an HLA-identical sibling and are under the age of 50 years. Even if older patients are accepted for transplantation, it is clear that the optimal form of therapy is available for only a minority of patients.

HLA-identical related donor transplantation in accelerated phase (AP) CML carries a greater risk of relapse and procedure-related mortality than BMT in CP1, but the four-year DFS may be as high as 43% [72]. Patients transplanted in second CP (CP2) have an intermediate prognosis whereas there are very few survivors of transplantation in blast crisis [73]. T-cell depletion in the HLA-identical related donor setting substantially increases the relapse rate and is not routine practice [74]. In terms of conditioning regime,

a randomized study of busulphan/cyclophosphamide against TBI/cyclophosphamide showed equivalent DFS [75].

Results of unrelated donor BMT

CML is the most frequent indication for unrelated donor BMT. Several groups have reported results in CP1 with a DFS of between 35% and 52% at two years [76–81]. These results have included a substantial number of transplants with known donor-recipient HLA mismatches at one or more loci. The use of single-antigen mismatched donors in the Seattle series of unmanipulated (non-T-cell-depleted) transplants was associated with increased procedural mortality, a lower rate of relapse and an equivalent overall DFS [82] to the HLA-matched group. The incidence of significant (grades II–IV) acute GvHD and chronic GvHD was 70% in this series. This group recently reported an update on 204 patients with CML in CP—the largest series to date [71]. The median age of the patients was 35 years (range 6–55 years), and disease duration prior to BMT was 1.5 years. Conditioning was with TBI/cyclophosphamide, and GvHD prophylaxis consisted of cyclosporin A and methotrexate but no T-cell depletion; 77% of donor—recipient pairs were HLA-matched at the A, B and DRB1 loci. The overall survival at five years was 55%. Adverse factors included DRB1 mismatching, increasing patient age and body weight, and a long interval from diagnosis to relapse. Interestingly, a degree of class I (A or B) mismatching was tolerated, with no adverse effect on survival or GvHD. In the subgroup of patients under the age of 50 years transplanted with an HLA-matched donor within one year of diagnosis, the estimated five-year overall survival was 74%—comparable to the results with matched related donor BMT. The transplant-related morbidity is, however, higher in the unrelated setting, particularly that due to chronic GvHD.

The role of T-cell depletion in unrelated donor BMT for CML is unclear. Results from the EBMT Registry data suggest that T-cell depletion increases the relapse rate and reduces the DFS [83]. However, this analysis is weakened by the variety of T-cell-depletion methodologies used by different centres. Data from the Campath users group has shown that *in vivo* T-cell depletion with the Campath-IgG antibody results in a lower incidence of GvHD but a higher rate of graft failure compared to published non-T-cell-depleted data [84]. The actuarial two-year relapse rate in the Campath group was 17%—lower than in the T-cell-depleted matched related setting, but higher than the non-T-cell-depleted unrelated setting [71,74].

In a report of 86 patients with CML CP1 receiving T-cell-depleted transplants using Campath antibodies (mostly *in vivo*) by the Hammersmith Hospital [85] the LFS was 41% at three years. Factors predicting improved survival included recipient seronegative cytomegalovirus (CMV) status and complete HLA matching (A,B-isoelectric focusing; DRB1, DQB1 restriction fragment-length polymorphism analysis). A low-frequency CTLp assay correlated with a reduced incidence of severe acute GvHD in this study. T-cell depletion with the narrow-spectrum T10/B9 antibody (which preferentially depletes αβT-cells) has also been shown to reduce the risk of GvHD. However, this was not associated with an apparent increase in relapse rate at three years [86] possibly relating to the preservation of donor γδT-cells associated with the use of this antibody. Data from the IBMTR on 231 leukaemia recipients of T-cell-depleted unrelated donor marrow

further supports the concept of the use of narrow-spectrum antibodies with a superior DFS over the broad-spectrum antibody group.

The role of T-cell depletion in unrelated donor BMT for CML remains unclear. Data in the HLA-matched related setting suggest a major role for infused T-cells in augmenting the GvL effect. Although the relapse rate is also higher using T-cell depletion, in the unrelated setting this effect may be considerably less marked. Also, the older age of most patients diagnosed with CML renders them more susceptible to the complications of GvHD [81]. At present, although there is evidence of a reduction in procedural-related mortality with all methods of T-cell depletion, effects on survival are not clear. Controlled prospective studies are required, although in practice clinicians and patients may find this conceptually difficult.

The approach to individual patients with CML

The decision to offer allogeneic BMT to patients with CML should involve full discussion of the risks involved and alternative therapeutic approaches available. In general, patients less than 50 years old with a fully HLA-matched related donor available and no other contraindication to BMT should proceed to an allograft within one year of diagnosis. Many centres have extended the upper age limit for such transplants to 60 years (or higher) if patients are in good general health. For older patients with CML (>40 years) it may be preferable to offer a trial of interferon-α for 6–12 months prior to proceeding to allogeneic BMT if there is no major or complete cytogenetic response with this therapy.

In patients with CML under 55 years of age and who lack a matched family donor, a search for an unrelated donor should be instituted at diagnosis. A trial of interferon-α for up to one year should probably be offered to all such patients and transplantation postponed in the relatively small number with a major cytogenetic response (<35% Ph^+ metaphases), who have an estimated five-year survival of 90%. Other patients should be offered unrelated donrf4or BMT within one year of diagnosis if a fully matched (A, B, DRB1) donor is identified. A mismatch at one of these loci may be tolerated, particularly if this is a minor serological A or B (CREG) mismatch, or if T-cell depletion is used. Survival figures may, however, be compromised, particularly with a DRB1 mismatch and in older recipients. Most centres would exercise extreme caution in proceeding with a mismatched unrelated BMT in patients over 35–40 years of age. The role of molecular typing/matching for class I loci (including C), DQB1 and cellular proliferation assays such as the CTLp assay requires further definition though some centres have found these to be of significance in predicting outcome [28,71,85].

Autologous transplantation in CP1 offers an alternative therapeutic strategy and may be better tolerated in older patients or those not prepared to undergo allografting. The chronic phase may be prolonged, particularly in interferon-responsive patients [87,88]. Re-infusion of cells purged by interferon or Ph-negative cells mobilized by chemotherapy or purified by culture techniques may be superior to the use of unpurged grafts, but long-term follow-up is required to fully assess the value of these interventions. A randomized prospective trial comparing autografting with conventional interferon-based therapy is currently underway (UK MRC CML IV/ECOG E7995 trial).

The results of allogeneic (related/unrelated) BMT in CML beyond first chronic phase are inferior. However, a proportion of patients in accelerated phase are long-term disease-free survivors. There are difficulties in comparing published studies, both because of differing definitions of accelerated phase and the inclusion of variable numbers of patients with CML in blast crisis. However, 15–46% of patients with advanced phase CML are disease-free survivors at least two years following UD BMT [77,78,81,85]. There are very few survivors of UD BMT for CML in blast crisis, and it may be preferable to try and induce a CP2 before considering offering this form of treatment to such patients.

Acute leukaemias in adults

Background: the role of allogeneic BMT

In assessing the value of allogeneic BMT in the acute leukaemias, the following factors should be taken into account:

- Patient age.
- Leukaemia subtype including cytogenetics.
- Phase of disease and previous treatment received.
- Donor type—matched/mismatched and related/ unrelated.
- Potential for alternative therapy, e.g. chemotherapy or autologous transplantation.

Unlike the case in childhood ALL, ALL in adults is associated with a poor prognosis with chemotherapy treatment, with an approximately 20% five-year DFS [89]. Patients with high-risk cytogenetics, particularly t(9;22) and t(4;11) are effectively incurable by chemotherapy alone. For such patients, HLA-related allogeneic BMT is clearly indicated, with a five-year DFS as high as 40–60% [89–91]. The role of HLA-related allogeneic BMT in adults with ALL lacking these high-risk features remains uncertain. While single-centre data are encouraging, with DFS of around 40–60%, a study by the IBMTR suggested equivalent survival with chemotherapy alone—a lower relapse rate being offset by a higher treatment-related mortality [92]. One randomized prospective study reproduced these findings [89]. The role of autologous BMT (both purged and unpurged) in standard-risk ALL CR1 is also uncertain. Further data are required from large prospective randomized trials. One example is the current MRC UKALL XII/ECOG 2993 trial comparing post-induction chemotherapy and autologous BMT in patients lacking biological randomization to allogeneic BMT by virtue of the availability of an HLA-matched related donor [89].

Adults with ALL who relapse have a very poor prognosis with chemotherapy alone. Although a second remission can be achieved in approximately one-third of cases, the long-term DFS is under 10%. HLA-related allogeneic BMT is the treatment of choice in second (or higher) CR, with around 40% long-term DFS [90]. For patients lacking a matched family donor, the choice is between autologous and unrelated donor BMT. There are very few survivors of relapsed chemoresistant ALL with any form of transplant, although allografting may have a role in primary refractory disease.

In first remission of acute myeloid leukaemia there are also difficulties in defining the role of both allogeneic and autologous transplantation. Long-term DFS of around 50% has been described for both allogeneic [93] and autologous BMT [94–96]. However, over the last decade, the use of more intensive post-remission chemotherapy has lead to improvements in DFS from approximately 20% to 40–50% without transplantation [97]. Clearly, results from large prospective randomized trials are required to determine the place of BMT in AML CR1. One such study from the EORTC/GIMEMA groups [98] showed significantly better four-year DFS for both allogeneic (55%) and autologous (48%) BMT than chemotherapy (30%). However, this study was criticized for the young age of patients entered (median 33 years; range 11–59 years) and the fact that a maximum of only three courses of chemotherapy were used, which may be suboptimal. Also, AML is increasingly being considered as a heterogeneous disease group with substantial biological variability between subtypes. It is probable that not all patients should be considered for transplantation in CR1 and a risk-directed approach may be appropriate. This is illustrated by data from the largest prospective AML study to date—the UK MRC AML X trial [93]. Three risk groups are defined in this study:

1. Good risk (the cytogenetic presence of t(15;17), t(8;21) and inv(16).
2. Standard risk (20% blasts after the first course of chemotherapy and without good risk cytogenetics).
3. Poor risk (>20% blasts after the first course of chemotherapy).

The five-year DFS in these three risk groups was: good risk (71%), standard risk (45%) and poor risk (16%) irrespective of the actual treatment received.

In a recent analysis [99], there was a survival advantage in favour of allogeneic BMT in the standard-risk group, no advantage in the poor-risk group and an inferior outcome in good-risk patients. In terms of the randomization between autologous BMT and chemotherapy alone, there was a reduced relapse rate (37% versus 58%) and improved DFS (53% versus 40%) in the autograft arm. With prolonged follow-up, an advantage also in terms of overall survival has become apparent (57% versus 45%). Current conclusions are that transplantation may not be appropriate for certain patients in CR1 (e.g. good-risk patients and younger age group) and that a further reduction in the procedural mortality of autologous transplantation may be required in order to realize a maximal benefit.

After relapse of AML, long-term cure with chemotherapy is uncommon. Allogeneic BMT from a matched family member provides the optimal approach, with a five-year DFS of around 40% [89,100]. Using this approach it may not be necessary to induce a second remission prior to transplantation [101]. Autologous BMT may also allow long-term DFS in a proportion of patients in CR2/untreated first relapse (20–45%) [102]. For the (approximately) 20% of younger patients with primary refractory AML, allogeneic BMT offers the only prospect of cure.

Results of unrelated donor BMT in adult acute leukaemia

There are difficulties in interpreting published results because of the inclusion of children in some reports and mixed disease categories (AML/ALL) and status (remission/relapse) in others. A summary of the results of key studies is shown in Table 26.8. Clearly the

results of unrelated donor BMT are dependent upon the disease stage prior to transplantation. For those patients with primary refractory acute leukaemia lacking a matched family donor, unrelated donor BMT offers a small chance of long-term DFS (approximately 10–20%) [105,106]. Patients who relapse following initial chemotherapy require a transplant approach for cure. Most centres attempt remission re-induction to allow time for identification/confirmation of an unrelated donor. There are few survivors of chemoresistant relapse from any modality of transplantation. This is particularly the case in patients who continue to have circulating blasts and transplantation should probably be withheld in these circumstances. If a second (or subsequent) remission is achieved, the choice in patients who lack a related HLA-identical donor is between unrelated donor and autologous BMT. There are currently no results from prospective randomized trials although data are available from two retrospective analyses. The Seattle group have compared the outcome in 43 patients with

Table 26.8 Results of UD BMT in acute leukaemia

Diagnosis (*n*)	Age range	Disease-free survival (2–3 years)	Study type/reference
ALL (83)	1.2–49.8 years	CR1/2 = 45 ± 13%	Multicentre NMDP survey [81]
AML (70)	Median 13.5 years (ALL), 27.9 years (AML)	(CR > 2, rel) ref = 21 + 9%	
ALL (28)	2–51 years	In CR ≥ 2 = 33 ± 19%	Multicentre survey [103]
AML (27)	median 25 years	Active disease = 15 ± 14%	
AML/ALL (174)		CR1=55±29%	study [104]
		CR2=31±16%	
		CR≥3=26±22%	
		Relapse=9±5%	
AML (18)	4.8–31 years, median 12.8 years	CR1/CR2=78±21%	Single-centre study [54]

AML/ALL receiving an unrelated donor BMT with 77 patients (disease, disease stage and age-matched) undergoing autologous transplantation [106]. There were no statistical differences in DFS between the two groups: 33% (UD) versus 25% (autotransplant) for patients in CR; 12% (UD) versus 5% (autotransplant) for patients in relapse.

In a similar but larger study from the EBMT/IMUST databases which also matched for year of transplant, the results of 206 UD transplants were compared with 412 autologous transplants [107]. There were no significant differences in two-year DFS:

AML 38% (UD) versus 39% (autotransplant); or ALL 39% (UD) versus 30% (autotransplant). The overall survival was also equivalent. In this study the relapse rate was significantly lower in recipients of unrelated marrow, but this was offset by a higher transplant-related mortality. The EBMT has recently initiated a prospective randomized trial of unrelated versus autologous BMT in second-remission acute leukaemia.

The role of unrelated donor BMT in first-remission acute leukaemia is limited to patients with high-risk disease—particularly ALL with adverse cytogenetics, and AML with co-existing myelodysplasia or monosomy 7 (Figure 26.5) [8,54,106].

Myelodysplasia and secondary leukaemia

The term 'secondary leukaemia' usually refers to cases of AML occurring after an antecedent phase of myelodysplasia or following exposure to prior chemotherapy, radiotherapy or other toxic chemicals. Abnormalities of chromosomes 5 and 7 are common: −5, −7, del(5q), del(7q). Other cytogenetic abnormalities occur in therapy-related secondary AML, notably translocations involving 11q23 and 21q22 (associated with the use of topoisomerase II inhibitors), t(15;17) and inv(16). Cases of *de novo* MDS with abnormalities of chromosomes 5 and 7 and/or excess blasts and patients with AML (primary or secondary) with these cytogenetic abnormalities have a very poor outlook in the absence of allogeneic BMT. Whether to proceed to allografting in MDS/secondary AML depends on several factors: availability of a matched HLA-related/unrelated donor, patient age, disease risk factor assessment (e.g. cytogenetics, percentage blasts), response to chemotherapy and patient/clinician choice. In a report from the EBMT of 400 patients receiving HLA-matched related donor BMT, the overall survival and disease-free survival at three years were 42% and 36%, respectively [108]. This was highest for MDS-

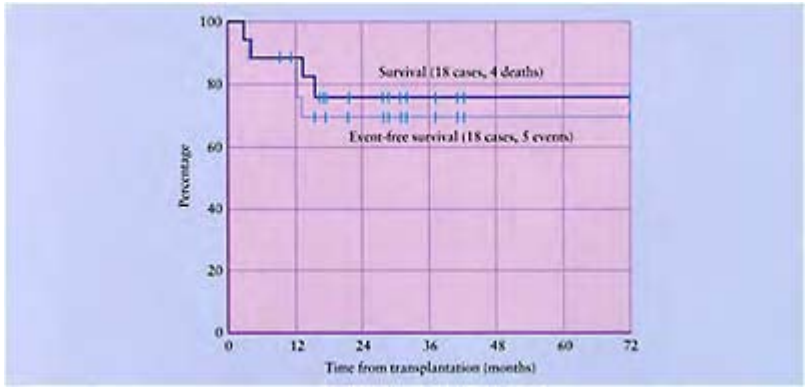

Figure 26.5 Survival (76%) and event-free survival (70%) of 18 patients, AML in CR post-unrelated donor BMT. After S Chown et al. *Blood 1995; 86:99 and personal communication.*

RA/RARS (52% DFS), intermediate for MDS with excess blasts (RAEB/RAEB-t) and lowest for secondary AML (28% DFS). In the ten years from 1985 to 1995, 67 patients with MDS/secondary AML (median age 26 years; range 0.3–53 years) received transplants from unrelated donors and were reported to the EBMT [109]. The overall survival at three years was 30%, with no difference between disease subgroups. In this study (largely on non-T-cell-depleted transplants), the procedure-related mortality was high at 60% and age-related (reaching 90% in the over-35 years age group).

Overall, it would seem reasonable to consider unrelated donor transplantation in younger patients with poor prognosis MDS and secondary AML. In the EBMT survey, the majority of patients received chemotherapy prior to allogeneic BMT. However, this is of uncertain value—the CR rate is low and infectious complications can develop which may reduce the chance of successful transplantation. Directly proceeding to transplantation is an alternative in patients with low levels of circulating blasts [110]. Patients with secondary leukaemia and favourable karyotypes—t(15;17) and inv(16)—respond well to intensive chemotherapy with similar survival as *de novo* AML sharing the same cytogenetic changes. Those patients with translocations involving 11q23 and 21q22 tend to respond well to induction chemotherapy with a high CR rate, but go on to relapse. These patients are therefore candidates for allogeneic BMT in CR1.

Severe aplastic anaemia

This is discussed in the paediatric section.

Other indications for unrelated donor transplantation

In the 1995 EBMT survey there were 764 reports of unrelated donor transplants: 698 were performed for the indications discussed above (CML, ALL, AML, MDS, severe aplastic anaemia, Fanconi's anaemia). Of the other 66 UD transplants, the indications (number) were as follows:

- Inborn errors of metabolism (30)
- Immunodeficiencies (11)
- Non-Hodgkin's lymphoma (8)
- Myeloma (3)
- Chronic lymphocytic leukaemia (3)
- Thalassaemia (1)
- Others (10)

There were no reports of UD transplantation in patients with other solid tumours. The rationale for allogeneic (including unrelated donor) BMT in the subgroups listed above is discussed in the disease-specific chapters.

Complications of unrelated donor BMT

The toxicity of any individual allogeneic BMT is primarily dependent upon the intensity of conditioning, HLA disparity between donor and recipient and the performance status of the recipient. Early studies of UD BMT were characterized by a high transplant-related mortality although this was largely due to the inclusion of relatively high numbers of patients with heavily treated advanced leukaemias. A more accurate picture of the relative toxicity of UD BMT is provided by a study conducted by Bearman *et al.* [111]. In this age and disease-matched cohort study, morbidity and mortality were compared between 104 sibling recipients and 52 recipients of phenotypically matched UD BMT. The authors concluded that whereas severe regimen-related toxicity was more common after UD BMT, this was not statistically significant. More recently, a number of groups have shown that DFS using unrelated donors is comparable to that seen after matched related donor transplantation in patients with acute leukaemia and CML. Thus, absence of a matched family donor should not necessarily be considered a contraindication to allogeneic transplantation if a matched unrelated donor is available.

Graft failure

Rejection of the graft is mediated by immunocompetent host T-cells which survive conditioning. The frequency and functional activity of residual host cells is related to the intensity of conditioning, degree of presensitization of the recipient to foreign antigens and HLA disparity between donor and recipient. Therefore, unrelated donor BMT would be expected to carry a greater risk of rejection than HLA-identical sibling BMT. However, two single institution studies of over 50 patients describe similar rates of graft failure after unmanipulated serologically matched unrelated donor BMT to that seen after fully matched sibling transplants. Both groups have also highlighted a significantly increased risk of graft failure after serologically mismatched UD procedures [112,113]. Also, it is well established that T-cell depletion increases the risk of rejection in the unrelated donor setting though this effect may be reduced by the use of pretransplant *in vivo* T-cell depletion in the recipient.

Failure of the graft leads to prolonged pancytopenia and substantial mortality and morbidity. An immune-based rejection should be differentiated from relapse or poor graft function due to infection or drug toxicity. In addition to morphological marrow examination, molecular and cytogenetic assessment of the degree of donor-recipient chimaerism may be helpful. The initial approach to a falling neutrophil count or slow engraftment should include cessation of potentially myelotoxic drugs and a search for viral infection including CMV, parvovirus and HHV-6. The administration of growth factors such as G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF) may produce a response in up to 50% of patients who have not previously received them during the transplant period [114]. Attempts to modify the process of immune rejection including high-dose corticosteroids and serotherapy with T-cell antibodies are usually unsuccessful. Although infusion of donor lymphocytes at times of progressive mixed chimaerism may represent an attractive approach, immunologic rejection is often very rapid. Where complete rejection occurs (with documented loss of donor cells) the choice is often between infusion of the recipient's autologous back-up harvest and a second

allogeneic transplant—usually from the same donor and after repeat but reduced intensity conditioning. There is increasing interest in the potential use of allogeneic peripheral stem cells in this situation for their enhanced engraftment potential.

GvHD and the need for T-cell depletion

Early data from Seattle and the NMDP suggested that more than 70% of patients receiving unmanipulated transplants from serologically matched donors develop grades II–IV acute GvHD despite standard cyclosporin and methotrexate prophylaxis [8]. This high incidence of GvHD may be reduced to 45% by the use of molecular matching for class II loci, in particular HLA DRB1. Matching for DQB1 may also be important. The impact of molecular matching for the class I loci (A, B, C) has yet to be assessed. While molecular matching offers the prospect of reduced rates of GvHD and graft failure, it may limit the number of available donors and prolong the search-to-transplant time. This may be problematic in a patient with a high-risk malignancy. Moreover, many groups believe that the risk of severe GvHD is unacceptable in the non-marrow-manipulated unrelated setting even with class II molecular matching, and have therefore used T-cell depletion for prophylaxis. This reduces the rate of significant acute and chronic GvHD and may allow the selection of one-locus mismatched donors if no alternative (fully matched) donor can be found. With regard to adverse effects, T-cell depletion may be associated with a slower rate of immune reconstitution leading to an increased risk of viral infections [79,115] and post-transplant lymphoproliferative disease. This latter complication occurs more frequently in association with the use of ATG than with Campath antibodies for *in vivo* T-cell depletion.

The large multicentre analysis of the first 462 unrelated BMT from the NMDP revealed a major decrease in moderate-to-severe GvHD in patients receiving T-depleted grafts, and significant decrease in early transplant-related mortality [8]. There was also a clear association between graft rejection and T-cell depletion. No difference in leukaemia relapse was seen between the depleted and non-depleted groups. T-cell depletion with the narrow specificity T10/B9 antibody may abrogate the effect of a DRB1 mismatch and result in a significant reduction in GvHD when compared to unmanipulated UD transplants. No increase in rejection was seen, reflecting additional pretransplant immunosuppression of the recipient with high-dose steroids. At three years, this type of depletion was not associated with an increased rate of relapse in patients with CML [77]. However, the risk of relapse was higher in this group than that seen after unmanipulated one-antigen mismatched UD BMT in Seattle. The decreased risk of relapse in this cohort was offset by increased toxicity secondary to GvHD [78].

Thus, the information available to date suggests that T-depletion reduces the risk of severe GvHD to levels similar to those seen after non-depleted sibling grafts and allows a widening of the donor pool. However, opinion remains divided as to whether T-cell depletion is advisable in the context of UD BMT. The crux of this issue is what degree of alloreactivity must be preserved to ensure GvL while minimizing the risk of uncontrollable GvHD and rejection. This is likely to be dependent on several variables, namely:

- *Transplant indication.* GvL appears pivotal to the eradication of CML but may be less relevant in acute leukaemia. Unmanipulated BMT may be necessary in alloimmunised patients (e.g. with severe aplastic anaemia), for advanced malignancy states or for second transplant procedures.
- *Patient age/performance status.* T-cell depletion may be preferred in older/sicker recipients in an effort to reduce transplant-related mortality.
- *Conditioning regimen.* Modification of accepted protocols including pretransplant T-cell depletion of the recipient and addition of other agents may favour engraftment of T-cell depleted marrow.
- *Degree of HLA disparity between donor and recipient.*
- *Stem-cell dose.* An increased stem-cell dose may be associated with a reduced rate of graft failure. Transplant outcome may be improved, with higher cell doses infused. This raises the possibility of using allogeneic mobilized peripheral blood.
- *T-cell dose/selection.* There is evidence for a threshold effect with respect to T-cell dose and GvHD. The optimal approach would be to remove cells responsible for GvHD while preserving GvL and cells promoting engraftment. Such an approach may also limit the impact of T-cell depletion on immune reconstitution after BMT but has not yet been achieved.

An alternative concept is to perform a relatively 'safe' T-cell-depleted transplant and subsequently attempt to augment GvL by sequentially increasing doses of 'prophylactic' T-cell infusions given post-transplant. Preliminary evidence suggests that this may be a successful strategy in avoiding severe GvHD in the matched sibling setting, although cell doses/infusion schedules require better definition.

Treatment of disease relapse following unrelated donor BMT

Recipients of allogeneic transplants who suffer disease relapse may attain complete remission with transfusions of leukocytes from the original donor. Experience to date is largely in the HLA-matched related setting—large surveys from the EBMT [116] and the USA [117] have been published recently. Factors primarily affecting outcome to donor leukocyte infusion (DLI) include:

- *Disease type.* Complete response rates are highest in CML at approximately 70%. These are largely durable responses with 80–90% remaining in remission at two to three years. Responses have also been described in a minority of cases of AML, ALL, MDS and myeloma.
- *Disease stage.* In CML, response rates are highest in cytogenetic and chronic phase relapse, intermediate in accelerated phase relapse and lowest in blastic phase relapse. Some patients with acute leukaemia have been given chemotherapy in an attempt to induce remission prior to DLI. It is unknown whether this will improve the response rate to DLI.

• *Numbers of T-cells infused.* It is clear that the majority of patients with early stage relapse of CML will respond to DLI consisting of $\leq 1\times10^7$ T-cells/kg recipient body weight, and that at this level the complication of GvHD is reduced [118].

The major complications of DLI include acute and chronic GvHD which occur in around 60% of cases (receiving around 10^8/kg T-cells) and pancytopenia in 20%. This latter complication is potentially predictable by assessing the proportion of donor chimaerism pre-DLI. In the majority of cases pancytopenia is reversible, either spontaneously or with growth factor support. Permanent pancytopenia which occurs more frequently in advanced disease relapse (with a consequent low level or absent donor haemopoiesis) may require further donor stem-cell infusion.

Response to DLI correlates strongly with the presence of acute and chronic GvHD. The association of other factors with response rate (including GvHD following initial BMT, T-cell depletion status of original BMT, timing of DLI with respect to initial BMT, selected T-cell subset therapy, addition of α-interferon treatment) requires further definition.

There is less experience of DLI in the unrelated donor setting. Three reports have included brief details on 28 recipients of UD DLI [116–119]. Responses appear to be at least as good as for the matched related setting. It is not clear from these reports whether the complications of DLI are higher in the unrelated donor setting. However, 7 of 17 evaluable cases suffered severe (grades III–IV) acute GvHD [117,119]. A cautious approach to UD DLI may be warranted—in CML, responses have been described with T-cell infusions at a level below 10^7/kg recipient weight [120].

The introduction of DLI therapy in the unrelated donor setting has implications for donor agencies. It is probably preferable that donors, after full medical review and consent, have a single-machine leukopheresis and that the cells are stored in multiple aliquots (according to the intended regime for the recipient) to avoid the need for repeat donation. It is hoped that the use of DLI will reduce requests for second donations of stem cells (marrow or blood derived) with the consequent risks involved in this.

Alternative stem-cell sources for unrelated donor transplantation

Umbilical cord blood

Umbilical cord blood contains haemopoietic stem cells capable of long-term engraftment in the allogeneic setting. The first human cord blood transplant was performed in 1988 by Gluckman and colleagues. A six-year-old boy with Fanconi's anaemia received cord blood from his HLA-identical sibling and the transplant remains a long-term success [121]. Since this time, many more cord blood transplants have been performed in the HLA-matched related setting [122]. Most of the recipients have been children suffering from a wide variety of malignant and non-malignant haematological disorders. Engraftment has occurred in excess of 90% of recipients, although the tempo of platelet and neutrophil regeneration is slower than with marrow. This may relate to the lower numbers of measurable CD34 cells and total mononuclear cells. There is a suggestion

that the rate and severity of GvHD is lower with cord blood than with comparable bone-marrow transplants. This may be because of both lower numbers, and reduced alloreactivity of T-cells. One worry is that this may also reduce the GvL effect, leading to a higher relapse rate. Resolution of this will require further comparative analyses.

This early success using matched sibling cord blood has predictably led to enthusiasm for establishing banks of cryopreserved cord cells for use in the unrelated donor setting. The first cord blood bank was set up in the New York Blood Center in 1992 and subsequently in several other countries. The first clinical reports of UD cord blood transplants were reported in 1996 [123,124]. An update on 63 patients transplanted between 1993 and 1996 at Duke University was presented at the American Society of Hematology meeting in December 1996 [71]. The majority of the recipients were children though 12 weighed in excess of 40 kg. Two-thirds of the transplants were performed for malignant disease, predominantly ALL and AML. Matching was analyzed at A, B and DR (or DRB1) loci: all except two donor-recipient pairs were mismatched at one (n= 18), two (n=33) or three (n=8) loci. The conditioning regimen and GvHD prophylaxis used was variable, although the majority of recipients received steroids in addition to cyclosporin. Initial engraftment occurred in 95% of patients, with a neutrophil count of 0.5×10^9/litre reached by a median of 20 days (range 12–59 days) and platelet transfusion independence by day 60 (35–96 days). Rates of acute grades II–IV (28 patients) and chronic GvHD (2 of 20 evaluable patients) were low considering the degree of mismatch in this series. The long-term success of this programme awaits further follow-up with respect to relapse rate/overall survival, but it is clear that matched and mismatched cord blood transplants are feasible, at least in children.

The potential advantages of cord blood compared to marrow relate to the potential for immediate availability (subject to satisfactory testing), low rate of CMV transmission and apparent reduced alloreactivity. Potential disadvantages include absence of a mechanism for obtaining further cells from the original donor, worries about the possibility of potentially transmissible genetic diseases, GvHD from contaminating maternal cells and a reduced GVL effect leading to increased relapse rates when transplantation is performed for malignant disease. There are also a number of ethical issues which are not fully resolved including those relating to the legal ‘ownership’ of cells, and issues concerning infectious and genetic disease screening.

Engraftment may be suboptimal, particularly for older children and adult recipients, unless *ex vivo* expansion of progenitors capable of long-term haemopoietic reconstitution can be demonstrated. Further clinical results are eagerly awaited. Cord blood may be an important source of stem cells for children (often from ethnic minorities) in whom a fully matched or one locus mismatched UD bone-marrow donor cannot readily be identified.

Peripheral blood progenitor cells (PBPC)

The use of PBPC in place of bone marrow is now well established in both the autologous and matched related allogeneic setting. The latter was considered in detail at an EBMT symposium held in Geneva in October 1995 and in a published review in 1996 [125]. It is clear that the increased cellular content of PBPC grafts (especially CD34 and T-cells) may lead to an engraftment advantage without an apparent increase in the rate of acute GvHD. This may be due to a threshold effect (with no additional GvHD beyond a set

number of T-cells) or a functional difference between marrow- and blood-derived T-cells. Currently, it is unclear whether the rate of chronic GvHD will be increased although some centres have reported a different pattern with more visceral involvement. Also, it is unknown whether the increase in T-cells will augment the GvL effect, resulting in a reduced relapse rate. These issues are being addressed by a large prospective EBMT multicentre randomized trial of BMT versus PBPC transplant.

Escalation of cell doses by the addition of mobilized PBPC to bone marrow has allowed successful engraftment in the haplotype-mismatched related setting [126]. This approach requires vigorous T-cell depletion to prevent severe GvHD. Initial results have been promising, stimulating interest in the use of PBPC transplant in the unrelated donor setting. Understandably, progress has been slow to date because of the complex ethical issues involved. However, several reports have demonstrated that PBPC transplant can be used to overcome initial graft failure following allogeneic BMT [127–129].

A limited number of *de novo* UD PBPC transplants have also been reported. These have shown satisfactory engraftment, with no apparent increase in GvHD [130,131] although differing approaches were used with respect to GvHD prophylaxis. Demonstration of potential advantages for recipients will require increased numbers of procedures to be performed, possibly in the context of a randomized trial. At present, the numbers of such transplants are being restricted by donor agencies, although pilot studies are underway in Sweden and planned in the UK and other countries. In North America the NMDP will consider requests for PBPC donation as an alternative to a second bone-marrow harvest in the setting of initial graft failure.

With regard to donor ethics, the major concerns relate to possible short-term effects and worries about the theoretical long-term risks of G-CSF administration. However, at a recent consensus meeting on unrelated donor transplantation [37], a view was expressed that provided a full explanation was offered and informed consent obtained, a consistent approach should be used for both related and unrelated donors. Ultimately this may involve giving all donors the right to opt for either bone-

Table 26.9 Factor adversely affecting outcome of UD tranplntation

Factor*	Comments
Advanced recipient age	The EBMT UD guidelines recommend an upper age limit of 45 years. Many centres would argue that this can be increased to 55 years in selected cases.
Advanced disease state	This illustrated most clearly in CML with improved results in CP1 of less than 1 years duration. In acute leukaemia, outcome is substantially worse in patients with excess blasts at the time of transplant (especially >30% marrow blasts).
Donor-recipient HLA mismatch	Whilst some degree of mismatch is tolerable, evidence is accumulating that DRB1 mismatching has an adverse effect. DQB1 and class I molecular matching may also be important. A simplified hierarchy of matching importance may be: DRB1> DQB1>class I (A, B, C) though the full impact of class I molecular matching cannot be interpreted at present. Functional assays (CTLp, HTLp) require further evaluation.
Donor-recipient	Recipient cytomegalovirus (CMV) seropositivity is an adverse risk factor

CMV status	particularly if the donor is CMV-negative. A CMV-negative recipient should ideally receive a CMV-negative transplant if choice is available.
Donor-recipient sex mismatch	Sex mismatching (particularly female donor to male recipient) is an adverse factor in some reports.
Total nucleated cell dose	There is evidence emerging of improved outcome in patients receiving higher total marrow cell doses. This may support introduction of PBPC transplantation.
Stem-cell source	UD PBPC/umbilical cord blood transplantation may provide an alternative to BMT and early results are promising but further experience is required.
Donor age	Advanced donor age is an adverse factor in some reports.
T-cell depletion	This can have a profound effect on rates of GvHD but there is no clear evidence of improved overall survival due to increased rates of graft failure and relapse.
Pretransplant serotherapy	This reduces the rate of graft failure in TCD UD BMT.
Conditioning regime	Some reports have indicated adverse outcome with respect to chemotherapy-alone conditioning.

*Parameters with potentially a major effect on outcome are shown in italics.

marrow harvesting or PBPC collection on an individual basis.

Summary

Many clinicians involved in the field of transplantation believe that the toxicity of unrelated donor BMT is steadily being reduced, and that this modality of therapy should be offered to all patients who are considered to be candidates for allografting but lack a family matched donor. Fortunately, with the increasing size of donor registries a suitable unrelated donor can be found for the majority of Caucasian patients, although further recruitment of donors from ethnic minorities is urgently required.

Molecular typing is already established for the class II loci (DRB1, DQB1) and is currently being introduced for the class I loci (A, B, C). This will lead to increasing recognition of donor-recipient mismatches. It is hypothesized that certain mismatches will be more ‘permissible’ than others, leading to a strategy of ‘intelligent’ donor selection. There is some evidence to support this, for example DRB1 mismatches appear to be on average less well tolerated (with respect to GvHD) than DQB1 or class I mismatches. However, it remains to be seen whether specific amino acid differences within one HLA antigen locus will have reproducible or predictable clinical effects. Moreover, there are many other variables which may dictate transplant outcome other than donor-recipient HLA disparity. These are summarized in Table 26.9.

Donor selection is therefore a complex process, especially considering the time constraints implicit in this clinical area. Clinicians, advisers to the donor agencies and HLA scientists should continue to work together in order to identify the important factors which dictate unrelated donor transplant outcome.

Unrelated donor transplants from alternative stem-cell sources (umbilical cord blood, and growth factor mobilized peripheral blood) look set to continue increasing in numbers. However, ethical issues are not yet fully resolved, and it will be important to conduct comparative trials with bone-marrow transplantation. The use of post-transplant donor leukocytes, effective in the treatment of relapse of CML and some other leukaemias, is currently being introduced into the unrelated donor setting. It is important that studies are conducted exploring issues related to cell dose and schedules of infusion in order to minimize toxicity of this treatment.

Finally, we are aware that we have not discussed the economic aspects of unrelated donor transplantation, nor organizational matters concerning standards of care, centre accreditation, complete data registration and so on. In Europe, the EBMT is currently addressing all of these issues along with national organizations in individual countries.

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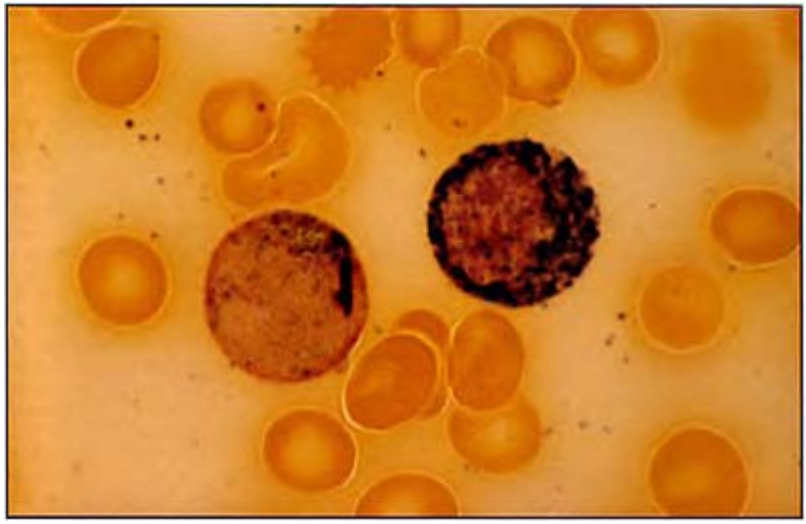

Chapter 27
Allogeneic related partially mismatched transplantation

P.Jean Henslee-Downey

Introduction

While allogeneic stem-cell transplantation (allo-SCT) represents a potentially curative therapeutic option and is considered the treatment of choice for an individual patient, there is only an approximately 25% chance that a human leukocyte antigen (HLA)-identical matched sibling donor (MSD) will be available. Therefore, the majority of patients who could benefit from allo-SCT require an alternative donor [1–5]. The prospect of using a haploidentical, partially mismatched related donor (PMRD), identified among family members, will be considered in this chapter and comparisons made to unrelated alternative donors who can sometimes be found in adult volunteer registries or from cord blood unit (CBU) banks. While availability and logistical differences between alternative donors can be defined, a clear preference in selecting between donor types for transplantation awaits prospective randomized trials.

The use of alternative donors compared to an MSD for allo-SCT involves various degrees of increasing genetic disparity defined by major and minor histocompatibility antigens that govern immunologic reactions between a host and donor. Immune responses

evoked by genetic disparities interfere with successful transplantation, often through impairment of engraftment [6–8], production of graft-versus-host disease (GvHD) [9–12] and/or prolonged disruption of the immune system [13–15]. In general, these complications are associated with higher rates of transplant-related mortality and have resulted in lower rates of long-term survival [7,11,16]. It is apparent that the successful use of genetically disparate donors will require alterations to the methods that have generally been employed to perform a transplant from an MSD. Technological and therapeutic advances which have affected the outcome following mismatched allogeneic transplants will be reviewed and discussed.

Donor compatibility and access

Genetics of histocompatibility

Histocompatibility between an allogeneic donor and recipient occurs through inheritance of identical 6th chromosomes which contain the major histo-compatibility complex (MHC), encoding the HLA specificities in man. These genes, whose products are polymorphic cell-surface molecules and serum factors, are responsible for immune responses through the creation of three major classes of antigens designated as classes I, II and III. The class I and II antigens, particularly alleles for HLA-A, -B, and -DR, are considered primary to immune reactions that govern organ and cellular transplant. The class I antigens (including HLA-A, -B, and -C) were initially known as 'transplantation antigens' for their role in activating $CD8^+$ cytotoxic T-cells responsible for rejection of foreign tissue [17]. The so-called 'immune response genes' produce class II antigens containing alpha- and beta-chains, which confer specificity for the HLA-D alleles (including HLA-DR, -DQ and -DP) [18]. These molecules activate $CD4^+$ T-lymphocytes (Figure 27.1) which have either helper or cytotoxic functions. Thus, HLA-restricted antigen presentation of immunogenic peptides, orchestrated through binding of class I and II molecules, to T-lymphocytes initiates cellular reactions responsible for rejection and/or GvHD [19] (Figure 27.2). Class III antigens correspond to components of the complement system. Although the class I and II major HLA antigens are considered prominent in evoking immune responses, a wide range of so-called 'minor' histocompatibility antigens are also felt to be operative in stem-cell transplantation [20–23]. The genetic information for minor histocompatibility antigens, some known and others still undetermined, is thought to be dispersed on many chromosomes in addition to those found within the MHC on the short arm of chromosome 6.

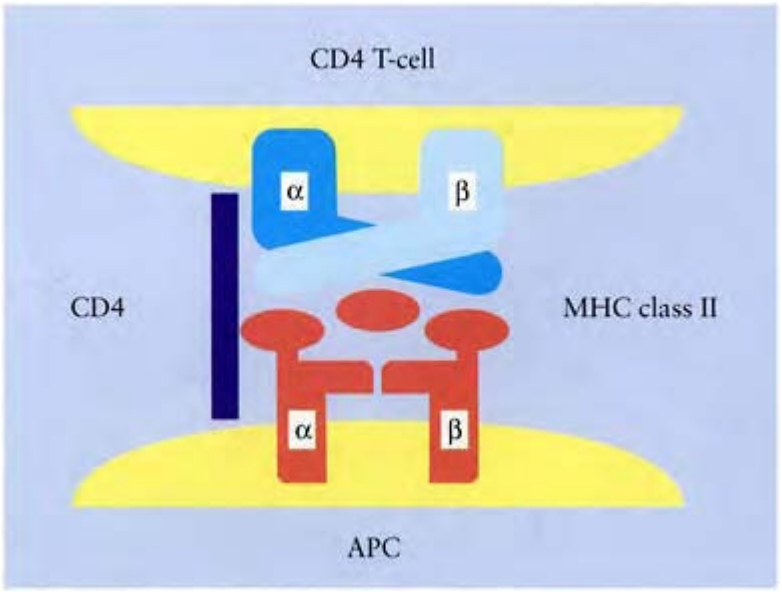

Figure 27.1 Schematic representation of a CD4$^+$ T-cell.

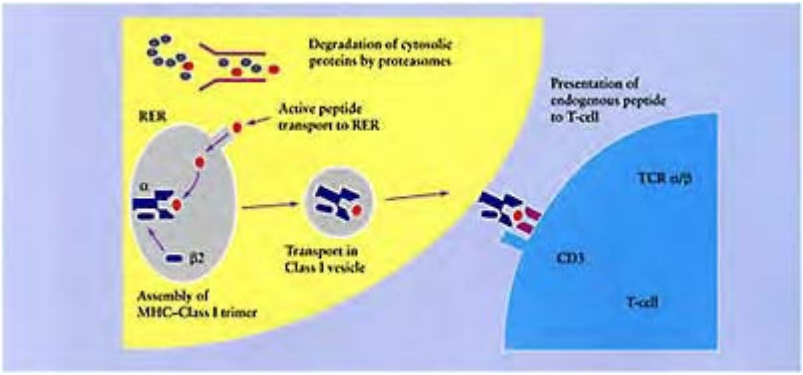

Figure 27.2 The class I antigen-processing pathway.

HLA allele identity is designated by numbers to denote specificities within each letter-designated locus (major HLA example: A1,30; B7,41; DR4,11). The MHC is inherited *en bloc*, and thereby HLA information is referred to as a haplotype [24]. Each individual has two HLA haplotypes, one from each parent. Given a constant parental pair, each offspring could inherit four distinct, sometimes full genotypically or partially phenotypically, similar haplotypes. In accordance with simple Mendelian laws governing

dominant genetic inheritance, each offspring will have a 25% chance of inheriting the same haplotypes as another offspring. There will be an approximate 75% chance for siblings to inherit one haplotype in common (genotypic haplo-identity) and a 25% chance to inherit no haplotype in common (complete mismatch). Unless a recombination event occurs, whereby genetic information crosses over from one chromosome to another, all parents and children will be haplo-identical with each other.

Genetic HLA haplo-identity can be found in many extended family members. When any parental pair or family member shares HLA antigenic specificities, additional phenotypic 'matching' of HLA antigens on the chromosome not inherited in common, or the appearance of homozygosity, which reduces disparity, can occur. This is similar to the sharing of phenotypic similarity for major HLA antigens between unrelated donor-recipient pairs that is referred to as 'matching' although it is not comparable to the genetic matching that occurs between a related donor-recipient pair as shown in Figure 27.3.

Definition of donor and host matching

A haplo-identical family member may share genotypic and phenotypic matching for major HLA antigens (6 of 6 match, i.e. zero-antigen mismatch) but usually the unshared haplotype is mismatched for one to all three major HLA antigens (5 of 6, i.e. one-antigen mismatch; 4 of 6, i.e. two-antigen mismatch; or 3 of 6, i.e. three-antigen mismatch). The number of major HLA-mismatched antigens in the patient (GvHD direction), as shown in Figure 27.3, rather than the number of mismatches in the donor (rejection direction), is conventionally used to define the degree of mismatch (zero- to three-antigen mismatch). For that reason, when a bidirectional descriptor is used, the recipient mismatch is given first. Recipient-donor pairs can thus be denoted based on bidirectional immunological barriers defined by the number of major HLA mismatches presented by the recipient followed by that found in the donor. This determination is helpful in the process of selecting the optimal donor for a patient.

Matching between unrelated individuals involves phenotypic sharing of major HLA only, primarily detected by serologic techniques, and is usually defined as a 6 of 6 or fewer match. However, with the use of sequence-specific oligonucleotide probes or DNA-sequencing techniques, serological (phenotypic) 'matched' antigens are often shown to be mismatched and these differences adversely affect clinical outcome [25,26]. In other words, the term 'match' is not a correct term when referring to histocompatibility between unrelated individuals. Techniques are not yet developed to measure non-major HLA and minor histocompatibility antigens, which are also most likely to be mismatched between unrelated persons.

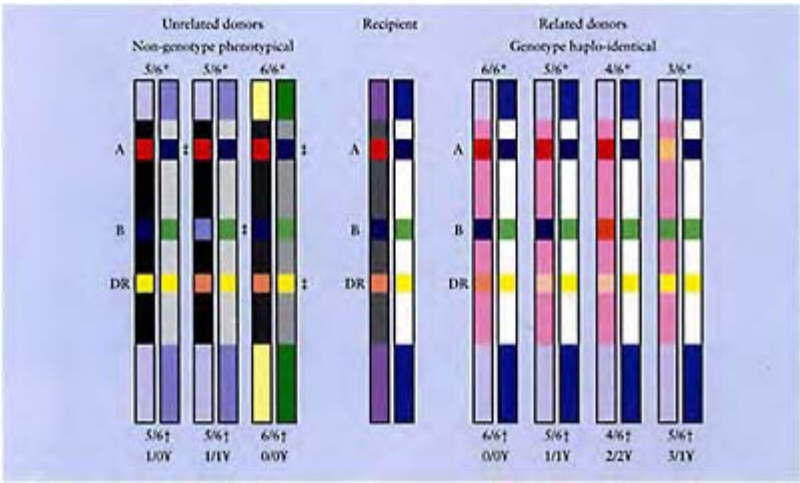

*Figure 27.3 Genetics of histocompatibility. *=Number of shared major HLA antigenic sites of the recipient (graft-versus host disease direction); ‡=molecular differences; †=number of shared major HLA antigenic sites of the donor (rejection direction); ¥=number of mismatched major HLA antigens in the GvHD vector (recipient) over the rejection vector (donor).*

Donor and host compatibility

Due to the existence of genetic haplo-identity and the possibility of inheriting HLA variants, MHC non-HLA, and minor histocompatibility antigens in common, mismatched family members may be more ‘compatible’ than ‘matched’ unrelated donors who are shown to be phenotypically similar for major HLA loci. The genetic probabilities of potential similarities between a recipient and various donors has been compared by Beatty and are shown in Table 27.1 [27]. We have developed a compatibility score that is assigned giving the greatest weight to the influence of major HLA antigens, moderate weight to minor histocompatibility antigens and less, but equal, weight to non-major HLA antigens [4]. This system illustrates that genetic inheritance will increase compatibility while known major HLA mismatch, in the absence of genetic chromosomal sharing, can result in profound incompatibilities between unrelated individuals.

Alternative donor access

The probability of finding allogeneic donors from family and unrelated donor pools is shown in Figure 27.4. Since only approximately 25% of patients will have a genotypic MSD, unrelated donor registries and

Table 27.1 Compatibility score of stem-cell donors

Donor type**	Major HLA	HLA variants	MHC non-HLA	Minor HLA	Compatibility score*
Syngeneic	100	100	100	100	8
MSD	100	100	100	50+	7–7.5
PMRD	50 to <100	50+	50+	50+	4–6
PMUD	<100	0+	0+	0+	3–5
PMMUD	<80	0+	0+	0+	2.2–4.2

*Compatibility score calculated by assigning a weight of four to the major HLA category, two to minor HLA categories and one to HLA variant and MHC non-HLA categories. Expressed as a range to show 25% upward adjustment for the potential for additional matching (indicated by +).
**Abbreviations: MSD=matched sibling donor; PMRD=partially mismatched related donor; PMUD=phenotypically matched unrelated donor; PMMUD=phenotypically mismatched unrelated donor.

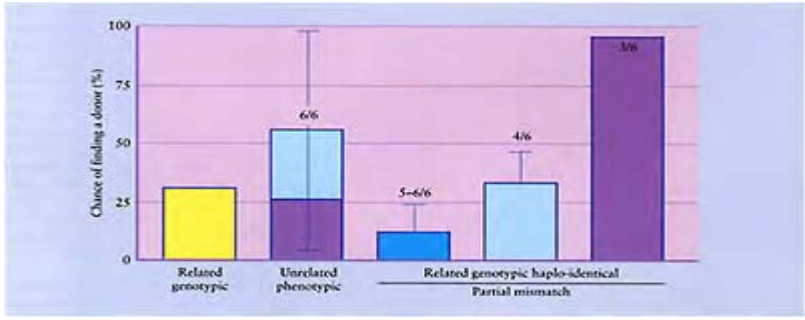

Figure 27.4 Donor availability. Coloured bars indicate general probabilities and extension bars indicate potential patient-specific probabilities. Darkened portion of bar for unrelated donor pool indicates current general probability of transplant.

cord blood banks have been developed as sources of alternative grafts [28,29]. The availability of a major HLA phenotypically matched unrelated donor (PMUD) can vary drastically amongst patients. It is dependent on the existence of all possible human HLA-haplotype combinations as found in those in need and those willing to donate, and thus ranges from zero to 100% [30]. Unfortunately, unless both patients and their donors are from a restricted population base, many patients, particularly from ethnic or racial minorities, are unlikely to find donors due to genetic preservation and vast disparity [31]. For some patients, many donors are available and for others, it will be impossible to identify a PMUD, regardless of the size of registries. In current practice, slightly over 25% of patients who have initiated a search for an unrelated donor have received this type of transplant. The use of molecular techniques to improve identity of major HLA loci between unrelated individuals in an effort to improve post-transplant outcome will, unfortunately, further decrease donor availability.

Greater donor accessibility might be possible using an unrelated CBU, particularly if increasing major HLA disparity is permissible due to a greater naivety of the immune cells obtained in this fashion. This will require demonstration that acceptable rates of stable engraftment can be achieved, as well as lower rates of GvHD that correlate with comparable rates of disease-free survival. As an advantage, cryopreserved CBU could be immediately available when needed, whereas the ability to obtain stem cells from unrelated volunteer donors can be considerably delayed or even disappear, although such a donor is initially identified in a registry.

To create immediate access to transplant, the most readily available donor is a genotypic haplo-identical family member. Almost every individual who can identify biological family members will have such a donor and thus, immediate accessibility is probably in excess of 95%. However, taking advantage of this almost universal donor pool has been limited in many centres by the acceptance of only zero- or one-antigen-mismatched genotypic haplo-identical related donors. Even if that criterion were adopted, there is evidence that such a donor is more available than the 10% likelihood that has often been estimated. Using a generalized probabilistic model, Kaufman predicted that there was an approximately 25% chance of finding a 6 of 6 PMRD through in-depth typing of family members beyond parents and siblings, including children, blood-related aunts/uncles, first cousins, first cousins once removed and nephews/nieces [32],

Of interest, parents of children with either acute lymphoid leukaemia or acute myeloid leukaemia have been shown to have greater sharing of HLA, especially at the DR locus, when compared to couples with healthy offspring [33,34]. This translates into an increased likelihood for such parents to be homozygous or phenotypically identical at the DR locus with their children. Under this circumstance, the probability of complete HLA-DR identity between a patient and either parent increases from <10% to 35 %, and the likelihood of HLA-DR identity between a patient and their siblings increases from 25 to 36%. Vowels and coworkers performed in-depth donor searches within the families of their patients, and in 46% a zero- or one-antigen PMRD was identified [35].

The ability to identify a two- or three-antigen PMRD is almost always possible when only first-degree relatives are typed, although occasionally, a closer match is found amongst second-degree relatives [32]. Clinical techniques have been developed to overcome major HLA haplodisparity between genotypic haplo-identical family members,

and thus donor availability no longer limits access to allo-SCT [36–40]. Usually family members are extremely willing donors and can be available for harvesting when needed.

Other considerations in choosing a donor

When multiple donors are available from an adult volunteer unrelated pool, clinical results indicate that high-resolution molecular typing should be employed to select the donor with the highest-resolved identity for major HLA loci [10,26,41]. The absence of cytotoxic donor T-cell activity between unrelated recipient-donor pairs has also been associated with reduction in post-transplant complications and mortality [42]. Marrow or T- and B-cell crossmatch studies should be performed and positive donors avoided. When multiple donors remain, consideration should be given to cytomegalovirus (CMV) status, age, sex and health of the donor. When selecting a CBU, information for consideration includes sterility, HLA and red blood cell typing, infectious disease testing and cell dose; however, clear recommendations await further study.

Often numerous donors are available from within a family, and prioritization of a choice of donor may change as additional information becomes available. Again, antibody crossmatch positivity is generally avoided, although immunomodulatory techniques have been successful in averting humoral rejection [43]. The degree of recipient major HLA mismatch (GvHD direction) has previously been an overriding consideration. However, based on current information, the donor with less major HLA mismatch (rejection direction) should be favoured (see Figure 27.3). Other considerations can also be weighed in the decision. For example, if the patient is CMV-seronegative, the CMV status of all potential related donors should be determined in favor of selection for CMV negativity. Whenever possible, a younger donor who is the same sex as the recipient is preferred and multiparous female donors avoided. Donor health or psychosocial consideration also plays a role in donor selection.

Clinical transplantation using alternative donors

Early complications

Engraftment and regimen-related toxicity

- **Engraftment**

  The most important consideration in an allogeneic major HLA partially mismatched SCT is avoidance of graft failure, the most lethal early complication [6,44,45]. When graft failure is an immunological event as a result of host-mediated rejection, it is extremely difficult to achieve secondary engraftment consistent with long-term survival [46,47]. Although the use of secondary conditioning therapy and haematopoietic growth factors can overcome rejection, death often occurs due to

infection, multi-organ failure, or severe acute GvHD, particularly if larger numbers of alloreactive lymphocytes are given in the secondary graft. When graft failure is a result of persistent malignant disease, death from refractory disease usually occurs. However, stable engraftment and long-term survival can be achieved if graft failure is a result of infection, or secondary to potential toxins by use of various therapeutic measures including haematopoietic growth factors [48]. Engraftment of allogeneic stem cells occurs as a result of an immunological competitive balance of challenge and competency between a host and donor where T-lymphocytes command a central role [49]. In general, higher graft-failure rates, influenced by transplant techniques, have been observed in alternative donor recipients and are shown to adversely affect survival [6,7,16,45]. Both host and donor immunoablation, achieved through pretransplant conditioning therapy and graft manipulation, modify immune responses influenced by histocompatibility disparities. These, ultimately, determine rejection or the establishment of a functional haematopoietic chimaeric state. Graft manipulation using methods of T-cell depletion (TCD), the details of which are described in Chapter 32, will be discussed conceptually in the context of developing complimentary therapy to ensure engraftment while achieving the aim of preventing GvHD.

- **Influence of conditioning therapy**

To enhance the success of engraftment, many investigators have increased host conditioning therapy, particularly when donor immunoablation by TCD is used [36–40,50–54]. While a higher dose of total body irradiation (TBI) and/or combination high-dose chemotherapies and immunosuppressants have resulted in improved engraftment in both animal and human studies, an increase in organ toxicity and infection rates can be observed. In addition, many of the clinical trials using PMRD have primarily included patients who are inherently at higher risk for toxicity and infection due to advanced or refractory disease for which they have received extensive therapy. It is, therefore, often difficult to assess a direct correlation between conditioning therapy and post bone-marrow transplant (BMT) mortality. Despite the combined use of high-dose TBI, chemotherapy and immunosuppression in a large series of TCD PMRD recipients, Henslee-Downey and coworkers observed a 7% risk of early (<60 days' post BMT) regimen-related mortality involving major organ failure, which occurred exclusively in high-risk patients [40].

In practice, TBI is more commonly used as part of the conditioning regimen for transplants using a PMRD compared to a MSD [16]. The total dose of TBI and the method of delivery have been associated with the success of achieving a full donor haematopoietic chimaera, particularly following TCD [6,40,54–58]. In some studies, additional total nodal irradiation has been shown to improve engraftment [59–61]. The use of TBI and cyclophosphamide as a backbone to conditioning therapy, combined with additional myeloablative drugs, e.g. thiotepa, dimethylmyleran and busulfan, or immunosuppressants, e.g. anti-T-cell antibodies or antithymocyte globulin, has also been helpful in improving engraftment of TCD marrow grafts [36,37,39,40,50–52,58,60–70]. Non-TBI busulfan-containing regimens have been associated with higher graft-failure rates after non-genotypically matched grafts [71]. However, Godder and coworkers have now demonstrated consistent engraftment using a non-TBI regimen combining busulfan, thiotepa, cyclophosphamide and methylprednisolone prior to partial TCD PMRD transplant [72,73]. Recently, Terenzi and coworkers suggested that the addition of a potent immunosuppressant, fludarabine, to the conditioning regimen may enhance engraftment of haplo-identical stem cells [74]. Early clinical studies have indicated a reduction in both the rate of rejection and regimen-related toxicity [75].

- **The influence of graft manipulation**

Clearly, there is an inverse relationship between the potency of TCD and the success of engraftment, particularly, using an HLA-mismatched graft [76]. Using highly effective methods of TCD such as agglutination with soybean lectin and E-rosetting, successful engraftment is possible in immunodeficient patients who inherently offer less graft resistance; however, graft failure can increase to up to 50% in patients with haematological malignancies or metabolic disorders [39,44,46,77–80]. Use of less rigorous methods of TCD, or T-lymphocyte subset depletion, resulting in a graft with a higher content of competent lymphocytes, has been associated with more consistent engraftment. However, this can be accompanied by increased rates of acute GvHD [39,81].

Based on the hypothesis drawn from animal work that a mega-dose of stem cells improves engraftment across HLA-barriers, Aversa and coworkers transplanted lectin-agglutinated and E-rosette TCD haplo-identical marrow and peripheral blood stem cells (PBSC), the latter

collected following recombinant human granulocyte colony-stimulating factor (rhG-CSF) stimulation [38,82]. Initial results indicated prompt engraftment but unfortunately, late episodes of rejection resulted in a >25% rate of graft failure [5]. These investigators are making attempts to further enrich the graft with primitive stem cells using methods for $CD34^+$ selection. This could potentially enhance donor tolerance and thereby facilitate HLA-mismatched engraftment [83]. A secondary benefit of $CD34^+$ selection may be further reduction in the number of mature $CD3^+$ lymphocytes which would help avoid acute GvHD. Although preliminary results are encouraging, rejection has occurred and additional study is required to determine the probability of graft failure and acute GvHD [5]. Severe acute GvHD has been seen by other investigators using this approach [84].

- **Other features associated with graft failure**

Greater HLA disparity, as well as crossmatch positivity, appears to be the most difficult deterrent to successful engraftment following all types of alternative donor marrow transplantation [6,8,16,39,40]. However, the ability to achieve consistent engraftment despite major HLA disparity is shown in a recent analysis of 210 genotypic haplo-identical recipients at the University of South Carolina as shown in Table 27.2 [85]. This suggests

Table 27.2 Differences in graft characteristics and clinical outcome following T-cell depletion with T10B9 versus OKT®3

Monoclonal antibody	Patients *(n)*	MNC (10^8/kg)	T-cell (10^4/kg)	Engraftment probability	aGvHD II–IV	III–IV
T10B9	100	1.45	6.77	0.91	0.21	0.11
OKT®3	110	0.87	4.27	0.97	0.32	0.16
P values		<0.001	<0.001	0.0001	0.38	0.56

Abbreviations: MNC=mononucleated cells, aGvHD=acute graft-versus-host disease.

that therapeutic measures can abolish a recipient's potent capabilities to respond to efficient antigenic presentation of donor-mismatched HLA. A salient caveat is the observation made by Bishop and coworkers, who showed an increased risk of graft failure following PMRD SCT in patients with chronic myelogenous leukaemia, particularly in those with advanced disease [90]. In their evaluation of PMRD graft rejection,

Lamb and coworkers also found the diagnosis of CML to be significantly associated with graft failure [91,92]. In their study, three-antigen major HLA mismatch in the donor was also associated with graft rejection but graft characteristics, including nucleated cell, T-lymphocyte, and colony-forming cells of the granulocyte-macrophage lineage (CFU-GM)/kg doses, were not. Study of peripheral blood from patients experiencing graft rejection revealed a predominance of $CD3^+CD8^+$ cytotoxic cells. These findings parallel those originating from experience with unrelated donors [93].

Graft failure is also increased following the use of CBU, particularly when obtained from a HLA-mismatched, related or unrelated, donor [86–89]. The mechanism of graft failure is less well understood following CBU transplant. However, there appears to be an association with lower cell dose [89].

Acute Graft-versus-Host Disease

Overview A high incidence of acute GvHD, particularly moderate or severe disease, which can exceed 70% and 50%, respectively, has been described following transplant from alternative marrow donors and is considered a major impediment to successful transplantation [4,7,9–12,16,39,94–99]. When compared to the use of a MSD, the difference in acute GvHD rates can be completely eliminated through the use of highly efficient TCD which can reduce the incidence to ~10%; however, as previously discussed, this can be at the expense of successful engraftment [100,101]. In pooled analysis of alternative donor transplantation, TCD is the only factor that appears to decrease the probability of moderate-to-severe acute GvHD [7,12,96]. The use of TCD has resulted in inferior disease-free survival in recipients of MSD transplants primarily due to higher relapse rates. This is thought to be due to an interference with a graft-versus-leukaemia (GvL) effect [102]. This remains controversial in T-depleted alternative donor transplantation [99,103–105].

The development of acute GvHD is of greater concern following alternative donor transplants as the disease is more difficult to manage and more often becomes refractory to therapy, and is hence associated with a higher risk of complications and death [16,106,107]. Effective management appears to require prompt institution of lympholytic, as well as broad-spectrum immunosuppressive therapy, which can be difficult to discontinue [108–110]. At the University of South Carolina, PMRD recipients are treated for early clinical manifestations of acute GvHD. In addition to on-going immunosuppressive therapy, pulse, high-dose methylprednisolone therapy is given at a dose of 500 mg/m^2 recipient weight every 12 hours for two doses and repeated in 48–72 hours if symptoms persist. Such prompt intervention often appears to abrogate the evolution of acute GvHD.

Use of T-cell depletion The dose of alloreactive T-lymphocytes in a SCT is directly correlated with the development of GvHD [111–113]. Thus, to balance engraftment and

GvHD control, new strategies have involved the use of less rigorous methods of TCD combined with post-transplant immunosuppression [3,36,37,39,40,50,52,66,67,69,70,72,73,76,81,84,85, 90,104,114–120]. Combining T-cell lytic therapies aimed at donor lymphocytes *ex vivo* and *in vivo,* used in sequence, Henslee-Downey and coworkers demonstrated that engraftment could be achieved in over 90% of patients while significantly reducing the risk of grades II–IV acute GvHD to <40% and grades III–IV to <20% [39]. In a matched cohort study of patients with advanced acute lymphoblastic leukaemia, Fleming and coworkers, using similar techniques, demonstrated that recipients of PMRD versus MSD SCT experienced comparable rates of engraftment (91% versus 94%, respectively) and grades 0-II and III–IV acute GvHD (71% versus 73% and 29% versus 27%, respectively) [119].

A subsequent clinical trial, shown in Figure 27.5, was conducted at the University of South Carolina to re-evaluate the anti-αβ heterodimer monoclonal antibody, T10B9, for *ex vivo* TCD. This was now combined with a Food & Drug Agency (FDA)-approved lymphocyte-specific anti-sera (Atgam®)

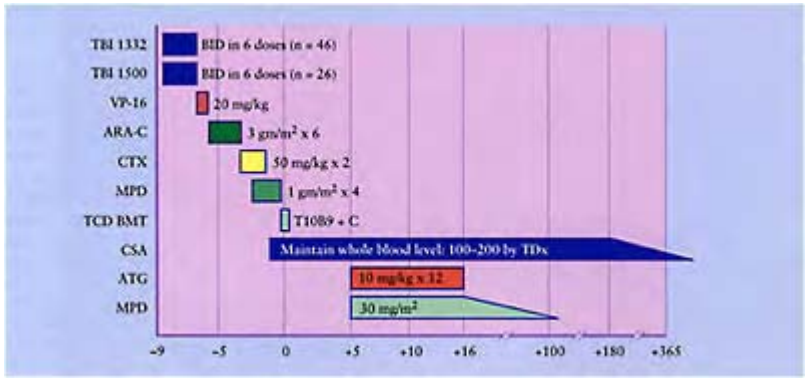

Figure 27.5 PMRD clinical protocol. Abbreviations: TBI=total body irradiation; BID=twice daily; VP-16=etoposide; Ara-C=cytosine arabinoside; CTX=cyclophosphamide; MPD=methylprednisolone; TCD BMT=T-cell-depleted bone-marrow transplant; C=complement; CSA=cyclosporin; ATG=antithymocyte globulin.

given after moderate-dose methylprednisolone for *in vivo* T-depletion [40,120]. Previously shown to be additive in successful transplantation of full haplodisparate marrow grafts, low-dose cyclosporin was also added to the post-transplant treatment protocol [37]. Sixteen percent of engrafted patients developed grades II–IV acute GvHD;

8% developed grades III–IV disease. In a multivariate analysis, the only significant prognostic factor for an increase in acute GvHD was a higher graft T-cell dose and no correlation was seen with the degree of major HLA mismatch [40]. Other investigators have used T10B9 for *ex vivo* TCD of unrelated donor grafts and reduced the risk of grades II–IV acute GvHD to between 33 and 46% [104,114,115].

In an ongoing trial at the University of South Carolina, an FDA-approved anti-CD3 monoclonal antibody, Orthoclone (OKT®3), was substituted for *ex vivo* TCD and the dose of TBI reduced to 1400 cGy with the addition of antithymocyte globulin (Atgam®) to the conditioning regimen (Figure 27.6) [70]. As shown in Table 27.3, successful engraftment was significantly improved and the incidence of acute GvHD remained low [85]. The majority of patients in these studies were major HLA two- and three-antigen mismatched. Taken together, current results indicate that techniques are available to effectively control acute GvHD following haplo-identical PMRD SCT. Questions remain as to whether T-cell-specific therapy is more beneficial when utilized *ex vivo*, *in vivo* or combined [40,116]. Further research in animal and human studies indicates that more potent immunosuppressant agents, e.g. trimetrexate and rapamycin, or the use of ultraviolet irradiation could be effective in modulating lymphocyte reactivity to prevent severe GvHD and facilitate tolerance across histocompatibility barriers [121–123]. The challenge of the future will be designing the least toxic but, probably, multifaceted approach which will abrogate GvHD while allowing expansion of GvL reactivity.

Other features associated with acute GvHD The use of molecular typing to more closely match major HLA antigens between unrelated recipient-donor pairs has been associated with a reduction in the incidence of acute GvHD [42,124]. Transplant techniques that are effective in preventing acute GvHD appear to obscure a correlation with HLA mismatch [40] that is often observed with less effective prophylaxis [10,97–99]. Dissimilar to MSD SCT, a younger recipient age does

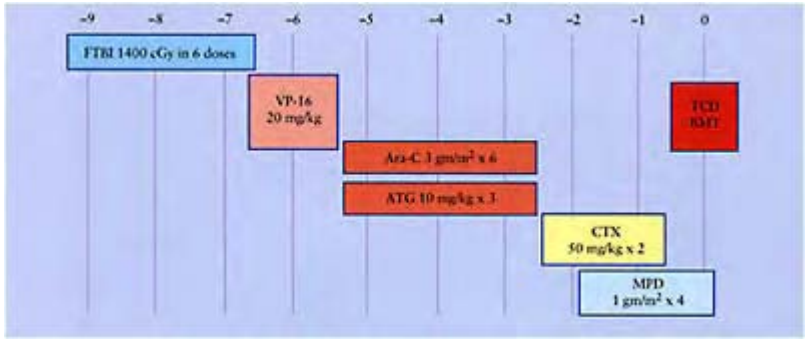

Figure 27.6 Enhanced immunoablative conditioning regimen. Abbreviations: FTBI=fractionated total body irradiation; VP-16=etoposide; Ara-C=cytosine arabinoside; ATG=antithymocyte globulin;

CTX=cyclophosphamide; MPD=methylprednisolone; TCD BMT=T-cell-depleted bone-marrow transplant.

not appear to protect children from acute GvHD following alternative donor SCT [96,97]. In adult patients, older age of either recipient or donor and advanced disease stage appear to increase the risk of GvHD [99]. Similar to MSD SCT, severe regimen-related toxicity and infection are thought to play a role in GvHD development.

There has been great optimism that the use of CBU would be associated with significantly lower rates of acute GvHD. Using post-transplant immunosuppression, this has been true using MSD CBU [86]. However, acute GvHD still appears to have an association with HLA mismatching, particularly in recipients of mismatched unrelated CBU [87–89]. The development of severe and fatal acute GvHD has occurred. Further study is required to understand the probabilities of moderate-to-severe acute GvHD following mismatched CBU SCT. If additional GvHD prophylaxis is required, it may be difficult to effectively modify a CBU, and the use of more aggressive post-transplant immunosuppression may result in higher rates of fatal complications.

Comprehensive approach to PMRD BMT

In considering primary immune-mediated early complications, the best results from PMRD trials with long-term follow-up show high rates (≥90%) of stable engraftment achieved concurrent with low rates (<20%) of moderate-to-severe GvHD [40,85]. This has been achieved using sequential immunomodulation as follows:

- A broad conditioning regimen containing TBI and combination chemotherapy coupled to immuno-suppressant agents;
- An approximate 2 log TCD of marrow alone;
- Post-transplant immunosuppression [39,40,70,119].

These studies are instructive in our understanding of the therapeutic goals for treatment of the pre-BMT patient, the graft, and the post-transplant chimeric recipient as outlined in Figure 27.7. Therapeutic modalities that may be employed to achieve specific goals require careful balance. There can be significant interactive dependency between therapies so that slight alterations in one portion of the treatment plan can result in significant changes to major post-transplant clinical endpoints.

Infection and lymphoproliferative disorders

Overview of life-threatening infection Fatal opportunistic infections, primarily of viral or fungal aetiology, are often encountered in recipients of T-replete and T-depleted alternative donor transplants [3,4,7,13,14,125–133]. While such recipients tend to have advanced disease and, due to heavy pretreatment with antineoplastic agents, are extraordinarily exposed,

Table 27.3 Comparison of major transplant endpoints using matched siblings and alternative stem-cell donors

Authors/ Year	Reference	Patients *(n)*	Engraftment			Acute GvHD			Chronic GvHD			Low-risk disease-free survival		High-risk disease-free survival	
			TCR	TCD	MTD	TCR	TCD	MTD	TCR	TCD	MTD	2 years	>3 years	2 years	>3 years
Matched sibling donor															
Szydlo *et al.* 1997	[16]	1224	99			29			42				66		12
Mitus *et al.* 1995	[203]	23	–			–			–				62		
Brown *et al.* 1995	[204]	40	–			48			48						29
Lynch *et al.* 1995	[205]	56	–			48			47						21
Rowlings *et al.* 1994	[184]	1640	–		–			–				50		18	
Range				99			29–48			42–48			50–66		12–29
Partially mismatched related donor															
Szydlo *et al.* 1997	[16]	340			84–91			44–56			52–60		25–33		15–22
Beatty *et al.* 1985	[9]	105	81			70			–				~37*		
Ash *et al.* 1991	[7]	470			89			41–45			44				
Henslee-Downey *et al.* 1996	[39]	40		93			36				–		40**		
Fleming *et al.* 1996	[119]	32		91			–				–				~38
Henslee-Downey *et al.* 1997	[40]	72		88			16				54	53		23	
Range				81–93			16–70			52–60			25–53		15–38
Phenotypically															

matched unrelated donor																		
Szydlo *et al.* 1997	[16]	491			91			54–63			62–73			26–41			11–17	
Beatty *et al.* 1993	[10]	112	~95			78–94			61–74			–			–			
Kernan *et al.* 1993	[12]	462			86–94			64			55	40					19	
Drobyski *et al.* 1994	[104]	48		94			40				74	52			46			
Davies *et al.* 1995	[206]	211						64			53			37*			30*	
Balduzzi *et al.* 1995	[97]	88	93			83–98			74					47–56			21–30	
McGlave *et al.* 1996	[99]	1000			85				–			54	40				1–21	
Range				85–95			40–98			53–74			26–56			1–46		
Cord blood unit																		
Wagner *et al.* 1995	[86]	44	85			3			6			46†						
Kurtzberg *et al.* 1996	[87]	25	88			43			–			48†						
Gluckman *et al.* 1996	[89]	87	49***			21–60			12			56			8			
Range				49–88			3–60		6–12				46–56			8		

Abbreviations: TCR=T cell replete, TCD=T-cell depleted, MTD=mixed T-cell dose, –=not available.
*Only survival data available.
** Event-free survival (includes non-malignancies).
***Refers to full donor chimaerism.

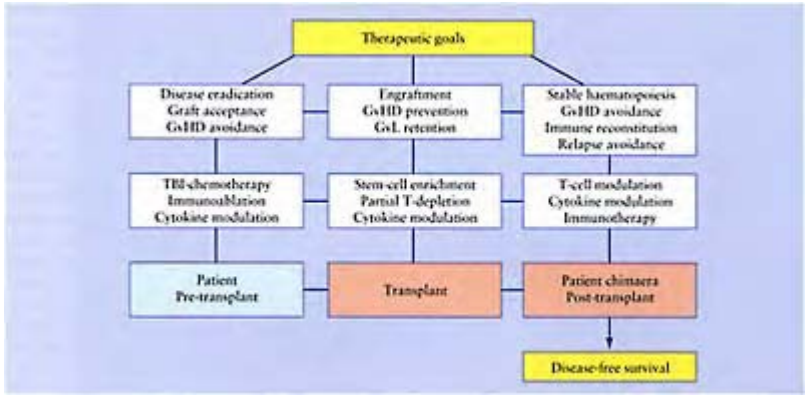

Figure 27.7 Crossing histocompatibility barriers using sequential immunomodulation.

so that they are often thought to harbour life-threatening infectious agents, they also appear to be more vulnerable to succumbing to opportunistic infections than MSD recipients. This may be explained by slow and incomplete immune reconstitution which is hampered by both ineffective antigen processing and intensive, as well as long-term immunosuppressive therapy [134]. Furthermore, it is common for alternative donor-recipients to have multiple co-existing infections which can display multidrug resistance. For this reason, these patients require careful infection surveillance, prophylaxis, and prompt institution of highly effective, broad-spectrum therapeutic agents.

Evaluation of infection Surveillance cultures for bacteria, virus and fungus should be obtained from blood, nasopharyngyl mucosa, stool and urine prior to transplant and at least weekly until engraftment is established. The sensitivity of polymerase chain reaction (PCR) techniques to identify infectious agents may be too exquisite to be meaningful for surveillance, but should be utilized in diagnostic endeavours. Precise radiological inspection of sinus, lung and major organs should also be performed prior to transplant. Continued chest surveillance is recommended weekly during hospitalization, and biweekly-to-monthly throughout post-transplant steroid administration; sinuses and abdominal surveillance should be performed as indicated. Aggressive diagnostic measures are always indicated, although empirical therapeutics remains critical to successful management of life-threatening infections. When opportunistic infection is suspected or proven, prompt resection of fungus and broad-spectrum therapeutic coverage, including high-titre intravenous immunoglobulins, may improve survival [135–139].

Prophylaxis of infection Respecting the limitation of therapeutic measures, prophylaxis against invasive opportunistic infections is mandatory. Prophylactic agents of choice are partly dictated by pretransplant history, physical, culture and serological surveillance. Injury to gastrointestinal mucosa increases the need for oral and gut mucosal decontamination often achieved with both local and systemic therapies. In addition to antiseptic rinse, Peridex, nystatin, Diflucan, non-absorbable antibiotics and

quinolones, many investigators use local or systemic prophylactic amphotericin-B for high-risk patients [140–143]. Godder and coworkers have shown that amphotericin can be administered in an Intralipid emulsion and safely given at a prophylactic dose of 0.5 mg/kg recipient weight without interference with the use of other nephrotoxics, e.g. acyclovir, cyclosporin and aminoglycosides [144]. When treating known invasive fungal infections, the ability to increase drug delivery of amphotericin-B to doses that can exceed 5 mg/kg recipient weight daily is now possible with amphotericin-lipid complex products that are less likely to cause renal failure [145–148].

While administration of broad-spectrum antibiotics may be disadvantageous in producing drug resistance and allowing fungal overgrowth, it is essential in order to avoid the rapid physiological decline that can result from generalized bacterial sepsis, particularly when absolute neutropenia, acute gastrointestinal injury or central line contamination significantly increase the risk for bacterial dissemination. Whenever a central line is suspected as a source of infection, all ports should be utilized in a rotating pattern for administration of antibacterial drugs. In the case of serious bacterial or fungal infections, haematopoietic growth factors are often used to enhance neutrophil recovery and macrophage function when they are not being utilized prophylactically [149–151].

Acyclovir remains the choice for prophylaxis of herpes simplex. However, ganciclovir is preferred for cytomegalovirus (CMV), particularly if invasive disease is evident [152]. When drug resistance is suspected, foscarnet is often chosen as second-line therapy. Although the use of intravenous immunoglobulin therapy, primarily for multiviral prophylaxis, is controversial in MSD or autologous transplantation, it is still felt to be additive in the protection of high-risk alternative donor recipients. Exciting new approaches to the control of virus replication have involved the production of virus-specific lymphocyte clones produced by *ex vivo* clonal expansion of donor lymphocytes exposed to viral antigens [153–155].

Virus-associated lymphoproliferative disorder Polyclonal and monoclonal lymphoproliferative disorders (LPD), thought to be induced through uncontrolled B-cell stimulation by the Epstein-Barr virus (EBV), are seen at a higher frequency in allo-SCT recipients when TCD is used for GvHD prophylaxis, an HLA-mismatched or unrelated donor is used, and when patients require intensive immunosuppressive therapy for acute or chronic GvHD management [156–159]. A polyclonal proliferation may behave more like an infectious disease and respond to aggressive antiviral therapy, including antiviral drugs, intravenous immunoglobulin and interferon, when given promptly in combination with a reduction in immunosuppressive therapy. In contrast, a monoclonal proliferation may behave more like a malignancy and become rapidly fatal. The development of EBV-associated LPD appears to be associated with profound deficiency in EBV-specific T-cell-mediated immunity which can be overcome by infusion of donor leukocytes [160,161]. This approach has been used to prevent as well as treat EBV-associated disease using EBV-specific cytotoxic T-cells that are prepared from donor cells collected during marrow harvest [162,163].

Of interest, Chiang and coworkers performed a retrospective review of 183 consecutive PMRD recipients who had experienced a modest rate (<20%) of greater than or equal to grade II acute GvHD and found an unexpected low incidence (3.3%) of LPD despite the use of TCD combined with administration of antithymocyte globulin [164]. The absence of GvHD activity in these patients, which avoided the use of intensive

immunosuppressive therapy, may have also avoided physiological factors that favour B-cell proliferation. Moreover, it was encouraging that half of the patients who did develop EBV LPD were surviving long-term following reduction of low-dose immunosuppression and administration of aggressive antiviral therapy.

Late complications

Chronic Graft-versus-Host Disease, late infections, and relapse

Features and therapy of chronic GvHD

In general, concomitant with an increased risk of acute GvHD, there is an increased risk of chronic GvHD following alternative donor transplantation [10,13,16,96,104,114,115,131]. While some data suggest a correlation to degree of mismatch, other studies have seen no association [10,16,40,97, 98,114,115,124]. Although Nademanee and coworkers could effectively control acute GvHD using T-replete unrelated donors who were DRB molecular matches, the incidence of chronic GvHD remained high (78%) and the majority of patients developed extensive disease [124]. The incidence of chronic GvHD appears to be higher following PMUD compared to PMRD SCT which may simply be a function of TCD, more frequently used in the later group. In a comparative matched cohort study, Fleming and coworkers showed a significant reduction in extensive chronic GvHD in recipients of T-depleted PMRD grafts compared to T-replete MSD grafts (11% versus 29%, respectively; P=0.005) [119]. In recipients who received similar GvHD prophylaxis following PMRD SCT, Henslee-Downey and coworkers found that the only significant prognostic factor that increased the relative risk of chronic GvHD was a higher total dose of TBI. In their study, only 10% of patients developed extensive disease [40]. The same TCD method using PMUD grafts also resulted in lower rates of severe chronic GvHD [104,115].

Chronic GvHD causes significant morbidity and mortality, particularly following non-genotypic and mismatched allo-SCT [13,14,97,98.133]. It is more likely to be progressive in nature, and more extensive and resistant to therapy [13]. However, the presentation of chronic GvHD may be limited to features that represent dysfunctional and/or incomplete donor tolerance. For example, the development of autoimmune antibodies may lead to haemolytic anaemia and/or thrombocytopenia [165]. Early recognition and prompt institution of therapy may help avoid a persistent auto-antibody production. Of great concern is the extensive, severe fibrotic changes that can occur in skin, muscle, joint, pulmonary, hepatic and renal systems severely diminishing the value of rendering a patient disease-free [166,167].

Little progress has been made in the therapy of chronic GvHD, particularly following alternative donor SCT. Delay in therapy may result in poorer response and refractoriness over time. Initial ‘induction therapy’ using high-dose lympholytic drugs may increase the success of subsequent ‘maintenance therapy’ using combination immunosuppressive agents. Long-term therapy is usually required and must be tapered very slowly [168].

When re-emergence of symptoms occurs during reduction or after discontinuation of immunosuppression, a repeat of induction and maintenance therapy is usually required. New approaches to management are needed to avoid the untoward side-effects of long-term steroid therapy. Although further study is required, the use of thalidomide, clofazimine, hydroxychloroquine, mycophenolate mofetil, etretinate, and extracorporeal chemophotopheresis are promising approaches with reduced side-effect profiles [169–175].

Late infections and delayed immune reconstitution

Over and beyond the correlation between chronic GvHD and late infection, the likelihood of developing late opportunistic infection is higher after alternative donor SCT [13,14,131,176]. Functional asplenia, T-and B-lymphocyte abnormalities and hypogamma-globulinaemia contribute to the increased risk of infection. Long-term infection prophylaxis, including penicillin, trimethoprim-sulfamethoxazole, acyclovir and immunoglobulin replacement, are recommended [13,139,177]. However, it is possible that prolonged use of antiviral therapy could interfere with the recovery of viral-specific T-cell response [178,179]. Restoration of virus-specific immunity by transfer of T-cell clones from donors has been previously discussed and appears promising [153–155,180]. However, if HLA-mismatched donor lymphocytes are to be used for this purpose, it will be necessary to demonstrate that significant GvHD does not occur. Vaccination may not be helpful until patients can be successfully weaned from immunosuppressive therapy.

Development of chronic GvHD further delays the significant lag in immune reconstitution seen after alternative donor SCT. In general, recovery of a normal number and distribution of lymphocytes occurs beyond one year following transplant and functional studies do not begin to return to pretransplant levels for over two years [15,181]. Also, alternative donor recipients who have received TCD marrow grafts generally demonstrate an early appearance of a predominant population of $CD56^+$ natural killer (NK) cells and a low T-lymphocyte count with an inverted CD4:CD8 ratio. There is little evidence of T-cell activation indicated by minimal, less than 10%, CD25 expression. Restoration of a normal CD4: CD8 ratio and recovery of $CD20^+$ B-cells is delayed beyond one year (see also chapter on TCD). Kook and coworkers [15] showed an association between younger recipient age and higher T-lymphocyte counts post-transplant. In their study there was no association between marrow-cell doses and hastened lymphocyte recovery. As expected, the development of GvHD or its treatment significantly delayed immune reconstitution.

Recurrent leukaemia

The higher incidence of chronic GvHD may benefit some patients, particularly those with chronic myelogenous leukaemia (CML) or advanced acute leukaemia where there is evidence of an associated antileukaemic effect [102,182]. This may be the best explanation for the lower relapse rate often ascribed to alternative donor SCT, even in patients who receive T-depleted grafts [16,95,96,99,104,105,123]. In chronic-phase CML where GvL effects are most beneficial, the use of an PMUD graft produced a 3% five-year estimate of relapse and was independently correlated with lower relapse compared to

the use of a MSD graft [183]. The use of TCD may block this effect [99]. In a pooled analysis of MSD compared to alternative donor SCT, Szydlo and coworkers found a higher relapse rate in CML patients associated with the use of increasingly mismatched PMRD grafts that appeared to be reduced by the use of a PMUD graft. However, patients with acute leukaemia appeared to have a significant reduction in relapse with an increasingly mismatched PMRD graft as well as PMUD graft [16]. Though difficult to understand, the risk of relapse appeared to be increased in PMRD recipients who were transplanted in early disease status and the inverse was the case for advanced disease. It is almost impossible to draw conclusions from these observations since most patients were referred for transplant prior to 1990 when use of mismatched alternative donors was not readily accepted and, therefore, disease stage alone may not have determined significant differences in patients. Most patients referred for PMRD SCT in early disease status tend to have multiple poor prognostic features.

In published studies with long-term follow-up, relapse rates have not been higher following TCD PMRD SCT when compared to published rates in large series of similar patients who received MSD SCT [40,119,184]. In TCD PMRD recipients, Henslee-Downey and coworkers found that the risk of relapse was significantly correlated to the disease status at time of transplant. Low-risk patients had an estimated 21% risk of relapse compared to a 58% risk in high-risk patients (P=0.03). These results are similar to the relapse rates reported in a large series of MSD recipients from the International Bone Marrow Transplant Registry, where relapse rates ranged from 20 to 28% for low-risk and 59 to 77% for high-risk patients [40,184].

For patients who relapse after SCT, the use of donor leukocyte infusions (DLI) has become a promising new therapy [185,186]. This approach may be even more beneficial when given post-SCT prior to relapse [187]. The prophylactic use of MSD DLI is supported by evidence that a GvL effect can be achieved without significant GvHD [188]. Successful remission induction without severe GvHD was also seen after administration of PMRD DLI [189]. This initial experience and the observations made by Johnson and Truitt, demonstrating that delayed (>21 days) infusion of spleen cells could produce long-term antileukaemic effect without GvHD in an MHC-haplotype-mismatched transplant model, led us to explore the pre-emptive use of PMRD in high-risk patients using a dose-seeking trial beginning in the second month post-SCT [190,191]. Compared to an historical control group, a significant increase in GvHD occurred that was associated with an increasing total DLI dose. A GvL effect could not be separated from GvHD and there was no difference in the probability of relapse in the two groups. Although there was no significant difference in disease-free survival, there was a trend toward worse outcome in patients given DLI. Taken together, extreme caution is warranted in the use of HLA-mismatched DLI unless the alloresponsiveness can be modified. If lymphocytes obtained from donor peripheral blood can be programmed to express recipient tumour-specific reactivity and/or mutated so that reactivity can be terminated, their use may be feasible and an important strategy in preventing relapse post-SCT.

A second transplant is another approach used in patients who relapse after SCT. The Seattle Transplant Team reviewed their experience using the same donor for second transplant and found a high rate (70%) of relapse and poor disease-free survival (14%); outcome improved by the presence of acute GvHD [192]. As expected, higher rates of

regimen-related toxicity and early mortality were also seen. Godder and coworkers showed that PMRD SCT could also be successful salvage therapy when used for second transplant in relapsed patients [72]. Particularly in patients with acute myelogenous leukaemia (AML), many who had relapsed after autologous SCT, these investigators showed a 52% probability of disease-free survival at 16 months with follow-up that extended to three years [73]. The fact that these patients were all in refractory relapse at time of transplant suggests a potent GvL effect.

Quality of survivorship

Studies on survivors of SCT, including recipients of PMRD grafts, demonstrate that patients experience difficulties in a variety of domains and that recovery of physical, occupational, emotional and cognitive functioning appears to plateau within several years post-transplant [193–195]. Global self-assessment questionnaires indicate that survivors experience emotional distress, chronic physical symptoms, low self-esteem and less than optimal life satisfaction. In general, functional status worsens with increasing recipient age. Patients over the age of 30 years reported feeling and appearing less well than those under 30 years. Older patients were more likely to report that their lives were disrupted by pain and nausea and that they could not maintain usual recreation and leisure activities.

Of interest, SCT recipients studied at two centres did not suffer from an inferior quality of life when compared to renal transplant patients [196]. In this study, quality of life was shown to be adversely affected by a lower recipient education level, an older recipient age and a higher dose of TBI. In a subsequent study, TBI-related cognitive impairment was shown to involve slowed reaction time, reduced attention and concentration and difficulties in reasoning and problem-solving [197]. However, it is difficult to know the extent to which neuropsychological impairment is related to the transplant procedure itself or to previous exposure to various potentially neurotoxic agents or events prior to SCT. In fact, Andrykowski and coworkers demonstrated that neuropsychological impairment in adult patients studied prior to SCT was strongly associated with patient disease and treatment history [198]. The risk of impairment increased as the number of risk factors (i.e. history of cranial radiation, prophylactic intrathecal chemotherapy, CNS disease and intrathecal chemotherapy, and high-dose cytosine arabinoside) in a patient increased. These investigators subsequently studied the physical and psychosocial status of patients prior to, and one or more years following, SCT [199]. In the study group as a whole, no significant differences were found in the number of physical symptoms or various measures of psychological and functional status. Paired analysis of specific variables indicated an increase in blurred vision, a superior vigour and a poorer sexual relationship. In addition, there was a trend toward superior functional quality of life at the follow-up assessment. Analysis of interpatient variability examining residual change scores indicated that significant differences did occur in some patients. Factors that predicted for detrements in both physical and psychosocial status included older age and male gender. An extended, multicentre study in 200 recipients confirmed variability in post-SCT quality of life with deficits most often seen in physical, sexual and occupational functioning which, contrary to previous findings, improved over time [200]. Older age, lower level of education and more advanced disease were risk

factors predictive for poorer outcome. Although not expected, factors that did not predict quality of life included disease diagnosis, TBI dose, chronic GvHD, type of GvHD prophylaxis and extent of marrow graft match.

A better understanding of the quality of survivorship should be helpful to the process of informed consent required of patients and families finalizing a decision to proceed with SCT. In an effort to understand expectations for returning to normal, 172 survivors were studied in five transplant centres [201]. A minority of patients considered themselves to have 'returned to normal' and few expected such. Of interest, survivors' evaluations of their decision to proceed with SCT were generally quite positive. Bryant and coworkers recommend a proactive approach to the psychosocial management of SCT candidates that appears to enhance the decision-making process, allowing patients to refuse or proceed with transplant. This process and on-going management, using anticipatory intervention, aids in the psychosocial adjustment of patients and their families [202].

Comparison of major transplant endpoints: engraftment, GvHD and disease-free survival

In the absence of prospective randomized trials, comparison of relevant post-transplant outcomes can be compiled from data published with substantive analysis. A composite of these data is summarized in Table 27.3, showing the average probability of engraftment, grades II–IV acute GvHD, and all chronic GvHD according to whether study patients received T-cell replete (TCR), T-cell depleted (TCD) or both, i.e. mixed T-cell dose (MTD) grafts, and the average probability of disease-free survival at two years or for three years or more in low-risk and high-risk patients.

Within a donor group or between groups, variability in the average outcome for a given endpoint may be the function of varying transplant techniques and/or status of recipients. Engraftment appears less successful following alternative donor transplants, potentially worst with the use of CBU, followed by PMRD, then PMUD, and best after MSD. Acute GvHD appears higher following the use of alternative donor marrow, potentially highest with the use of PMUD, followed by PMRD, then MSD, and lowest after CBU, particularly if unrelated CBU is excluded. Chronic GvHD appears higher following the use of alternative donor marrow, potentially highest with the use of PMUD, followed by PMRD, then MSD, and clearly least after CBU. Disease-free survival in low-risk patients tends to be worse following alternative donor SCT, without an apparent difference between types of alternative donor grafts; high-risk patients do poorly regardless of donor type.

Although specific conclusions cannot be drawn, certain trends, described in Table 27.4, are apparent. These observations indicate the importance of genetic histocompatibility that is not defined by major HLA antigens alone. In fact, recent data that show no differences in outcomes using two or three major HLA mismatched haplo-identical family donors are further confirmation of this principle [40,119]. In general, results obtained using grafts derived from PMRD compare favourably with those obtained from other types of alternative donors.

Advantages of using haplo-identical related donors

There are compelling reasons to chose a family member to serve as a SCT donor when patients do not have a matched sibling and are not appropriate candidates for autologous transplant. These are primarily related to three major areas of concern: donor availability, graft engineering and cost efficiency. How a family donor can be beneficial in solving these problems is summarized in Table 27.5.

Conclusion

Developing technology has made all forms of alternative donors acceptable for SCT. With rare exceptions, every patient should have almost immediate access to a donor. Since disease status is the single most important factor predictive of outcome, universal donor availability should allow SCT to be used earlier in the disease course when benefit is greatest. Based on accumulating data, patients should be offered transplant with the most readily available alternative donor. Despite risk involved with alternative donor transplant, Lee and coworkers developed a model to evaluate the quality adjusted, discounted, survival for patients with CML who could choose to undergo transplant using a PMUD graft and showed that the choice to proceed with transplant was superior to no transplant, particularly when transplant was performed within the first year of diagnosis [207].

Future prospective, randomized trials are needed to assist in donor selection when more than one type of alternative donor is available. Recommendations for

Table 27.4 Correlation between donor type and clinical outcome

Major endpoint	Favourable ranking by donor
Engraftment	MSD>PMUD>PMRD>CBU
Acute GvHD	MRCBU<MSD<PMRD<PMUD
Chronic GvHD	CBU<MSD<PMRD<PMUD
Disease-free survival	MSD>Alternative donor

Abbreviations: MSD=matched sibling donor; PMUD=phenotypically matched unrelated donor; PMRD=partially mismatched related donor; CBU=cord blood unit; MRCBU =matched related cord blood unit.

Table 27.5 Advantages using a family donor

Donor availability
• A family member is immediately available
• A family donor is equally available to all racial and ethnic groups
• Often able to select donor based on age, sex, parity and infection status

- Able to perform a full medical evaluation of the donor
- Able to obtain additional donor cells for immunotherapy

Cost efficiency

- Minimal additional HLA typing required
- Cost of stem-cell graft is the same as from a matched sibling
- No additional cost related to a donor search
- Avoids registry and/or banking expenditures

Graft engineering

- Ability to perform stem harvest directly from donor
- Ability to control number of stem cells obtained
- Ablility to alter cell content and/or cellular function to enhance transplant outcome

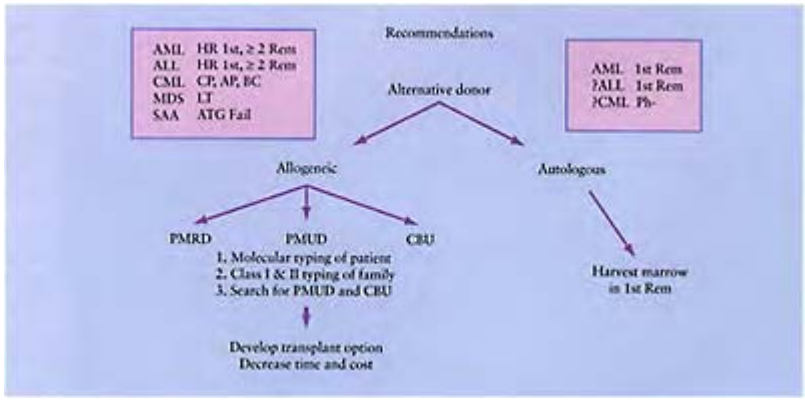

Figure 27.8 Recommendations for patients without matched siblings. Abbreviations: AML=acute myelogenous leukaemia; HR=high-risk; Rem=remission; ALL=acute lymphoblastic leukaemia; CML=chronic myelogenous leukaemia; MDS=myelodysplastic syndrome; LT=leukaemic transformation; SAA=severe aplastic anaemia; ATG= antithymocyte globulin; Fail=failure; Ph^-=Philadelphia chromosome-

negative; PMRD=partially mismatched related donor; PMUD=phenotypically matched unrelated donor; CBU=cord blood unit.

developing a transplant option in patients without a MSD should prioritize consideration of the diagnosis, disease status, time to transplant and cost, as indicated in Figure 27.8.

On-going progress in technology and clinical management should provide the basis for continued improvement following the use of alternative donors. This will enable every patient in need to develop access to potentially curative therapy. At this time, there is no justification for delaying or denying transplant when a haploidentical family member is a willing stem-cell donor.

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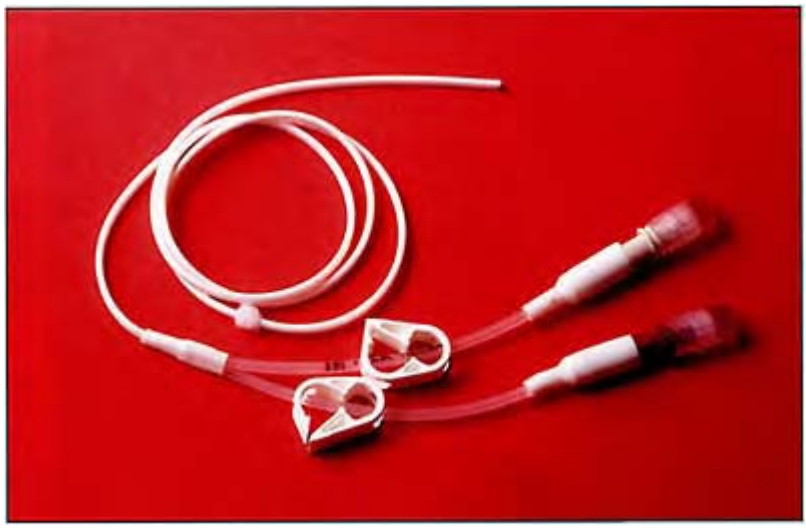

Chapter 28
Venous access

Clair Nightingale and Jacqueline Filshie

Introduction

Patients undergoing bone-marrow transplantation (BMT) require reliable venous access since their survival depends on the use of prolonged and intensive intravenous therapy. Vascular access presents a physical and psychological problem for these patients, many of whom describe the number of venepunctures endured as the most memorable and unpleasant part of their hospital stay [1]. Table 28.1 lists indications for reliable venous access in patients undergoing BMT. Venous access is needed initially for high-dose cytoreductive therapy and subsequently for reinfusion of harvested marrow. Fluid and electrolyte infusions are required to replace gastrointestinal losses following total body irradiation (TBI) or high-dose chemotherapy. Complications of high-dose cytoreductive therapy include mucositis, alterations in taste perception and nausea and vomiting, often necessitating nutritional support in the form of total parenteral nutrition for one to two months following transplantation [2]. A more recent indication for venous access is for peripheral blood stem-cell (PBSC) harvesting prior to treatment. Following treatment,

neutropenic patients frequently become febrile and require antibacterial and antifungal therapy until the pyrexia and neutropenia resolve [3]. Pancytopenia usually persists for two to five weeks post-transplant

Table 28.1 Indications for reliable venous access

High-dose chemotherapy
Marrow transfusion following total body irradiation/high-dose chemotherapy
Fluid and electrolyte replacement
Nutritional support
Peripheral blood stem-cell harvesting
Antimicrobial therapy
Marrow support with red cells and platelet transfusions
Blood sampling
Treatment of graft-versus-host disease

requiring support with red blood cell and platelet transfusions [4], Indwelling venous access allows repeated, painless blood sampling which is necessary to monitor the progress of therapy during BMT. Finally, when engraftment has occurred, the patient may still need intravenous support should graft-versus-host disease (GvHD) develop.

Venous access is particularly challenging in patients undergoing BMT as they have often received extensive treatment beforehand and may have few accessible peripheral veins [2,5]. The morbidity associated with central venous cannulation may be further increased in this patient population by thrombocytopenia and dehydration. Available forms of venous access comprise:

- Peripheral venous cannulation
- Arteriovenous fistulae
- Non-tunnelled central venous catheters
- Implantable devices
- Tunnelled central venous catheters.

Venous access for peripheral blood stem-cell harvesting

Peripheral blood stem-cell harvesting is usually undertaken on an outpatient basis and may require several (3–13) collections over a period of 3 to 14 days [6]. Separate venous outflow and return ports are required, either by using two separate vascular access sites or a double lumen catheter with separated outflow and return ports. Flow through a cannula is proportional to the fourth power of the cannula radius, and inversely proportional to its length [7]. Since the outflow catheter is required to conduct typical blood flows of 70–90 ml/min, this is achieved by using a large lumen (e.g. 14 French) short catheter which is

relatively rigid to prevent it abutting against the vessel wall. Commonly used sites for access are the basilic and brachiocephalic veins of the antecubital fossa, the internal and external jugular, the subclavian or the femoral veins. On account of insufficient flow, an indwelling central venous catheter is usually unsuitable for use as the outflow port, but can usually be used for blood return. A variety of routes have been described for small children requiring PBSC harvesting [8].

Complications of venous access include occlusion, thrombosis and infection. Goldberg *et al.* experienced catheter occlusion requiring thrombolytic treatment or replacement of catheter in 15.9% of PBSC harvests, but more with 13.5 French Davol than with smaller 11.5 French Vascath soft-cell catheters; 85 % of occluded catheters were unblocked using urokinase [6]. Catheter thrombosis was not related to the underlying diagnosis (haematological malignancy or breast cancer), the stem-cell mobilization protocol or the number of collections required [6]. Hahn *et al.* [9] reported a 5% incidence of thrombosis in internal or external jugular placement of large-bore central venous catheters in a series of 183 insertions. Haire *et al.* [10] used a translumbar inferior vena caval route together with aspirin prophylaxis against thrombosis in 31 patients and achieved a low thrombosis rate of 3%.

An important consideration in choosing the route of venous access in these patients is whether to renew the cannula for each collection or to allow the patient home with the cannula *in situ;* to some extent this is dependent on hospital policy and on the patient's attitude and capability.

Peripheral venous cannulation

This is the standard form of venous access in the hospital population, and at the Royal Marsden NHS Trust it is the most commonly used access for PBSC harvesting. The main problems are limited vessel size, short catheter lifespan, venous thrombosis, risk of extravasation of irritant infusates and unsuitability for injection of drugs requiring central venous administration.

Arteriovenous fistulae and grafts

Arteriovenous fistulae, used with success in patients undergoing dialysis, have been used in the past for vascular access in patients with malignant diseases. The advantages were low infection rates, and no maintenance requirement when not in use. Forearm fistulae were often hampered by previous peripheral chemotherapy resulting in sclerosed veins which were unable to dilate. In addition, the hypercoagulability associated with malignancy meant the thrombosis rate was unacceptably high. High-flow polytetrafluoro-ethylene (PTFE) arteriovenous shunts have been placed between the brachial artery and axillary vein in the upper arm, or between the femoral artery and vein in the upper thigh. Due to high blood flow allowing rapid dilution of chemotherapy, the durability of PTFE and its stability in the presence of infection, these grafts were reasonably successful (24% associated with treatable complications, 17% losing their function) [1]. The technical expertise and time required to insert and revise these shunts, and the development of more suitable alternatives renders them unsuitable now for the large number of patients requiring intravenous access.

Central venous catheters

The majority of bone-marrow recipients rely on indwelling central venous catheters situated in the great veins or right atrium, and placed percutaneously via the subclavian, external or internal jugular or femoral veins. A translumbar approach to the inferior vena cava has also been described for Hickman line placement [11]. The site of insertion often depends on operator preference. If the patient has a coagulopathy, the jugular or femoral veins offer a safer approach as any haemorrhage can be stopped by local compression. The jugular route is less popular for tunnelled lines because flexibility of the neck causes increased movement of the catheter within the tunnel. Drugs infused into central veins are quickly diluted by the large volume of circulating blood, reducing the chance of local adverse effects. The lines may be tunnelled and may have a subcutaneous injection port. They may have single, double or triple lumens. Catheters are usually made of polyurethane (Vialon, Tecoflex) which is relatively rigid, fluoroethylene (Teflon) or silicone (Silastic), which is the most flexible. The different materials may be associated with differing rates of thrombosis, but rates of *in vivo* bacterial colonization are the same [12]. The choice of catheter depends on the type and duration of use, whether it is to be used in the out-patient setting, the number of lumens required and patient acceptability. In a randomized prospective study comparing tunnelled and non-tunnelled central lines in a variety of patients with solid and haematological tumours, the rates of sepsis and thrombosis were similar [13]. Differences between the lines lay in the way they were used: non-tunnelled lines were removed after each course of treatment, while the tunnelled line was retained until the end of therapy. However, the multiple subclavian venepunctures used for the non-tunnelled lines were associated with a 4% rate of pneumothorax, compared with none in the tunnelled group [13]. Keohane *et al.* showed in a prospective study a higher incidence of infection in non-tunnelled lines (11.5%) compared with tunnelled lines (28%) until a dedicated nurse was employed to look after the lines; thereafter the infection rate fell to 4% for both groups [14]. Single-lumen catheters last longer than double-lumen ones, although this may reflect the greater severity of illness for which double-lumen catheters are employed [15]. Smaller (2.2 mm) dual-lumen catheters may be associated with a lower complication rate than larger (4.5 mm) dual-lumen ones [1]. Non-tunnelled central venous catheters made of silicone rather than polyurethane, inserted via either the antecubital fossa or subclavian vein, have been shown to have longevity and complication rates comparable with tunnelled silicone catheters and they offer the advantages of being cheaper and easier to insert [16].

Further advantages and disadvantages of each catheter type are considered below.

Non-tunnelled central venous catheters

Non-tunnelled catheters are usually made from polyurethane and may be impregnated with an antiseptic or antibacterial agent. Insertion is relatively straightforward, and may be performed in the ward using local anaesthesia. Large-lumen catheters (12 to 14 French gauge) may be used where high blood flows are required. It is recommended that these more rigid catheters are placed with their tips superior to the cephalic limit of the pericardial reflection to avoid the risk of erosion of the catheter through the vessel into the pericardial sac with resulting cardiac tamponade [17]. Non-tunnelled catheters are

preferably confined to the in-patient setting, and usually only survive for a limited period, resulting in multiple central venepunctures if access is needed for prolonged therapy. In intensive-care patients, the incidences of infection and thrombosis increase the longer the non-tunnelled catheter is *in situ* [18,19]. It is possible that the stiff materials used in manufacture contribute to thrombus formation by causing intimal damage [20]. Thrombus may then form a nidus for infection, in much the same way that abnormal heart valves predispose to endocarditis [21]; this results in higher rates of infection for thrombosed lines [3,19]. Polyurethane, non-tunnelled lines are mainly used where the need is short-lived, for example, for patients receiving autografts or for PBSC harvesting when peripheral access is limited.

Implantable devices

These consist of a subcutaneous reservoir with a silicone rubber diaphragm attached to a tunnelled central venous catheter [15]. Such devices were first used in the early 1980s in an effort to decrease the risk of infection and increase patient acceptance [22,23]. Access is via a Huber-tipped needle, which cuts a slit through the skin and silicone diaphragm rather than taking a core, allowing more punctures without leakage. (Figure 28.1). The advantages are reduced incidence of infection compared with Hickman catheters [24,25], increased patient acceptability as the port is hidden, no need for routine dressing changes and that these lines only need flushing every two to four weeks. Some difficulties may be experienced with these devices: insertion in a thrombocytopenic patient may result in more bleeding than the insertion of a Hickman catheter [15]; likewise, access via skin puncture carries more risk of causing bleeding. The risk of needle dislodgement and extravasation is a possibility, and there is often a limit to the rate of fluid infusion [26]. The incidence of thrombosis is similar to that seen with Hickman catheters. Most of these devices are single lumen only. They are better suited to the out-patient who needs limited access for short periods of time, than to patients who need prolonged, intensive support.

Long-term tunnelled lines

These catheters are made of polymeric silicone rubber impregnated with barium to render them radio-opaque. They range from single to triple lumen and a Dacron cuff is attached to the catheter to help secure the line and obliterate the subcutaneous tunnel by stimulating fibroblastic ingrowth. Their use was first described by Broviac *et al.* in 1973 in Seattle for the artificial gut programme [27]. Use of the device for BMT was reported in 1976 [28]. The original 1.0-mm internal-diameter single-lumen line was referred to as the Broviac catheter. This was then modified by Hickman and his colleagues, who enlarged the lumen to 1.6-mm internal diameter and Raaf subsequently developed the multi-lumen catheters which carry his name [29]. There is now a wide variety of catheters available.

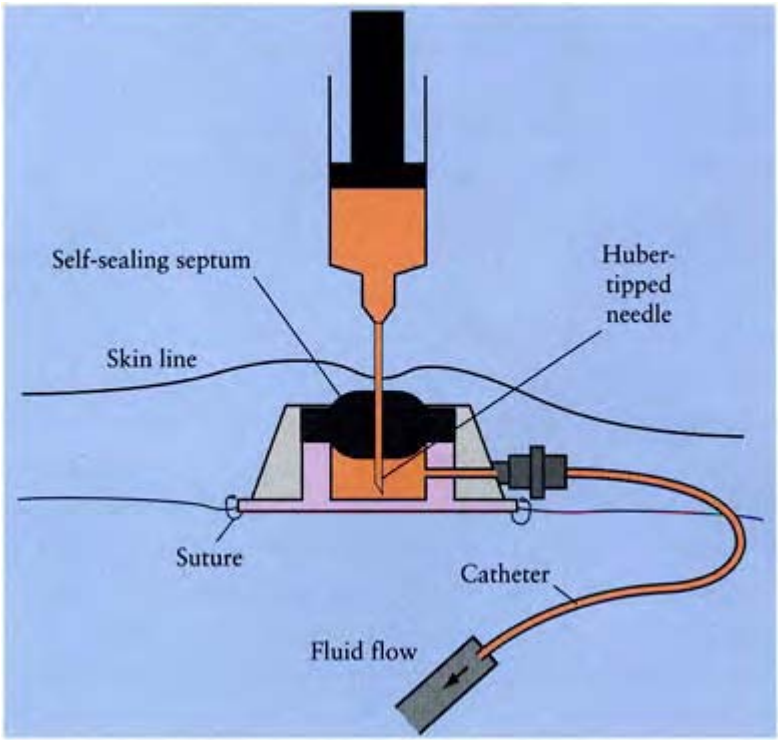

Figure 28.1 Diagrammatic representation of an implantable device.

Silicone rubber is an inert, antithrombogenic flexible material. Flexibility is thought to be important when sclerosant infusions are used since it allows the catheter to move with each heartbeat, thus minimizing contact with any single endothelial area [27]. The insertion guidelines for Raaf catheters recommend placement of the tip within the right atrium. The multiple lumens permit a dedicated parenteral nutrition lumen or simultaneous administration of incompatible infusions, the blood flow in the right atrium allowing sufficient dilution of the infusates to prevent interaction. The subcutaneous Dacron cuff serves to anchor the catheter; its role in preventing infection is less clear [30,31] although it was shown to decrease the incidence of peritonitis when used with peritoneal dialysis catheters [27]. The cuff does seem to limit exit site infections [3,26,27,32] unless they occur within one to two weeks of placement before there is sufficient fibroblastic ingrowth into the cuff.

Insertion of tunnelled catheters

Insertion of Hickman catheters should ideally be performed by experienced personnel, since this reduces both per- and post-operative complications [3,13,26,32]. Insertion may

be performed by surgeons, anaesthetists, physicians, radiologists or specialist nurses, depending on local policy. Either an open venous cut-down or a percutaneous technique, with or without imaging guidance, is used. In comparisons of percutaneous and surgical cut-down techniques, the former have shorter operating times and higher success rates for placement, with no difference in overall complication rate [33]. Using the percutaneous approach, the vein is conserved for future use, whereas surgical cut-down may result in obliteration of the vein [34]. Surgical cut-down may be necessary in patients with haemostatic problems [30,35], severe lung disease [30,34], or an inability to tolerate the head-down position [30]. Surgical cut-down is also the preferred technique in children below the age of four or five years, as the stiff dilators used in the percutaneous method can result in vessel perforation.

Preoperative assessment of all patients is essential. A chest radiograph should be taken to identify any mediastinal mass or obvious distortion that might make placement difficult. A coagulation screen and platelet count should be performed. Patients with suspected distorted venous anatomy or previous central vein thrombosis should be investigated with venography and ultrasonography. Most contra-indications to insertion are relative, and are influenced by the method and site of insertion. Disseminated intravascular coagulation need not be considered an absolute contraindication [32]; insertion has been performed using careful cut-down and heparin to control the condition [36]. In thrombocytopenic patients, percutaneous subclavian line placement is possible with platelet support. It is recommended that 6 to 8 units of platelets are given if the platelet count is less than 50×10^9/litre, or the bleeding time is prolonged [30]. If an open technique is used, platelets may be infused intra- or post-operatively if required to control haemorrhage [29]. Post-operatively, pressure dressings are a wise precaution to minimize tunnel haematomas. Uncontrolled bacteraemia or infection at the proposed site of insertion are contraindications, although fever, neutropenia and infection at other sites are generally not [3]. The trend is now towards subclavicular insertion into the subclavian vein, using a percutaneous Seldinger technique. This employs a split sheath introducer, adapted from pacemaker insertion sets [34,37,38].

Technique of percutaneous insertion of Hickman-type catheters

At the Royal Marsden Trust, insertion is carried out in the operating theatre under aseptic conditions using fluoroscopic imaging. Electrocardiographic (ECG) monitoring is used during placement to detect arrhythmias. Children receive a general anaesthetic, while the majority of adults are sedated and receive supplementary analgesia. Opinions differ concerning the value of prophylactic antibiotics [39]. We routinely use an anti-staphylococcal regimen. Patients are placed in a head-down position, the skin is prepared on both sides with 0.5% chlorhexidine in spirit or povidone-iodine from the chin to below the nipples. Sterile drapes are then applied.

Local anaesthetic (30 ml 0.25% bupivacaine with 1 in 200000 adrenaline) is used to anaesthetize the skin at the proposed subclavian entry site, the exit site over the fourth or fifth intercostal space between the nipple and sternum, and along a tract between the two (Figure 28.2). The subclavian vein is entered with an 18-gauge needle, through which a J-wire is introduced. The skin entry site should be one finger's breadth below and lateral to

the junction of the medial and middle thirds of the clavicle. If the approach is too medial, it may result in sharp angulation and kinking of the introducer sheath below the clavicle [37,39,40]. It may also lead to the catheter being pinched between the clavicle and the first rib, possibly resulting in malfunction or breakage [15,24]. If the approach is too

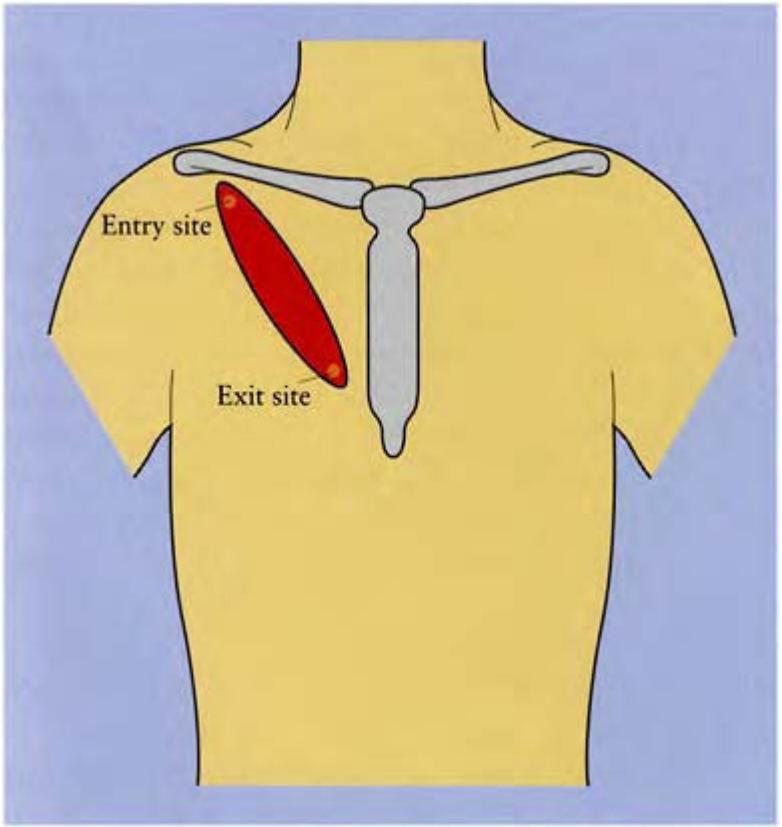

Figure 28.2 The area infiltrated by local anaesthetic, showing the entry and exit sites of the catheter.

lateral, the introducing sheath may be left short of the superior vena cava and difficulty may be experienced directing the catheter.

The guidewire should be advanced into the superior vena cava while observing the ECG monitor for ectopic beats. If these occur the wire should be withdrawn slightly. The position is checked with fluoroscopy. A small (5 mm) incision is made at the proposed catheter exit point and around the guidewire at the entry site to the vein. The catheter is tunnelled from the exit site to the vein entry site. The tunnel can be created using a variety of forceps [31,38,40] or an introducer to which the catheter is attached and pulled through the subcutaneous tissues [34,35,37]. We use a Redivac drain introducer (Figure

28.3). The tunnelling is facilitated by the use of a large volume of local anaesthetic which demarcates the tissue planes. In women with pendulous breasts, the tunnel should be kept as medial as possible in the immobile tissues near the sternum. This will prevent catheter retraction secondary to falling of the breasts when the woman assumes the upright posture [41]. It should be noted that the perforating branches of the internal mammary artery are at risk in this region if the tunnel is too deep.

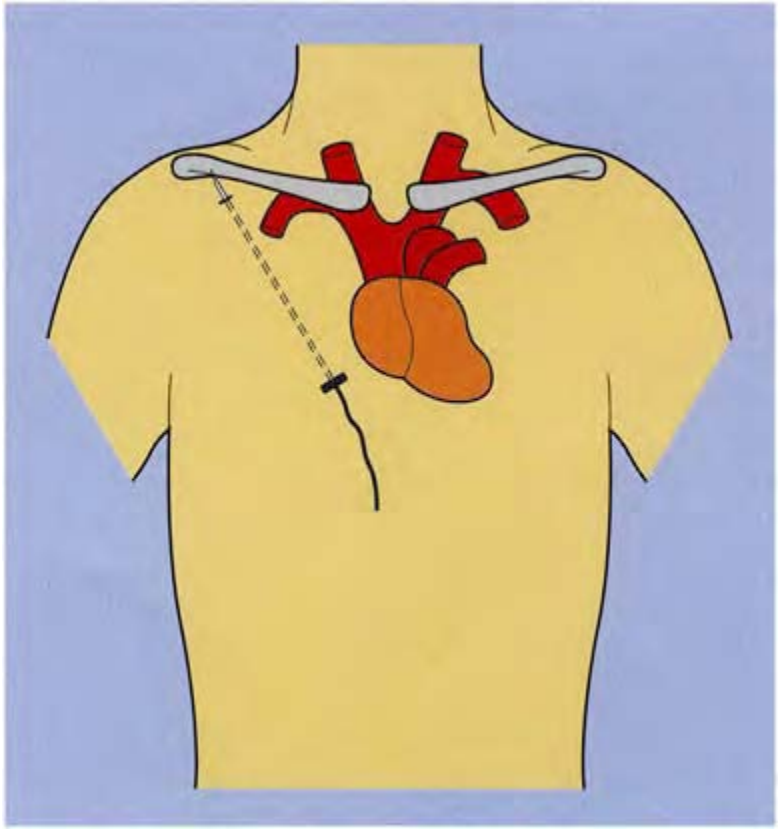

Figure 28.3 The line is attached to an introducer and pulled through the subcutaneous tissue.

The cuff is positioned in the tunnel at least 2 cm from the exit site, to reduce the risk of cuff extrusion [37,42] or infection [43]. The catheter is cut to the appropriate length for the tip to lie in the right atrium, and is primed with heparinized saline. The dilator and sheath are threaded over the guidewire into the vein. The guidewire and dilator are removed and the catheter is introduced into the right atrium through the sheath. The risk of air embolism is minimized by the trendelenberg tilt and asking the patient to hold his breath. Passage of the catheter through the sheath is eased by prior lubrication with liquid

paraffin [24]. When the catheter is correctly positioned, the sheath is removed by splitting the sides apart, while the position of the catheter is carefully maintained (Figures 28.4 and 28.5).

After confirming that blood is easily aspirable from each lumen and that the catheter is correctly placed on fluoroscopy, the two incisions are closed with a stitch to each. The exit site stitch is extended to secure the catheter and is left in place for three weeks to allow time for an adequate fibroblastic response to the Dacron cuff [27]. A post-insertion chest radiograph is taken to confirm position and checked specifically for pneumothorax.

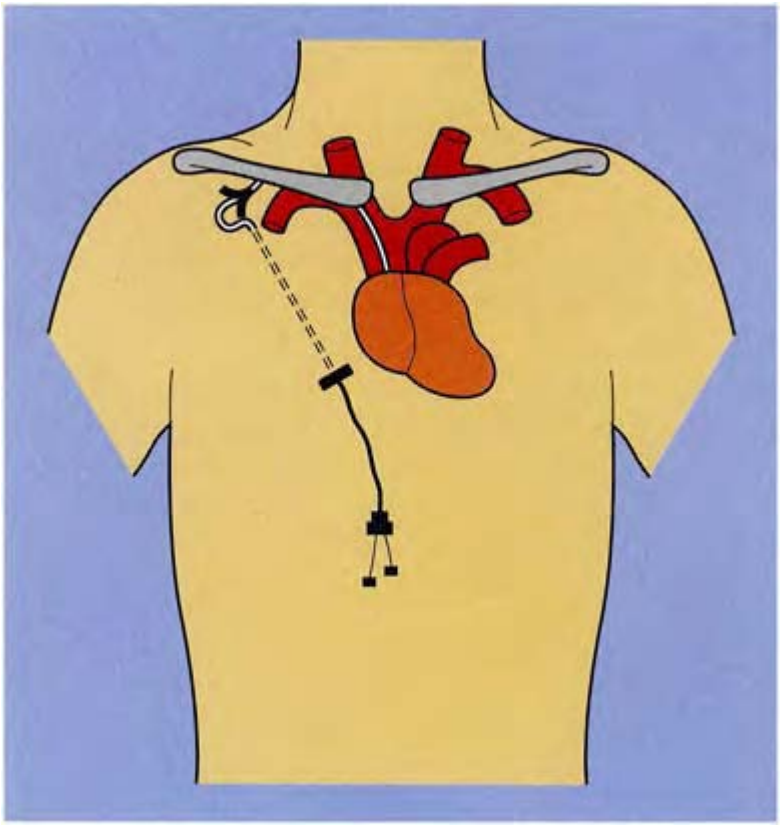

Figure 28.4 The sheath is carefully separated from the line by pulling the sides gently apart.

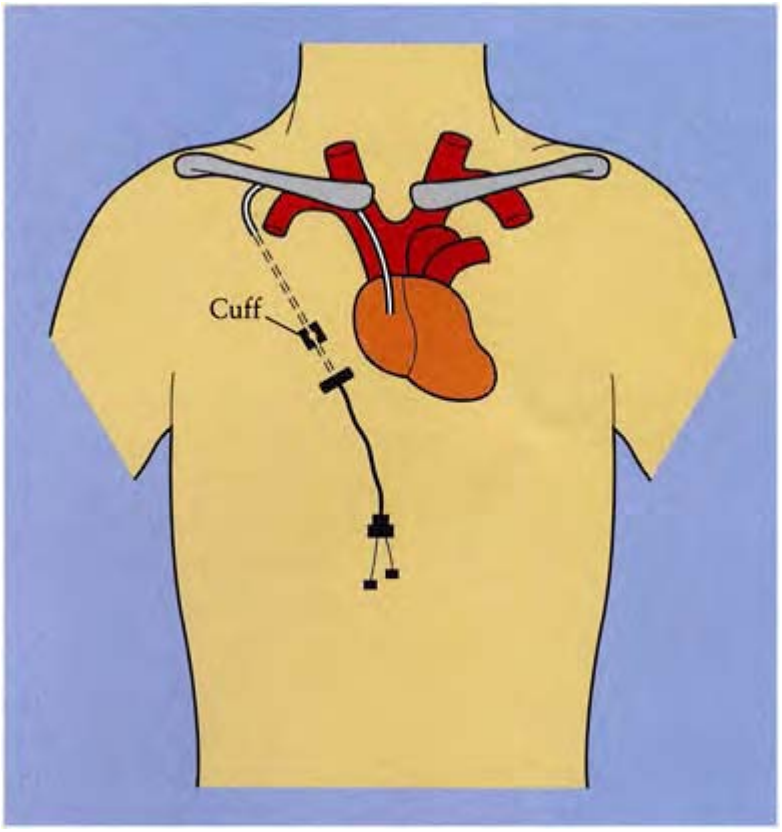

Figure 28.5 The Hickman line in its final position.

Catheter maintenance

Meticulous catheter maintenance is the key to maintaining function and avoiding complications [14,44,45]. At the Royal Marsden, most patients are educated to care for their own catheter by dedicated nurse specialists. The patients are assessed for competence in aseptic technique prior to being allowed home with their catheter *in situ* [46]. The catheter exit site should be cleaned daily using skin antiseptic until the wounds have healed. Once the wounds have healed, skin care ranges from daily cleansing and dressing [2] to no maintenance care in the absence of exit-site inflammation [47,48,50]. Occlusive dressings are thought to retain rather than exclude bacteria and moisture [15,20]. A dry dressing is generally applied [1,2,15].

Catheter hubs should be kept clean and free from debris as an important measure in avoiding infection [44,45]. Handling of lines should be kept to a minimum. The UK Department of Health recommends that giving-sets should be changed daily using aseptic

precautions. However, less frequent changes have not been associated with increased infective complications in published studies [45,47]. Patients may take showers provided that the dressing is changed afterwards. Some authorities allow swimming after one month [29].

The necessity for flushing the catheter regularly has been challenged, since thrombotic complications are infrequent in unflushed catheters [47]. Traditionally, heparinized saline is used, although suitable alternatives are normal saline or disodium EDTA in a concentration of 20 mg/ml. The latter has the advantage of possessing bactericidal properties against *Staphylococcus epidermidis,* the most common infecting organism [49].

A daily dose of 1 mg of warfarin has been shown to decrease the incidence of thrombosis associated with indwelling central venous catheters in patients with malignancy [50]. This regimen had no effect on prothrombin time, activated partial thromboplastin time or coagulation factor levels [50]. However, it is most commonly used for patients with solid tumours having ambulatory chemotherapy and is inappropriate for patients requiring bone-marrow transplantation. When treated with care, the catheter should last for the duration of treatment, in many cases months or even years.

Catheter removal

The indications for catheter removal are completion or cessation of treatment, or an untreatable complication. The decision to remove a catheter depends upon the gravity of the complication, the need for ongoing venous access, the risks of inserting a new catheter, and the life expectancy of the patient [4].

Removal of the line can be performed at the bedside by cut-down onto the Dacron cuff under local anaesthesia. The patient is positioned head-down to reduce the risk of air embolism. The cuff is carefully dissected out, taking care to avoid catheter damage. The catheter is clamped and cut distal to the cuff and the proximal part is removed first. The remaining catheter should slip freely from the distal tunnel and venous system. Children require general anaesthesia for this procedure.

Complications of long-term catheters

Complications of right atrial catheters may be divided into those due to the insertion of the line and those due to the continued presence of the line (Tables 28.2 and 28.3).

Table 28.2 Complications of percutaneous catheter insertion

Subclavian venepuncture
Failure to locate vein
Haematoma

Venous laceration
Arterial puncture
Pneumothorax
Haemothorax, haemomediastinum
Nerve injury
Tunnel-related
Haematoma
Breast tissue damage
Catheter insertion
Arrhythmias
Displacement or kinking of guidewire
Vessel perforation
Damage or kinking of introducing sheath
Air embolus
Catheter misplacement

Insertion complications occur less often with experienced operators [32]. The most frequent complications with percutaneous line placement are accidental arterial puncture and pneumothorax, occurring in 4% and 0.7–6% respectively [33,51]. The threshold for draining a pneumothorax should be lower than in the general patient population as, in those about to be profoundly immunosuppressed, a significant degree of lung collapse with its associated infection risk is unacceptable. Bleeding may result from injury to the subclavian vein or artery, depending on the site and extent of damage and competence of haemostatic mechanisms. Outcome may vary from haematoma to haemomediastinum or haemothorax. Haematomas may form along the tunnel, particularly in thrombocytopenic patients, predisposing to infection [35,39], and possibly precipitating seeding of tumour cells [52].

Catheter-related complications are relatively common, but if recognized and treated promptly, do not necessarily lead to catheter removal. The overall

Table 28.3 Catheter-related complications

Infection
Exit site
Tunnel
Thrombophlebitis
Bacteraemia and septicaemia
Endocarditis

Thrombosis
Catheter occlusion
Large-vessel thrombosis
Right atrial thrombosis
Pulmonary thromboembolism
Migration
Tip
Cuff extrusion
Accidental removal
Other
Positional and unidirectional functioning
Catheter damage
Extravasation
Arrhythmias

removal rate due to complications is reported as being between 7% and 43% [1,3,29,30].

Infection is the most common complication of long-term right atrial catheters [3,25], and exit site or tunnel infection, thrombophlebitis, bacteraemia or endocarditis may occur. The catheter may be colonized intraluminally by blood-borne seeding [27,49] or from contaminated hubs [24,53]. Predisposing factors are the presence of tunnel haematoma [32], thrombosis [3] or catheter damage [54]. Infections are twice as common in bone-marrow recipients, when compared to patients receiving induction chemotherapy for leukaemia [3]. This is due to the prolonged neutropenia in BMT patients. Patients with haematological malignancies have higher rates of infection than those with solid tumours [55]. Infections tend to occur during neutropenic periods, and also during exacerbations of GvHD [2,56]. In one prospective study of 189 leukaemia patients, those who were neutropenic experienced fewer catheter infections than did those with normal counts. The authors attributed this anomaly to stricter catheter maintenance given during these periods [3]. Other studies have also cited suboptimal maintenance as the cause of high infection rates [13,45].

Bacteraemia with no obvious source is extremely common in neutropenic patients. The intravascular catheter is often unjustifiably blamed, despite the fact that the incidence of septicaemia in this population has not increased since the introduction of indwelling catheters [2,3,32,57]. The majority of such bacteraemias are not catheter-related [26,32]. Coagulase-negative staphylococci are the most commonly isolated organisms [21,30,55]. Reasons for the predominance of this organism include its prevalence on the skin [58] and its ability to adhere to and degrade catheter materials [3,11], and the formation of an intraluminal slime. However, it should not be assumed that bacteraemias due to this organism always originate from the line, since it is increasingly isolated from the gut and respiratory tract in the neutropenic population [3,58]. Table 28.4 illustrates the

microorganisms isolated in one study of proven catheter-related infections. Polymicrobial infections are not usually secondary to the catheter, but commonly occur in the presence of disrupted mucosal surfaces, for example, secondary to perianal abscesses [3].

Table 28.4 The microorganisms isolated in proven catheter-related infections (exit site, tunnel, and bacteraemic) over 100 544 catheter-days (press et al. 1984 [3])

Organism	Frequency (%)
Staphylococcus epidermidis	54
Staphylococcus aureus	20
Candida spp.	7
Pseudomonas spp.	6
Corynebacteria	5
Klebsiella spp.	4
Enterococcus spp.	4

The management of suspected catheter infection requires prompt recognition and immediate treatment with broad-spectrum antibiotics administered via the catheter. In the majority of cases this enables salvage of the line. Exit-site infections usually resolve with a combination of antibiotics, daily cleaning and topical povidone-iodine ointment [47,55], while tunnel infections usually require catheter removal [26]. Press *et al.* found 15.4% of exit site infections, 69% of tunnel infections and 100% of patients with septic thrombophlebitis required catheter removal [3].

Persistent bacteraemia may be due to infected catheter-associated thrombus, and there are reports of successful treatment using combined antibiotic and fibrinolytic therapy [21,54]. If such therapy is used, it is important to establish therapeutic antibiotic levels before fibrinolytic therapy is commenced, in order to prevent seeding of microorganisms throughout the circulation. Fungal infections may be the cause of persistent fever in patients treated empirically with antibiotics. Amphotericin B has been used with good effect in febrile neutropenic patients who failed to improve after five days of broad-spectrum antibiotics [59]. Endocarditis is a rare complication, but has been reported in BMT recipients [60,61], as has osteomyelitis of the clavicle in a patient with a tunnel infection [62].

Thrombosis and catheter occlusion

Large-vessel thrombosis occurs in 8–63% of patients with indwelling central venous catheters [21,63–66], and clots have been found in 30% of samples taken from heparinized lines [67]. Right atrial thrombi have been described [68], on occasion large enough to obstruct the tricuspid valve [60,69]. The problem is enhanced in this patient

population as some chemotherapeutic agents degrade catheter surfaces, predisposing to platelet adherence and thrombus formation [70]. Following clinical suspicion, diagnosis may be confirmed by ultrasonography, contrast venography, X-ray after injection of contrast via each catheter lumen, or computerized tomography. Venous thrombosis, including superior vena caval obstruction, has been treated with high-dose fibrinolytic therapy, allowing the right atrial catheter to be retained [21,71].

Low-molecular-weight heparin has been used to manage residual thrombosis where the catheter has been removed [72]. It has the advantage of a reduced risk of haemorrhagic complications in the thrombocytopenic patient [72].

The incidence of catheter occlusion is approximately 0.6 per 1000 catheter-days [37,73], depending upon catheter size. Similar incidences have been noted whether or not routine flushing is performed [47]. Encasement of the endovascular catheter shaft by a fibrin sheath occurs in 75% of catheters as early as 48 hours post-insertion [13,21,28], and is probably present on all catheters by five to seven days [74]. The fibrin propagates from sites of intimal damage including the site of insertion and also the tip if it is in approximation with the vein wall. Infusates pass from the catheter lumen into the circulation via perforations along the length of the fibrin sheath. The fibrin sheath is likely to lead to increased resistance to infusion and decreased blood flow [74]. It has been suggested that the measurement of catheter resistance can be used to detect partial occlusion, permitting measures to prevent complete occlusion [75]. When faced with an occluded catheter, forceful injection with a small syringe should be avoided as it may result in catheter rupture [76]. An effective treatment is to introduce either streptokinase 250 U/ml or urokinase 5000 U/ml, in a volume equal to the catheter dead-space [76,77]. If this fails, a low-dose fibrinolytic infusion may be effective [73].

Two techniques of percutaneous fibrin sheath stripping have been described for occluded catheters: a J-wire is passed through the catheter until the curved tip just exits. It is then repeatedly rotated to strip the catheter tip of its fibrin sheath [78]. Alternatively, a snare loop is passed via a femoral vein and used to strip the fibrin from the distal 5 cm of catheter [78].

Catheter migration

Catheter-tip migration may occur in up to 10% of cases [57], most commonly into the ipsilateral internal jugular vein. Displaced lines should be repositioned, preferably with their tip in the right atrium, as thrombosis and intimal damage are more likely in vessels with lower blood flow. Malpositioned lines may be repositioned using a combination of a J-wire passed through the catheter and a snare passed up from the femoral vein [79]. Cuff extrusion usually occurs early in the life of the catheter [47]. Reported rates vary from 0.9% [1] to 6% [64]. Accidental removal due to traction is more common in children. It occurs in 1–3% of cases, usually shortly after insertion [1,4].

Other complications

Positional functioning, most commonly unidirectional, where fluid may be infused but not withdrawn, may be due simply to the catheter tip impinging upon the vessel wall. This happens more frequently if the tip is bevelled [48], a feature which has resulted in

perforation of the superior vena cava [80]. In the absence of a bevelled tip, the most common cause is likely to be fibrin sheath formation, with the sheath acting as a ball valve [74]. Alternatively, an excessive length of catheter in the right atrium may bend and impinge upon the endocardium [32]. Shoulder girdle movements may result in pinching of the catheter between the first rib and clavicle. This initially causes catheter malfunction, but may eventually lead to catheter breakage [24] and possible embolization. Breakage of the external portion has been quoted as occurring in 7% of catheters [24]. Repair kits exist for most of the currently marketed catheters.

Extravasation is a serious complication which has recently been reported in association with the split-sheath method of insertion. Venous thrombosis or fibrin-sheath formation usually precede extravasation, which is probably caused by excessive bolus flushing of clogged catheters where least resistance is encountered alongside the catheter [37,64].

Severe late onset shoulder pain occurs in 6.6% of patients having ambulatory chemotherapy for gastrointestinal malignancy at the Royal Marsden Hospital, and, in a proportion of these, this may be due to extravasation of chemotherapy along the fibrin sheath. However, this has less relevance to a bone-marrow transplant population. Investigation of these patients should include radiographic screening following the injection of contrast through each lumen of the catheter.

Arrhythmias have been reported where the tip of the catheter lies in the right atrium; withdrawing the catheter slightly may lead to resolution [80,81], The catheter tip should be electively positioned in the superior vena cava in patients susceptible to arrhythmias [32].

Conclusion

Procuring and maintaining long-term venous access is a vital part of the management of BMT patients. The choice of catheter and method of insertion often depend on hospital expertise and, to a great extent, tradition. Whatever the method of access, careful handling and aggressive treatment of complications are essential to maintain the catheter for the duration of therapy, thus avoiding repeated venepunctures. The Hickman-type catheters, despite infection risks and limitations upon lifestyle, are currently the best option for the intensive intravenous support necessary in patients undergoing BMT.

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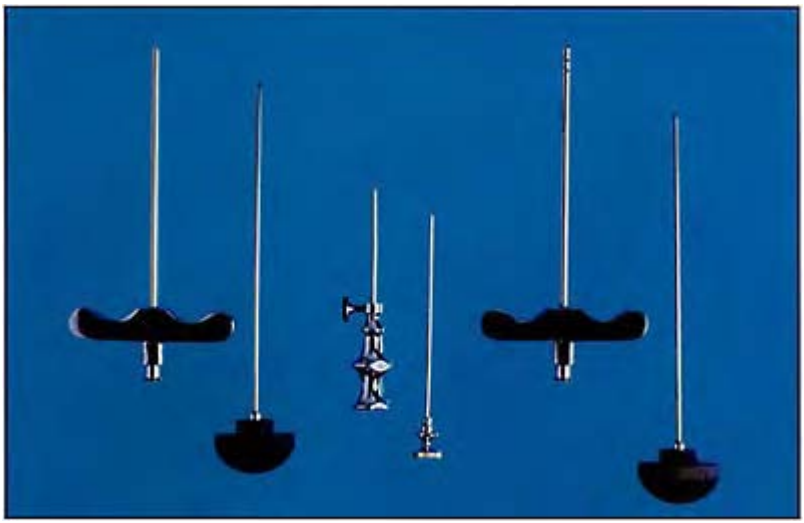

Chapter 29
Marrow collection and processing

Michele Cottler-Fox

Introduction

The technique described by Thomas and Storb in 1970 [1] has undergone few major changes, and is still the basis of marrow harvesting today. Historically, transplant physicians have aimed to acquire 2–4×10^8 nucleated cells/kg recipient body weight for an unmanipulated allogeneic marrow graft, and 1–3×10^8 nucleated cells/kg for an unmanipulated autologous graft [2]. In general, this number of cells may be acquired with a collection of 10–20 ml of marrow/kg recipient body weight, although donors under the age of 20 years may have significantly more cells per volume harvested, while those over the age of 60 years may have significantly less [3]. When deciding how much marrow to harvest, it is imperative to know whether a graft will undergo manipulation, and what the cell loss associated with processing is likely to be, in order to harvest sufficient cells to ensure an adequate graft.

Haematopoietic progenitor stem-cell grafts are currently obtained either from human marrow, peripheral blood or cord blood. Recently, a graft has also been attempted from

baboon marrow [4]. Collection of marrow from cadaveric human donors is also feasible [5,6], and cadaveric donor marrow has been documented to engraft in the related recipient [7]. It is also possible to derive a graft from foetal liver [8], or through tissue culture expansion of a minimal cell dose obtained from one of the sources listed above [9]. It is hypothesized that by using cord blood, foetal or xenogeneic cells, it will be possible to minimize or avoid the occurrence of graft-versus-host disease (GvHD) since cells from these sources may not respond to, or stimulate, alloresponses as do cells from a mature human host.

Marrow collection

When to harvest

Marrow from a related donor is generally harvested at the recipient's transplant centre on the day of transplant. Marrow from an unrelated volunteer donor may be harvested on the day of transplant if transport time to the recipient is no more than 24 hours. Alternatively, an unrelated marrow may be harvested the day before transplant in order to allow it to arrive on the planned day of infusion. Autologous marrows are usually cryopreserved, so that timing of the harvest is rarely a problem. However, with short conditioning regimens for transplant such as high-dose melphalan, some centres store the autologous marrow at 4°C in the liquid state for up to 48 hours [10].

Although frozen allogeneic grafts have rarely been used due to fear of stem-cell loss, successful engraftment has been reported using such grafts [7,11].

Risks of marrow harvest

General anaesthesia, spinal and epidural anaesthesia have all been utilized successfully. The major risks of harvest are associated with induction of anaesthesia. These include non-fatal cardiac arrest, pulmonary embolus, aspiration pneumonia, ventricular tachycardia and cerebral infarction [12]. At least one fatality in an older donor as a result of cardiac arrest is known but has never been fully reported [13]. The Seattle transplant team has reported that 27% of allogeneic donors have a complicating event, of which 92% may be considered minor, i.e. bacteraemia, local infection at harvest sites, post-operative fever, fractured iliac crest, broken aspiration needle requiring surgical removal, transient pressure neuropathies from haematomas at harvest sites and spinal headache [14]. Approximately half of the serious complications in this series were related to anaesthesia. Table 29.1 summarizes the most well-recognized problems associated with bone-marrow harvest.

Harvest technique

In general, an adequate harvest may be obtained from the posterior iliac crests. Alternative sites which may be utilized in addition include the anterior iliac crests, sternum and, in a donor less than one year old, the tibia. Thus, after anaesthesia is

induced, the donor is usually placed first in the prone position on pillows to support the hips and lower rib cage allowing the diaphragm free movement, or on an adjustable spinal frame such as is used for laminectomy. Harvest in the lateral position has also been described [15].

After a sterile field is prepared, the marrow is aspirated into a syringe which has been rinsed with preservative-free heparin or which may contain a heparinized solution. It has been suggested that no more than 3–5 ml should be collected per aspirate, as more than this is simply blood and increases the number of T-cells in a harvest and leads to a larger volume harvest to acquire an adequate number of stem

Table 29.1 Problems associated with bone-marrow harvest

Major risks associated with anaesthesia
Cardiac arrest
Pulmonary embolus
Aspiration pneumonia
Ventricular tachycardia
Cerebral infarction
More minor risks include
Bacteraemia
Local infection at harvest sites
Post-operative fever
Fractured iliac crest
Broken aspiration needle requiring surgical removal
Transient pressure neuropathies
Haematomas at harvest sites
Spinal headache

cells [16]. The skin over the iliac crests is quite flexible, so that an average harvest of 1–1.5 litres may only require three or four skin punctures per iliac crest. Many different styles of needle have been used for harvest with varying degrees of comfort and success [17], including disposable needles. The syringe used for harvest is usually 20–60 ml in volume in order to obtain good suction. It has become common to use disposable plastic syringes rather than glass as originally described, to avoid leaving the harvest field covered in splinters if a syringe weakens with use and breaks.

Aspirated marrow is expelled into a container holding heparinized fluid. Originally, tissue culture medium but more recently a buffered saline solution such as Plasmalyte-A® or Normosol® has been used. How much heparin to use per harvest, and whether it should be preservative-free or not, remain controversial since the range of what has been used successfully is wide. The container itself was originally a stainless steel or glass beaker,

but more recently a commercial collection bag with attached filters has become available (Baxter Fenwal, Deerfield, IL). If the Fenwal kit is not used, then some other means must be found to filter out bone spicules and to break up cell clumps after the harvest. After the harvest is filtered, many centres add citrate in a volume equal to 10% of the collection for further anticoagulation.

After the harvest

At the end of harvest, the aspiration sites are bandaged with a pressure dressing, and analgesia is supplied as needed. Ice applied in the immediate post-operative and recovery room period may be useful to reduce swelling and pain. A single dose of intravenous non-steroidal anti-inflammatory medication just prior to ending anaesthesia may also decrease post-operative pain. In any event, it is rare to need more than minor narcotic analgesics such as acetaminophen (paracetamol) with codeine or oxycodone for more than a few days following harvest.

Marrow harvests were in-patient procedures for many years following the development of marrow transplantation. However, the desire to decrease hospital costs led to the study of marrow harvesting as an out-patient procedure [18,19]. Today, if the patient is prepared to be admitted to hospital in the event of hypotension, excessive pain or unexpected complications of harvest, the majority of autologous and allogeneic donors may be discharged within 12 hours of harvest.

Alternative sources of marrow

Surgically resected ribs, cadaveric vertebral bodies and complete ilia are potential sources of marrow [20]. The principle in such a harvest is to open the bone, expose the marrow-rich cavity, and remove the marrow into a buffered salt solution containing DNase but no heparin [21]. Gentle agitation releases cells from the bone matrix, and the cell suspension is then washed, filtered and prepared for use or cryopreservation. Alternatively, marrow may be initially pressed out of the bone into a sterile fluid. Although only a single cadaveric marrow transplant has been reported [7], it is possible that there would be less graft-versus-host disease with cadaveric marrow since it contains fewer T-cells than marrow aspirated from a living donor [22]. This approach may be of importance in the future as a means of inducing tolerance for solid organ transplants [23].

Marrow transport

Marrow arrives from the operating room to the processing laboratory in a standard blood transfer bag of 600–2000 ml volume, bearing a label identifying the donor and recipient (if it is a related donor), as well as the fluid and anticoagulant solution into which it has been collected. Unrelated donor marrow is labelled with an identifying number, rather than the donor name, and is hand carried by a courier from the operating room at the harvest centre to the processing laboratory at the transplant centre. Unrelated donor marrow may be transported on wet ice or without coolant in an insulated container, and

should arrive at the recipient's transplant centre within 24 hours of harvest. Since marrows are collected and transported worldwide, it is common for the marrow courier to arrive outside of regular working hours. Thus, if processing is required, the receiving laboratory may need to have personnel on call 24 hours a day.

Volume reduction

In many centres, an ABO-matched allogeneic marrow is transported directly from the operating room after harvest to the bedside, where it is infused through a central line. In such cases, the only potential complications to the recipient are related to the volume infused, the dose of heparin it contains, and the possibility that, despite filtering, cell clumps may cause respiratory difficulty. In cases where the volume collected would be enough to cause problems with fluid overload, the volume is reduced by centrifugation and removal of plasma. This will increase the haematocrit of the final product, but should not present a clinical problem as long as the marrow is infused over an appropriate time period (3–4 hours). In cases where the marrow donor plasma contains a clinically significant antibody against cellular elements of the recipient (i.e. alloagglutinins against ABO or other red blood cell antigens in red blood cell mismatched transplants), donor plasma may be removed.

Red blood cell depletion

In the case of an ABO major mismatched allogeneic transplant (where the recipient has alloagglutinins against a donor red blood cell antigen), the simple removal of red blood cells will prevent acute haemolysis. In the past, it was feared that this marrow manipulation might interfere with engraftment, for which reason recipient alloagglutinins were removed by vigorous plasmapheresis of the recipient, by plasma exchange over an immunoadsorbent column, or by infusing a small amount of incompatible red-blood cells to adsorb out the recipient alloagglutinins *in vivo* just prior to marrow infusion. However, as red blood cell removal has been shown to involve minimal white blood cell (i.e. stem cell) loss, it is now more common to remove red blood cells using one of the following methods [24]. Red blood cells removed from an allogeneic or autologous marrow may also be reinfused into the donor after the harvest.

Starch sedimentation

Marrow containing heparin is mixed with 6% hydroxyethyl starch at a ratio of 8:1, after which the red-blood cells are allowed to sediment as a function of gravity with the blood transfer bag in the inverted position (entry port, side down). After 30–180 minutes, the sedimented red blood cells are removed into a secondary bag. The original bag contains approximately 75% of the original nucleated marrow cells for transplantation, as well as 5–25 ml of the original red blood cells. This volume of incompatible red blood cells is

still capable of causing a haemolytic transfusion reaction, for which reason other techniques are commonly preferred to sedimentation.

Repeated dilution with compatible red blood cells

After a white blood cell-rich cell concentrate has been prepared by sedimentation, the remaining incompatible red blood cells may be decreased by diluting the cells with a unit of recipient-compatible, irradiated, leukocyte-depleted red blood cells and 150 ml of recipient-compatible plasma, if possible from the marrow donor. The product is then allowed to resediment, reducing the total of incompatible red-blood cells to <10 ml while retaining 85% of nucleated white blood cells. This procedure is time-consuming, however, and most processing laboratories today would choose instead to go directly to a method for concentrating white-blood cells.

Buffy coat preparation

Buffy coat preparation is often the first step towards additional processing for T-cell depletion, tumour-cell purging or cryopreservation. A white blood cell-rich concentrate, the buffy coat, is prepared by centrifuging the marrow collection using a standard blood bank centrifuge, a blood-cell washer, or any of the apheresis devices currently available. Approximately 80% of nucleated cells from the marrow are retained, while simultaneously reducing the volume and red blood cell concentration by 80%. Although this is an adequate method of volume reduction for cryopreservation, and a good preliminary step towards a mononuclear cell separation, it is not recommended where red blood cell contamination may interfere with chemical or immunological purging methods.

Mononuclear cell separation

Mature myeloid elements may be removed from a buffy coat using manual or automated methods, with or without density-gradient materials [24] such as albumin, Ficoll and Percoll. Such agents remove essentially all red blood cell and myeloid elements, leaving only mononuclear (morphological lymphocyte +monocyte populations) portions of the graft. While this is of great importance for the economic feasibility and success of purging protocols for autologous transplantation, and of T-cell depletion for allogeneic transplantation, it remains a problem that upwards of 30% of the original progenitor stem-cell elements of a graft may be lost. In order to offset this loss, and to maintain adequate numbers of such cells after separation procedures, it is necessary to collect considerably more than are needed for the final graft.

Cryopreservation

Autologous marrow is usually cryopreserved for later use. Cells to be cryopreserved may be aliquoted into small vials, or they may be placed into freezing bags with a capacity of 30–200 ml. If the bag to be frozen requires heat sealing, it is imperative to remove all air prior to sealing, as air in the bag may cause it to explode on thawing, when the air expands rapidly but the bag has not yet become flexible.

Addition of cryoprotectant

Dimethylsulphoxide (DMSO) is the most widely used cryoprotective agent used to cryopreserve haematopoietic stem cells at present. DMSO is a universal solvent capable of stabilizing cell membranes under rapidly changing conditions, preventing intracellular ice crystal formation during freezing and heat release during the period of phase transition. DMSO has been described as toxic to stem cells at room temperature, for which reason investigators have emphasized the need to add it at 4°C to cells prior to controlled rate freezing and to begin cryopreservation quickly thereafter. A final concentration of 10% DMSO with albumin or human serum is commonly used, with some centres using hydroxyethyl starch to help stabilize cell membranes and reduce the amount of DMSO used to 5%.

Freezing technique

Freezing has traditionally been performed in a controlled rate freezer, using liquid nitrogen to decrease the temperature at a rate of 1–2°C/min until a phase transition occurs, during which heat is given off by the solution, followed by an extra burst of liquid nitrogen to prevent an increase in temperature. After this, the temperature drop is adjusted to 5–10°C/min until the mixture has reached a temperature of approximately −120°C.

An alternative to controlled rate freezing is so-called ‘dump’ freezing [25]. In this technique a freeze mix containing DMSO, albumin or human serum and hydroxyethyl starch is added to cells and the product is placed into a −80°C freezer.

Storage conditions

After cryopreservation, the products are removed from the freezing apparatus and stored either in the liquid (−196°C) or vapour (−156°C) phase of a liquid nitrogen freezer, or in a mechanical (−80°C) freezer. Marrow frozen with a controlled rate freezer and infused after more than eleven years in liquid nitrogen storage has resulted in good haematopoietic reconstitution [26], but products which have been subjected to ‘dump’ freezing and stored at −80°C may deteriorate more rapidly [25].

Thawing and infusion

A number of studies have shown that rapid thawing is desirable for the survival of haematopoietic progenitor stem cells [27]. Thawing may be performed in a temperature-controlled water bath, or in a sterile basin using sterile saline heated to 37–40°C prior to use. Once thawed, the cells are commonly infused directly, and rapidly, via a central venous access device. Each bag is infused over no more than 15 minutes to avoid the supposed DMSO toxicity to stem cells. Immediate side-effects associated with rapid infusion of stem-cell products may include volume overload, bradycardia (the result of cold cardioplegia), nausea and vomiting (the result of the unpleasant taste of DMSO via direct nerve stimulation from the product in blood), fever, tachycardia, hypotension or hypertension (the result of lysed granulocytes if the product is not a clean mononuclear product) and allergic reactions ranging from urticaria to anaphylaxis (related to plasma proteins and/or DMSO). Most, if not all of these problems may be avoided by volume-reducing the products after collection, and giving anti-emetics, diphenhydramine and/or hydrocortisone prior to infusing stem cells. Renal dysfunction related to red-cell haemolysis is a delayed problem which may be seen with infusion of stem-cell products, for which reason some centres prophylactically give mannitol and frusemide along with stem-cell infusions [28].

Alternatively, the thawed product may be processed to remove DMSO prior to infusion and to reduce the volume. This is not a widely accepted manoeuvre, however, due to concern for stem-cell loss. It is also possible to thaw rapidly and then dilute the product with a sterile buffered saline solution prior to infusion, and then infuse the product slowly at room temperature (E.Areman, personal communication, 1996). While this allows the processing laboratory to deal with potential bag breakage in the most efficient manner, it increases the volume infused and may require the use of DNase to avoid cell clumping.

Product assessment/quality assurance

Since there is thought to be a threshold dose for stem cells above which rapid haematopoietic reconstitution can be expected, it is important to evaluate graft quality. Grafts were originally evaluated on the basis of total nucleated cells infused per kilogram recipient body weight. Fewer nucleated cells are believed to be needed in autologous transplantation than allogeneic. However, the total nucleated cell count is a surrogate for those cells which reconstitute haematopoiesis, and much effort is currently being expended to define a threshold dose more appropriately. Until recently, the most commonly used assay for reconstituting ability of a graft was the growth of the day 14 granulocyte-macrophage colony-forming unit (GM-CFU) in semisolid culture media. Threshold doses for CFU-GM differ widely, but have been said to be in the range of 0.1–1×10^4/kg for marrow. Unfortunately, culture conditions vary among laboratories, making comparisons difficult. Further, since the cultures cannot be counted until 14 days after a harvest, realtime evaluation of a graft is impossible. For this reason, progressively more centres are now using flow cytometry-based evaluation of the CD34 cell-surface marker

found on progenitor stem cells to determine the suitability of a graft for transplantation. Here, too, technical difficulties make comparisons between centres difficult, and the threshold doses in the literature differ. However, it seems likely at present that a dose of at least 2.5×10^6 $CD34^+$ cells/kg will lead to granulocyte and platelet recovery to clinically acceptable levels within approximately 14–21 days of transplantation.

While all processing laboratories evaluate grafts in one of the above-named fashions prior to cryopreservation, some have argued that grafts should also be evaluated after thawing. This is not commonly done due to the expense and the fact that data are not evaluable until after the graft has been infused.

Finally, microbiological evaluation of a graft for contamination resulting from the collection or processing procedures may be performed. While some feel that all grafts should be subjected to bacterial and fungal cultures both initially and after processing, others have pointed out that: (1) results are often not available until after the infusion; (2) cultures are expensive; and (3) due to the small volumes most processing laboratories are willing to sacrifice for cultures, the results may be invalid. Infusion of contaminated grafts has been reported not to be associated with ill effects to the recipients [29].

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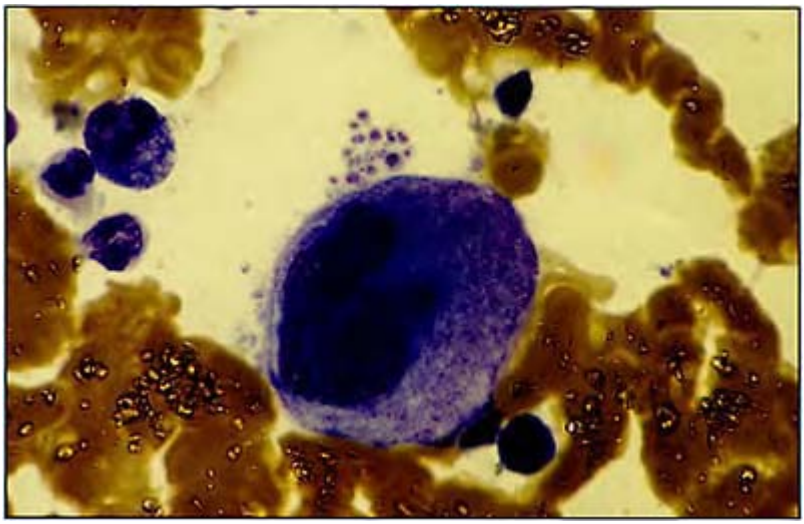

Chapter 30
Use of the cord blood

Hal E.Broxmeyer

Introduction

Many patients have disorders which currently can only be cured by a haematopoietic stem-cell transplant. Some disorders may be amenable in the future to treatment by specific gene modification utilizing a gene-transfer approach to a potential gene-therapy outcome. Patients in need of a stem-cell transplant have mainly three options. This can be a transplant using either:

1. Adult bone-marrow cells;
2. Adult blood in which stem cells have been mobilized from the marrow after chemotherapy treatment and/or the use of haematopoietically active growth factors;
3. Umbilical cord and placental blood retrieved at the birth of a newborn infant.

For stem-cell transplantation, the limitation has been access to appropriately human leukocyte antigen (HLA) -matched tissue. Even with computerized registries such as the National Marrow Donor Program in the United States, the majority of patients in need of

a stem-cell transplant cannot find a suitable donor. In some cases, a suitable donor may be uncovered, but perhaps not in time to treat a rapidly progressing terminal disorder. Bone marrow is still the major tissue source of stem cells. While mobilized peripheral blood is becoming a more utilized source for such cells, it is the author's opinion that the newly emerging field of cord-blood banking and transplantation offers the greatest hope for enhancing donor pool availability for stem-cell transplantation.

Until the mid to late 1980s, cord-blood was considered mainly a material that was to be discarded after sampling of these cells for routine tests on the newborn. The first cord-blood transplant was performed as recently as October 1988 [1–3] with only a few more such transplants performed in the following two years. Four of the first five transplants with cord blood were carried out for correction of the genetic disorder Fanconi anaemia [1–4], while one was done to treat juvenile chronic myelogenous leukaemia [5]. These first transplants used HLA-matched sibling cord blood based on a laboratory study that suggested that such cells were a potential source of transplantable haematopoietic stem and progenitor cells [6]. The cells used were first sent to the author's laboratory from a distant obstetric unit where they were assessed for content of haematopoietic progenitor cells, frozen and stored in the first of what has become an increasing number of cord-blood banks.

The field has advanced rapidly from clinical transplants utilizing only HLA-matched, or one HLA-antigen disparate sibling material [1–5,7–16], to one in which greater than one antigen disparate cells have been used [17]. Also, unrelated donor cell transplants have now come to the point where they outnumber those transplants using sibling cells [18–24]. The acceleration in clinical experience with cord-blood transplantation is due to the establishment of banks for storing unrelated cord bloods [25–28], the largest of which is at the New York Blood Center under the direction of Dr Pablo Rubinstein. The vast majority of unrelated cord-blood transplants have used donor cells from this bank [18–22]. Banks for the storage of autologous or related allogeneic cells are also being set up [29].

The broadness of applicability of cord blood for transplantation is still being assessed. Currently, over 26 different disorders for malignant and non-malignant states have been treated (Table 30.1). Most of the recipients of cord-blood transplants have been children of relatively low body weight, but more experience is now accumulating in the context of higher body-weight recipients and adults [18–22,30].

Clinical results are encouraging, with engraftment rates from a number of studies being reported to be in the 90% range. Relatively low levels of graft-versus-host disease (GvHD) have been noted even with one to three antigen disparate unrelated cord-blood donor grafts, compared to that expected based on previous results with similarly matched unrelated bone-marrow grafts [18–22]. However, the experience with cord blood is still small compared to that for bone marrow, and much more experience is needed, including results from controlled trials, before definitive conclusions can be drawn regarding the disorders best treated by cord blood, and a more accurate assessment of engraftment, GvHD and relapse rates can be made. Efforts in these directions are occurring throughout the world, and with a developing information network we should soon have a more conclusive idea of the advantages and disadvantages of cord-blood transplantation compared to transplantation using bone marrow or mobilized peripheral blood stem cells.

Table 30.1 Examples of malignant and non-malignant disorders for which cord-blood transplantation has been utilized

Malignant disorders
Acute lymphoid leukaemia—remission, relapse
Acute myeloid leukaemia—remission, relapse
Chronic myeloid leukaemia—accelerated/blastic
Juvenile chronic myeloid leukaemia
Myelodysplastic syndrome
Neuroblastoma
Non-malignant disorders
Acute megakaryocytic thrombocytopenia
Severe combined immunodeficiency
Common variable immunodeficiency
Wiscott-Aldrich syndrome
X-linked lymphoproliferative disease
Hurler syndrome
Hunter syndrome
Fanconi anaemia
Severe aplastic anaemia
β-thalassaemia
Sickle-cell anaemia
Leseh-Nyhan syndrome
Pure red-cell aplasia
Osteopetrosis
Globoid cell leukodystrophy
Adrenoleukodystrophy
Gunther’s disease—porphyrin accumulation
LAD syndrome—adhesive membrane glycoprotein deficiency
Kostmann’s syndrome
Diamond-Blackfan syndrome

This review will briefly cover what we know about the immunology, biology and gene transfer of cord-blood cells. It will be advancements in our knowledge in these areas that will hopefully set the stage for understanding the true potential uses of cord blood.

Immunology

The apparently lower levels of GvHD seen with donor cord blood compared to those seen with adult cell transplantation [1–5,7–24] has intrigued investigators as it suggests that cord blood may be more readily able than adult bone-marrow cells to cross some histocompatibility barriers. If this belief holds true, the obvious implications are that more recipients in need of a stem-cell transplant will have access to one, and that the number of cord-blood collections stored will be smaller than if stricter HLA-matching were required. Some information concerning the extent of matching required may derive from use of animal models. One such study has suggested that newborn blood is able to reconstitute the lymphohaematopoietic system of lethally irradiated adult mice without inducing GvHD against incompatible non-H2 antigens [31]. While mice have been very helpful in establishing information on immunogenetics, efforts on species more closely related to humans are warranted but no such studies have, as yet, been reported.

Orignally, a serious concern was the potential for massive GvHD because of maternal-cell contamination. While more sensitive techniques [32–36] have revealed higher levels of maternal-cell contamination than previously recognized by less sensitive procedures [1–3], the degree of maternal-cell contamination has not correlated with the degree of GvHD experienced by the recipient, leaving us, currently and until proven otherwise, with the belief that the amount of maternal-cell contamination of the cord blood may not have clinical implications.

Cord blood apparently contains normal frequencies of cytotoxic T-lymphocyte precursors and helper T-lymphocyte precursors as assessed against non-inherited maternal and paternal antigens [37]. However, the cytotoxic activity of cord-blood T-cells in primary, secondary and tertiary mixed leukocyte culture is minimal compared to that of adult T-cells [38,39], factors which may correlate with low levels of GvHD seen after cord-blood transplantation.

While cord-blood T-cells mount a vigorous proliferative response to primary allogeneic stimulation [38–40], an attenuated reaction occurs after secondary allogeneic stimulation. In this situation, cord-blood T-cells are induced to proliferate at less than 40% of the primary response level, compared to the sixfold enhancement of proliferation seen with adult T-cell cells after secondary allogeneic stimulation [40]. Similar decreased secondary responses have subsequently been reported by others [41]. The relationship of this dampened secondary response of cord-blood T-cells to the clinical GvHD situation remains to be defined, but understanding the mechanisms involved in this induced anergic state could be important. To this end, detailed analysis of intracellular signal transduction events from the receptor to the nucleus are clearly warranted. Interleukin (IL)-10 has been demonstrated to induce a long-term antigen-specific anergic state in human $CD4^+$ T-cells [42]. It would seem that IL-10 is a reasonable candidate cytokine that might be involved in the induced anergic state of cord-blood T-cell response to a

secondary allogeneic stimulus. Evaluation of a possible role for IL-10 and other immune-associated cytokine interactions is warranted.

Natural killer (NK) and lymphokine activated killer (LAK) cells have been implicated in graft-versus-leukaemia (GvL) effects. Currently, there is no firm evidence that the reduced severity of GvHD seen after cord-blood transplantation has resulted in a reduced GvL effect. While NK cell activity in cord blood is on the whole greatly reduced compared to that of adult tissue [43], NK cell-derived LAK cell activity is rapidly activated by cytokines such as IL-2 and IL-12 [43]. This activation is associated with release of the lytic molecules perforin and granzyme B [44]. Although $CD16^{+}CD56^{+}$ and $CD16^{-}CD56^{+}$ NK cell subsets typical of adult blood are present in cord blood, a significant population of $CD16^{+}CD56^{-}$ cells, not found to a significant extent in adult blood, is present in cord blood [43]. Evidence is available that these cord-blood $CD16^{+}CD56^{-}$ NK cells with low lytic activity may be precursors of mature NK cells [44].

Investigation of the reactivity of cord-blood immune cells has lagged greatly behind that of our knowledge of such events and their mechanisms with adult immune cells. Efforts in understanding cord-blood immune cell reactivity need to be increased. Information in this area has the potential to allow manipulation of both cord blood and adult cells for more effective crossing of HLA barriers during stem-cell transplantation.

Biology

Although a number of papers suggested that single cord-blood collections contained enough stem and progenitor cells to engraft adults [6,45–51], the assays used to come to this conclusion are not believed to detect the long-term marrow repopulating stem cell [52]. Based on the limited number of cells in a cord-blood collection, the inability to get many more of these cells shortly after birth [45], and the purported need for larger donor-cell inoculums as the weight of the recipient increases, it was considered by some that the amount of cord blood collected would not suffice for engraftment and long-term repopulation of adults or heavy body-weight children. It is now clear that adults and larger-sized children can be engrafted with single cord-blood collections [18–22,30]. However, whether single cord-blood collections will suffice for engraftment of all adults remains to be determined.

While some larger studies have not demonstrated a limiting cord-blood cell dose necessary for engraftment [7,19], other studies are suggesting that perhaps a limiting cord-blood cell dose is becoming apparent, which may be in the range of 10^{7} nucleated cells/kg body weight [18,23]. Cases of successful engraftment have been noted with administration of less than 10^{7} nucleated cells/kg body weight, but the overall likelihood of engraftment may be statistically less likely at the lower nucleated donor-cell numbers. A preliminary report has not indicated a correlation between nucleated cell count or progenitor cell levels in donor cord-blood inoculums with failure to engraft. However, a statistically significant correlation has been noted between progenitor cell content of cord-blood collections and time to myeloid cell engraftment [53].

Immature subsets of progenitors are highly enriched in cord blood [6,45–51]. These cells are in a slow- or non-cycling state [54–57], but they respond rapidly to stimulation with combinations of cytokines [54–56] and they have been expanded greatly in cell

culture *ex vivo* [45–51,55,56,58,59]. This expansion relates to the types and combinations of cytokines that are used. Those cytokines that have been used for expansion purposes include the potent co-stimulating cytokines that are ligands for tyrosine kinase receptors such as steel factor (also termed stem-cell factor, *c-kit* ligand, and mast-cell growth factor) [45,60] and the Flt3/Flk2 ligand that binds the Flt3/Flk-2 receptor [61], in combination with colony-stimulating factors (CSF) such as granulocyte-macrophage (GM)-CSF, granulocyte (G)-CSF or IL-3, including the GM-CSF/IL-3-fusion protein PIXY321 [61] and/or the interleukins: IL-1, IL-6 and IL-11.

Thrombopoietin has recently been found to be intimately involved in supporting the growth of early myeloid progenitors in addition to those of the megakaryocyte/platelet lineage [62], and a recent report has shown the extensive amplification and self-renewal of primitive cord-blood progenitor cells in stroma-free cultures containing only thrombopoietin and Flt3 ligand [63]. The combination of steel factor with a recombinant soluble IL-6 receptor has also been reported to enhance the *ex vivo* expansion of primitive progenitors in cord blood [64].

An unknown with the use of *ex vivo* expanded cells is whether those cells with long-term marrow repopulating capacity are being maintained, expanded or decreased, while those cells identified as immature progenitors (colony-forming cells of the granulocyte-macrophage (CFU-GM), erythroid (BFU-E) and multipotential (CFU-GEMM) lineage) and more mature stem cells (long-term culture initiating cells (LTC-IC), high proliferative potential colony forming cells (HPP-CFC) and subsets of CFU-GEMM)) are expanded. This concern is heightened by the lack of a quantitative assay for the human long-term marrow repopulating stem cell already mentioned [52], and a recent report suggesting that *ex vivo* expansion of murine marrow cells with IL-3, IL-6, IL-11 and steel factor led to impaired engraftment in irradiated hosts, with the engraftment defect being more profound at longer time intervals [65]. This offers the possibility that the greatest engraftment defect of *ex vivo* expanded cells may be on the long-term marrow repopulating stem cells.

Cell tracking has been utilized to estimate the presence of very early cells in *ex vivo* expansion cultures [58,66]. However, it is possible that studies of human cell engraftment of mice with severe combined immunodeficiency (SCID) [67,68], especially those SCID mice of the non-obese diabetic (NOD) type [69–72] or those carrying human cytokine transgenes [69], will offer us the opportunity to quantitate populations of long-term marrow repopulating human stem cells through *in vivo* analysis. The SCID marrow repopulating human cells (SRC) appear to be more immature than the LTC-IC and the immature progenitors, assessed in semi-solid culture medium (HPP-CFC, CFU-GEMM) [72]. Human cord-blood cells engrafting SCID mice give rise to primitive cells including LTC-IC [73,74] and cells with myeloid and lymphoid engrafting capacity after transplantation into secondary SCID mice [74].

It will be only a matter of time before we can assay and know that we can quantitate the ability to expand long-term marrow repopulating human stem cells. When this happens, we will probably not only be able to use single collections of cord blood to engraft larger-sized children and adults, but we will may also have the capacity to utilize a single cord-blood collection for engraftment of multiple recipients. Different cytokine combinations can enhance selected expansion of different lineages [49,75–77]. Having an assay for the long-term marrow repopulating human stem cell will allow us to determine

whether these cells are compromised during differential expansion of lineage-specific progenitor cell subsets. Cytokine receptor expression is beginning to be used to define subsets of stem and progenitor cells in mice [78] and it is possible that such approaches may be usable in the future to define the earliest human stem cells and the cytokines to which they will respond.

The migration and homing of stem cells is poorly understood. While a number of receptors, including those for cytokines and integrins, are believed to be involved in this process, this is clearly another area which needs investigation. It is possible that these and other receptors may be involved in the high frequency of primitive cells in cord blood at the birth of a child and in the drastic decrease in the circulating levels of these cells within days after birth [45]. Whether such migration/homing receptors are influenced in expression or activity during attempts at *ex vivo* stem-cell expansion requires definition.

Gene transfer

Gene therapy is a conceptually exciting, but presently unrealized, clinical treatment. The potential for correction of genetic defects, or for insertion of genes which may act to activate the immune system to fight infections or cancer is intriguing and is obviously worthy of investigation.

Haematopoietic stem cells have become a logical choice for gene transfer in a gene-therapy setting since the first rigorous study demonstrating the introduction of new genetic material into what are now known to be a more mature subset of mouse stem cells [79]. However, while it has become relatively straightforward to transduce new genetic material into early mouse stem cells, such has not been the case with early human stem cells [72,80].

Human stem cells from cord blood may be efficient vehicles for the delivery of new or modified genetic material, based on their rapid proliferative responses to cytokines, their extensive proliferative ability and the capacity of early cord-blood cells to be expanded [6,45–61,67–74].

Immature cord-blood progenitors are capable of being efficiently transduced by both retroviral- [81,82] and adeno-associated viral [83,84] vector-mediated gene transfer. Retroviral-mediated gene transfer of a gene for adenosine deaminase (ADA) has already been used clinically in an attempt to treat patients with ADA-deficient SCID with transduced autologous $CD34^+$ cord-blood cells [85]. While a clinically therapeutic dose of transduced cells has not been defined yet in this situation, it is clear that the procedure resulted in ADA gene-marked cells being identifiable in the circulation of transplanted recipients for years after the transplant, although the frequency of these cells and the expression of the transduced gene is low [85].

Immature progenitors in cord blood have been transduced to high efficiency with a gene for the complementation group in Fanconi anemia (FAC) by both retroviral [86–89] and AAV vectors [HE Broxmeyer, DW Clapp and A Srivastava, unpublished observations], and patients with FAC may be a potential patient population for future attempts at using cord blood for gene transfer/gene therapy.

Cord-blood cells can be stored in a cryopreserved state for over ten years with high-efficiency recovery of viable immature progenitors with extensive proliferative capacity

[90]. Recovered, thawed cells also have the capacity to engraft SCID mice [91]. Cryopreserved cord-blood progenitor cells can be recovered and transduced to high efficiency, and these cells expanded *ex vivo* [86,91,92].

Different situations can be envisioned in the context of gene-transduced cord-blood stem and progenitor cells based on recent studies. Transduction of a ribozyme gene into cord-blood progenitors has led to expression of the ribozyme gene in the macrophage-like cells that derived from the transduced progenitors, and this was associated with resistance of these cells to infection by a macrophage tropic human immunodeficiency virus (HIV) type 1 [93]. How realistic such an approach will be to treating patients with acquired immune deficiency syndrome (AIDS) remains to be determined, but perhaps such a gene therapy approach may be of value in combination with some of the newer treatments for this disorder including the use of proteases and/or chemokines.

The use of gene transfer to introduce genes for cytokines or for cytokine receptors in order to differentially modulate the processes of stem- and progenitor-cell self-renewal, proliferation and differentiation can also be envisioned.

Insertion of genes for either the erythropoietin [94,95] or IL-9 [95,96] receptor into populations of $CD34^+$ cells or into single isolated $CD34^+$ cord-blood cells has increased the capacity for differentiating cells towards the erythroid progenitor cell type when cells were plated in the presence of erythropoietin with a cocktail of other cytokines including steel factor, IL-3 and GM-CSF. Introduction of both the erythropoietin and IL-9 receptors into single isolated $CD34^+$ cells [95] resulted in even greater enhancement of the numbers of erythropoietin-dependent erythroid progenitor cells. Retroviral vectors have also been used to insert genes for intracellular signal-transducing molecules such as Syp, a protein tyrosine phosphatase [97] and the oncogene H-*ras* [98,99]. These manoeuvres have modulated the growth characteristics of the transduced cord-blood progenitors. Manoeuvres such as these may be of future use for treatment.

Even though immature cord-blood progenitors respond rapidly to the proliferative-inducing activities of combinations of cytokines and have extensive self-renewal and proliferative capacities, these cells need to be preincubated and prestimulated with cytokines for optimal efficiency retroviral-mediated gene transduction [100]. A potential concern is that the stimulation with cytokines necessary for high-efficiency retroviral-mediated gene transfer might differentiate the cells to the extent that the levels of long-term marrow repopulating stem cells decrease. One effort to enhance retroviral-mediated gene transfer has entailed the use of fibronectin fragments [80,101]. This has enhanced the gene-transduction efficiency of some retroviral vectors into immature progenitors of cord blood [72,101], but it is not yet clear whether it has enhanced the gene-transduction efficiency into earlier cells such as the SCID repopulating cell [72].

AAV vectors offer the possibility of transducing genes into non- or slowly cycling cells, which is a functional phenotype associated with the earliest stem cells. High-efficiency gene transduction using a marker gene has been noted in immature cord-blood progenitors which did not require preincubation with cytokines prior to AAV infection [83]. However, there is a concern that AAV vectors may not stably integrate into stem and progenitor cells. Some evidence has been presented that AAV transduction of cord-blood $CD34^+$ cells leads to stable integration in a population of the transduced cells [102], Efforts are under way to isolate AAV vectors at greater purities. Many previous studies with AAV vectors will require reproduction using the much more highly purified AAV

vectors before people in the field become more accepting of the future uses of this particular viral vector system for gene transduction of haematopoietic stem and progenitor cells.

It would be helpful if one could use a marking system to identify the earliest or later stem-cell populations that have been transduced and use this marker to isolate the selected stem/progenitor subset of choice. Efforts in this direction are under way using a multiparameter fluorescence activated cell-sorting (FACS) assay to assess the gene-transfer efficiency and the immunophenotype of transduced cells simultaneously [103,104]. A retroviral vector which expressed high levels of a truncated version of the low-affinity nerve growth factor receptor was used for such analysis.

Ultimately, with the development of better vectors and improved gene-transduction procedures, it is hoped that gene therapy will become a reality. In the meantime, the National Institute of Health of the United States has chosen three national gene vector laboratories [105] which will produce clinical grade vectors. Indiana University was picked to produce both retroviral and AAV vectors under the direction of Dr Kenneth Cornetta. The University of Michigan was picked to produce liposomes, naked DNA and DNA-coated pellets under the direction of Dr Gary Nabel, and the University of Pennsylvania was picked to produce adenovirus and DNA virus vectors under the direction of Dr James Wilson.

Summary

Cord blood, a recently identified source of transplantable stem and progenitor cells, has shown encouraging results for treatment of a variety of malignant and non-malignant disorders in the context of sibling and unrelated allogeneic transplantation. To date, engraftment rates are high and GvHD relatively low. The entire spectrum of use for these stem and progenitor cells continues to evolve. This will be enhanced by the development of cord-blood banks, and will be dependent, in part, on attaining additional laboratory and clinical information. This includes assessment of the immunological reactivity of cord-blood cells, the growth characteristics of the stem and progenitor cells and their capacity to be expanded *ex vivo* without loss of the long-term marrow repopulating human stem cell, an event which will require monitoring after development of a quantitative assay for these earliest stem cells. This also includes the development of the next generation of viral vectors with enhanced procedures for high-efficiency gene transduction and high-level expression of the transduced gene. Successful efforts in these endeavours will no doubt broaden our capacity to utilize cord blood for clinical treatment.

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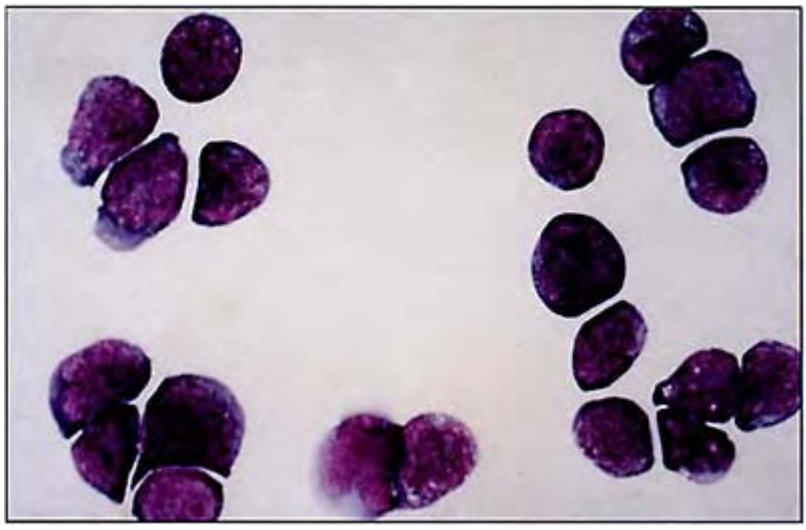

Chapter 31
Peripheral blood stem cells for autologous and allogeneic transplantation

Nigel H.Russell and Gail Miflin

Autologous PBSC transplantation

Introduction

In the last few years there has been a radical switch in the source of haemopoietic cells being used to support patients undergoing high-dose chemotherapy and autologous transplantation from bone marrow to peripheral blood (PB). This is most obviously apparent in an analysis of patients reported to the European Group for Blood and Marrow Transplantation (EBMT) which has shown that 65% of autologous transplants carried out in 1994 were from peripheral blood, whereas four years previously, this figure was only 15% [1]. These changes occurred as a consequence of developments in peripheral blood

stem-cell (PBSC) mobilization, particularly related to the use of haemopoietic growth factors which have made it possible to reliably harvest sufficient stem and progenitor cells to permit rapid haemopoietic engraftment following myeloablative therapy. This rapid haemopoietic recovery has translated into major clinical benefits, particularly in terms of reduced antibiotic and blood product usage, resulting in reduced hospital stay with subsequent economic savings when compared to autologous bone-marrow transplantation (BMT). These more rapid engraftment kinetics have now been demonstrated in a randomized clinical trial comparing PBSC with bone marrow for autologous rescue of patients undergoing high-dose chemotherapy for lymphoma [2], As PBSC transplants are associated with less morbidity and more rapid recovery of haemopoietic function following high-dose chemotherapy than bone marrow, they are now considered to be the most appropriate source of haemopoietic support for patients undergoing high-dose chemotherapy for many conditions. Despite this recommendation, there is so far little evidence from prospective randomized trials to demonstrate an improved outcome from PBSC transplant compared to BMT. However, the developments in PBSC which have resulted in increased safety are now allowing high-dose therapy to be evaluated earlier during the course of the patient's treatment when it is most likely to have a maximal antitumour effect.

In allogeneic transplantation, the use of PBSC instead of bone marrow has also been demonstrated to have major advantages, particularly related to rapid haematological and immune reconstitution, and this has led to the increasing use of PBSC in this setting, which is now being evaluated in the context of randomized trials.

The characteristics of blood stem cells

That large number of clonogeneic haemopoietic progenitors can be mobilized into the peripheral blood from cancer patients and from normal donors is clearly established. Committed progenitor cells including CFU-GM, BFUe, CFU-Meg and CFU-GEMM have all been cultured with peak values rising 10–100-fold over basal peripheral blood levels. Furthermore, long-term culture-initiating cells (LTC-IC) have been detected in human peripheral blood harvests [3] and mobilized murine peripheral blood contains cells with long-term repopulating capacity when transplanted into irradiated animals [4]. Phenotypic studies suggest that human LTC-IC are found in the $CD33^-$, $CD38^-$, $HLA\text{-}DR^-$ fraction of $CD34^+$ cells, and immunophenotypic studies of PB cells mobilized by granulocyte colony-stimulating factor (G-CSF) or by chemotherapy and G-CSF have established that these pluripotent stem cells are present in proportions similar to those found in bone marrow [5]. These data support the contention that mobilized PB contains not only $CD34^+$ cells with a mature phenotype ($CD34^+$, $CD33^+$, $CD38^+$) but also primitive cells with long-term repopulating capacity. These conclusions are supported by the recent demonstration that gene-marked $CD34^+$ cells from peripheral blood stem-cell harvests contain cells whose progeny could be detected in multiple haemopoietic lineages 18 months after transplant [6].

Important immunophenotypic differences between bone marrow and mobilized peripheral blood $CD34^+$ fractions have been reported. In particular, the fraction of $CD34^+$ cells co-expressing CD19 is much lower in peripheral blood (<1%) than in bone marrow (10–20%), which may be of importance as dual expressing CD34/CD19 lymphoid

progenitor cells have been implicated as being involved in the pathogenesis of certain types of low-grade non-Hodgkin's lymphoma [7]. The proportion of $CD34^+$ cells co-expressing different activation antigens and lineage-specific antigens in peripheral blood compared to bone marrow is shown in Table 31.1.

Table 31.1 Immunophenotype of $CD34^+$ cells in bone marrow compared to mobilized peripheral blood (adapted from To et al. [5])

Immunophenotype	Bone marrow (%)	G-CSF-mobilized peripheral blood (%)	Chemotherapy+G-CSF peripheral blood
CD38	96.1±0.8	88.0±3.0	97.0±1.1
$HLA\text{-}DR^+$	94.8±1.5	89.0±4.8	96.7±0.4
$CD33^+$	38.5±9.4	28.2±7.6	39.9±17.9
$CD13^+$	59.1±4.1	67.9±9.7	81.3±3.0
$CD15^+$	6.9±0.5	3.4±1.2	3.8±1.0
$CD10^+$	18.8±6.9	1.2±0.4	0.2±0.4
$CD19^+$	16.1±6.1	0.9±0.3	0.1±0.1
$CD7^+$	4.0±1.7	1.9±0.5	0.5±0.3

Mobilization and collection of blood stem cells

Mobilization with chemotherapy alone

In the steady state, relatively few stem cells circulate in the peripheral blood, and although it is possible to collect sufficient cells to support patients undergoing high-dose chemotherapy, multiple leukapheresis procedures are required. The demonstration that the numbers of circulating colony-forming cells of the granulocyte-macrophage lineage (CFU-GM) increased following chemotherapy [8] by 20-fold led to the development of clinical studies using peripheral blood stem-cell collections timed to coincide with the recovery of white-cell counts following chemotherapy-induced neutropenia. To *et al.* [9] used intermediate-dose cyclophosphamide (4 g/m^2) for mobilization and reported a mean peak CFU-GM elevation of peripheral blood 14-fold above baseline. With this mobilization protocol they were able to obtain sufficient cells to accelerate neutrophil and platelet recovery following high-dose chemotherapy compared to patients who received autologous bone-marrow support. The author reported that the time taken to reach a neutrophil count (ANC) of $>0.5\times10^9$/litre was 11 days for PBSC compared to 24 days for bone marrow. Likewise, platelet recovery to $>20\times10^9$/litre was significantly faster (13.5 versus 33 days) [10]. Although these engraftment results were impressive, following the

introduction of recombinant haemopoietic growth factors into clinical practice, the mobilization of PBSC with chemotherapy alone has largely been superseded by the combination of chemotherapy and growth factors or by growth factors alone.

Mobilization of PBSC with growth factors with or without chemotherapy

One of the unexpected benefits of the introduction of recombinant haemopoietic growth factors into clinical practice was the observation that the leukocytosis associated with treatment with either G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF) was accompanied by an increase in the numbers of CFU-GM and BFUe circulating in the peripheral blood [11,12]. Following the administration of G-CSF for four days, Sheridan *et al.* [13] observed a median 58-fold increase in the numbers of circulating CFU-GM in previously treated cancer patients, peaking at days 5 and 6 of G-CSF therapy. In this study, G-CSF (12 μg/kg/day) was administered from steady state, thus avoiding the neutrophil nadir and risk of sepsis associated with chemotherapy-induced mobilization. Furthermore, the kinetics of mobilization with growth factor are usually more predictable than are those following chemotherapy. Lower doses of G-CSF (5 μg/kg/day) have been studied but appear to be less effective for mobilization from the steady state [14]. The use of G-CSF to mobilize PBSC from normal donors is discussed in greater detail below.

The use of G-CSF and GM-CSF to enhance chemotherapy-induced peripheral blood stem-cell mobilization has also been extensively studied. Thus, Gianni *et al.* [15], using cyclophosphamide at a dose of 7g/m^2 in combination with GM-CSF, reported an up to 1000-fold elevation in circulating CFU-GM. Likewise, G-CSF when combined with conventional chemotherapy produced a 278-fold increase in circulating CFU-GM [3].

Cyclophosphamide at high doses (4–7 g/m^2) is associated with considerable morbidity and occasional mortality, and a number of groups have explored the use of lower doses of cyclophosphamide in combination with G-CSF. In this context, Jones *et al.* [16] reported that cyclophosphamide (1.5 g/m^2) combined with G-CSF could mobilize sufficient PBSC for successful engraftment following a median of just one leukapheresis. This dose of cyclophosphamide avoided significant neutropenia and there were no admissions for infectious complications during mobilization in 26 consecutive patients. The efficacy of mobilization with lower doses of cyclophosphamide (<4 g/m^2) which avoids severe neutropenia and thrombocytopenia has been confirmed by others [17]. In a series of 77 consecutive patients with lympho-proliferative diseases we were able to mobilize >2.5×10^6/kg CD34$^+$ cells from 46 patients (66%) with cyclophosphamide (3 g/m^2) and G-CSF (300 μg/d). In a further 26 patients the total number of CD34$^+$ cells harvested was between 1.0 and 2.5×10^6/kg (median 1.6×10^6/kg), these patients were all transplanted with PBSC alone. A total of 16 patients were considered to be stem-cell mobilization failures. Six of these patients finally underwent high-dose therapy following autologous bone-marrow harvest and combined bone marrow and PBSC support.

In a randomized trial of two different mobilization protocols using cyclophosphamide and G-CSF, patients who received high-dose cyclophosphamide (7 g/m^2) reached statistically higher peak values of peripheral blood CD34$^+$ cells than patients who received cyclophosphamide at 4 g/m^2. This resulted in fewer leukaphereses being

required to obtain >2.5×10^6/kg $CD34^+$ cells, suggesting that this dose of cyclophosphamide may be more efficient at mobilizing these cells, although it is also more toxic [18].

Kinetics of stem-cell mobilization

In patients or normal subjects mobilized with growth factors the peak blood levels of $CD34^+$ cells and CFU-GM occur after four to five days of G-CSF therapy [14,19]. This predictable release of progenitor cells facilitates the timing of apheresis and is one of the major attractions of using growth factors alone for mobilization. With the combined use of chemotherapy and growth factors the timing of leukapheresis is more difficult and can be monitored either by the recovery of the white-cell count following myelosuppression or by the daily measurement of peripheral blood $CD34^+$ cells. Pettengell *et al.* [3] initiated the first leukapheresis when the white-cell count reached between 5 and 10×10^9/litre. In this study, the peak progenitor cell release coincided with the exponential increase in the white-cell count. Likewise, the peak in progenitor cells coincided with the maximal release of primitive LTC-IC into the circulation.

In heavily pretreated patients, the timing of the initiation of leukapheresis is more problematic, particularly as these patients may have a slow and/or delayed recovery of white-cell counts following chemotherapy. In these patients, sequential monitoring of peripheral blood $CD34^+$ cell counts can be of value. In our experience, there is a good correlation between the number of $CD34^+$ cells in the blood and that finally collected in the leukapheresis product (Figure 31.1), and a peripheral blood $CD34^+$ count of >25/μl would predict a yield of $CD34^+$ cells and a stem-cell harvest of >2.5×10^6 $CD34^+$ cells/kg.

Engraftment following PBSC transplantation

Introduction

The major advantage of PBSC over bone marrow for the support of patients undergoing high-dose chemotherapy relates to the rapid engraftment of both neutrophils and platelets. Table 31.2 shows the results of selected studies of PBSC giving median days to both neutrophil and platelet recovery. These results have now been confirmed in a randomized study comparing PBSC to bone marrow for patients undergoing high-dose chemotherapy for lymphoma, with both groups of patients receiving post-transplant growth factor (G-CSF) support. In this study, the time required for both neutrophil engraftment to an ANC of >0.5×10^9/litre (11 versus 14 days) and engraftment of platelets to >20 ×10^9/litre (16 versus 23 days) was significantly more rapid in the PBSC group [2].

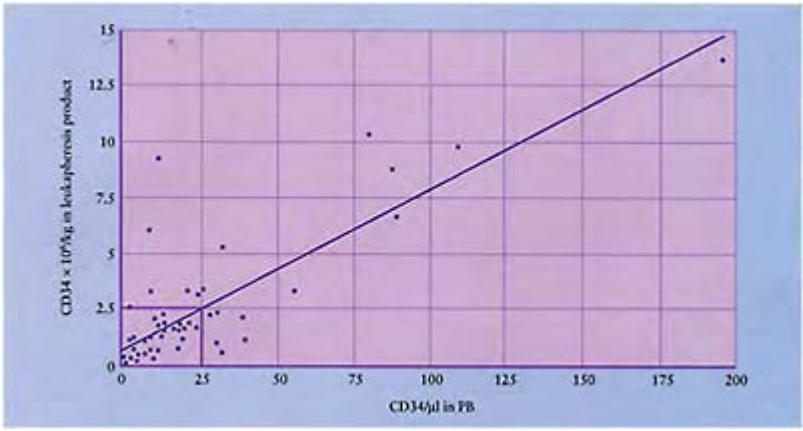

Figure 31.1 The relationship between CD34$^+$ cells measured in the peripheral blood (PB) and CD34$^+$ cells subsequently collected in the leukapheresis product shows a good correlation (r=*0.81*). *A count of >25/µl would predict a yield of >2.5×10^6 CD34$^+$ cells/kg in the stem-cell harvest.*

Table 31.2 Effect of number of CD34$^+$ cells on haematologic reconstitution following high-dose therapy

Study	CD34+ cells (×10^6/kg)	Days to ANC >0.5×10^9/litre	Days to platelets >20×109/litre
Schwartzberg *et al.* [21]	>2.5	12 (9–21)	9
	<2.5	14 (11–27)	15(6–43)
Haynes *et al.* [17]	>2.5	12 (9–17)	12 (8–40)
	<2.5	16 (11–26)	26 (11–118)
Haas *et al.* [20]	>2.5	<14	<14
	<2.5	17 (11–34)	31 (13–141)

Engraftment kinetics: relation to quality of graft

Over the last few years the importance of $CD34^+$ cell enumeration to monitor the quality of PBSC harvests has become well established. The CD34 antigen is expressed on the majority of haemopoietic precusor cells including CFU-GM and BFUe as well as being expressed on LTC-IC, and the detection of $CD34^+$ cells in stem-cell harvests has been used successfully as a surrogate marker for these haemopoietic progenitor cells. The actual number of $CD34^+$ cells required for rapid engraftment following high-dose therapy has been the subject of much debate. Schwartzberg *et al.* [21] reported that optimal engraftment kinetics were associated with the infusion of $>2.5\times10^6$/kg $CD34^+$ cells, a figure which has been confirmed by a number of other studies [17,20,22] (Table 31.2). However, others have suggested that numbers in excess of 5×10^6/kg $CD34^+$ cells are required for rapid engraftment [23]. These differences may be explained, at least in part, by methodological differences in $CD34^+$ cell analysis.

The importance in obtaining an optimal PBSC collection is illustrated by the delayed neutrophil and particularly platelet engraftment seen in patients who receive a sub-optimal graft. Thus, we have found that patients who received $<2.5\times10^6$/kg $CD34^+$ cells had significantly delayed neutrophil ($>0.5\times10^9$/litre) and particularly platelet ($>20\times10^9$/litre) engraftment (15 days and 26 days, respectively) compared to patients who received $>2.5\times10^6$/kg $CD34^+$ cells (11 days and 12 days; $P<0.05$) [17] (Figure 31.2). Furthermore, as shown in Table 31.3, patients receiving a sub-optimal graft had significantly increased requirements for antibiotic and blood product support. Similar results have been published using the CFU-GM assay to monitor stem-cell harvest quality. Thus, in a series of 118 patients with haematological malignancies undergoing PBSC transplantation, those patients receiving $>20\times10^4$ CFU-GM/kg had particularly prompt haemopoietic recovery [21]. The advantages of CD34 enumeration in this setting relate to its reproducibility and speed, thus allowing analysis of stem-cell harvests within an hour or two of completion of the leukapheresis, thereby permitting decisions to be made concerning the need for further collections. Clonogenic assays such as CFU-GM give delayed results and are not useful in this context but do permit a useful quality control of cellular viability of the leukapheresis product following the cryopreservation procedure. It is likely, then, that both assays should be used for quality assessment of the graft.

Although a $CD34^+$ cell count of $>2.5\times10^6$/kg is associated with optimal engraftment, transplantation can be undertaken with fewer cells but the patient will be at risk of delayed engraftment, and in particular of platelets. Thus, in our experience, patients receiving between 1 and 2.5×10^6/kg have delayed platelet engraftment $>20\times10^9$/litre at a median of day 26 and $>50\times10^9$/litre at a median of day 50 [17]. For patients who mobilize less than 2.5×10^6/kg there are a number

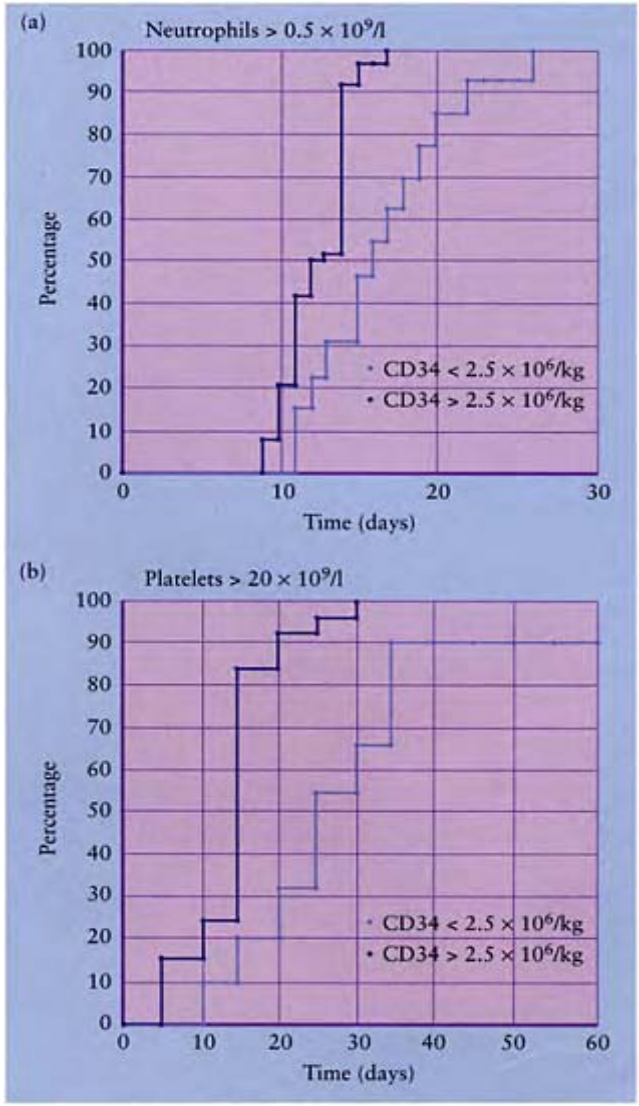

Figure 31.2 Kaplan-Meier probability of reaching 0.5× 10^9/litre neutrophils (a) (P<*0.005*) *or 20×10^9/litre platelets (b)* (P<*0.005*) *comparing patients receiving more or less than 2.5×10^6 $CD34^+$ cells/kg after high-dose chemotherapy.*

Table 31.3 Effect of $CD34^+$ cell dose on post-PBSC transplant support therapy requirement (adapted from Haynes et al. [17], with permission)

Parameter	$>2.5\times10^6$/kg (n=24)	$<2.5\times10^6$/kg (n=14)	*P* value
Post-transplant in-patient stay	15 (11–23)	20 (15–60)	0.02
Red-cell transfusions (units)	4 (0–14)	7 (2–31)	<0.05
Platelet transfusions (days)	2 (0–8)	5 (5–25)	<0.05
Febrile days	4 (0–12)	6 (0–12)	0.38
Days of i.v. antibiotics	6 (0–13)	10 (0–20)	0.06

of possible options. Our practice would be to proceed to high-dose therapy if the $CD34^+$ count were $>1.0\times 10^6$/kg. However, for counts $<1.0\times10^6$/kg we would either repeat the mobilization procedure or undertake a bone-marrow harvest to use as a combined PBSC plus bone-marrow transplant. For these true mobilization failures, there is evidence that bone-marrow harvesting can still allow successful completion of high-dose therapy [22,24].

Factors affecting PBSC mobilization and engraftment

Certain factors are predictive of poor stem-cell mobilization, the most clearly established being the quantity and type of preceding chemotherapy and radiotherapy. The amount of prior exposure to alkylating agents is particularly important. Haas *et al.* [20] calculated an average decrease of 0.2×10^6/kg CD34 cells per leukapheresis in malignant lymphoma per course of previous combination chemotherapy that patients had received, and that a course of large-field radiotherapy reduced the yield by an average of 1.8×10^6/kg $CD34^+$ cells. Likewise, Dreger *et al.* [22] found that more than one cycle of the myelosuppressive regimen Dexa-BEAM significantly reduced the yield of $CD34^+$ cells in PBSC harvests in lymphoma patients although this regimen did not affect the quality of bone-marrow harvests. The adverse effect of certain drugs on mobilization is well recognized. Thus, patients exposed to the cumulative effects of drugs such as busulphan, melphalan, BCNU are particularly at risk of being mobilization failures. In contrast. Pettengell *et al.* [3] did not find a reduction of circulating colony-forming cells during repeated cycles of the VAPEC-B regimen (vincristine, adriamycin, prednisolone, etoposide, cyclophosphamide and bleomycin), confirming that different chemo-therapeutic drugs vary in their capacity to affect the pool of haemopoietic progenitors available for mobilization. The effect of the four-drug combination regimen ABCM (adriamycin, BCNU, melphalan, cyclophosphamide) on the CD34 cell yield for PBSC harvests of myeloma patients is shown in Figure 31.3. The median yield of $CD34^+$ cells for patients receiving any ABCM therapy was 0.23×10^6/kg/leukapheresis. In contrast, patients receiving infusional vincristine, adriamycin, dexamethasone or methylprednisolone (VAD or VAMP) had higher yields of $CD34^+$ cells (median

3.04×10^6/kg/leukapheresis). It is clear that patients who are being considered for high-dose chemotherapy should be mobilized early in their treatment plan following initial disease control with cytotoxic regimens that are relatively stem-cell sparing. Thus, cumulative use of agents such as melphalan, BCNU and chlorambucil should be avoided.

Use of growth factors following PBSC transplant

Compared with autologous bone-marrow transplantation, haemopoietic reconstitution with PBSC is more rapid and in-patient stay is also reduced. Despite these advantages, there remains an obligate period of severe post-transplant neutropenia when the patient is at risk of sepsis. The infusion of a myeloid growth factor

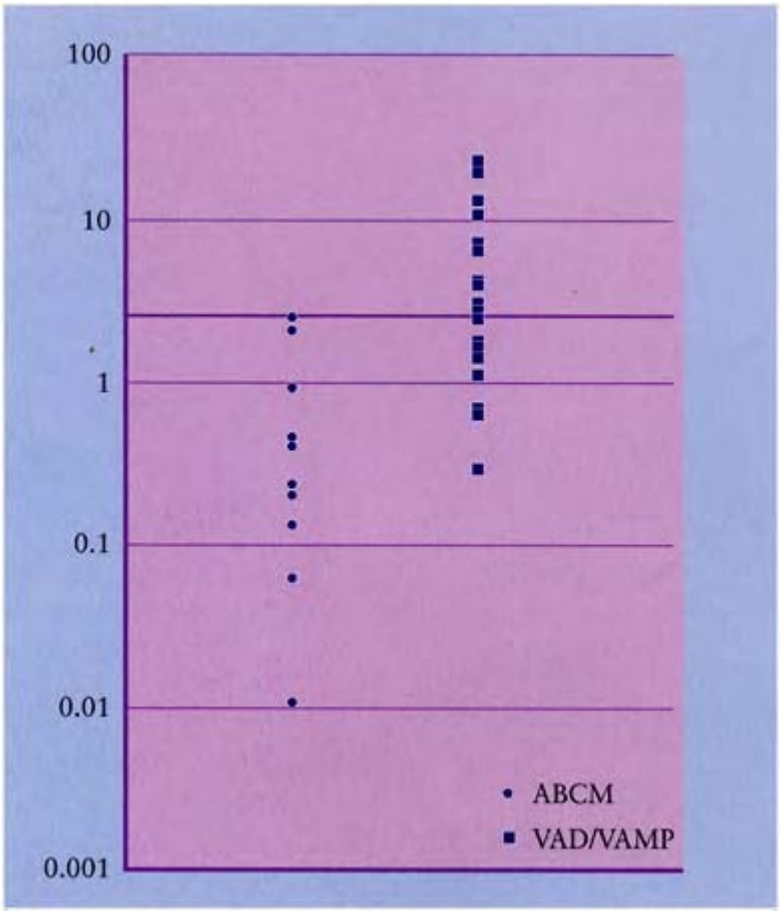

Figure 31.3 The effect of different previous regimens of chemotherapy on the yield of $CD34^+$ cells following

subsequent stem-cell mobilization. Previous ABCM chemotherapy results in lower numbers of $CD34^+$ cells/kg/leukapheresis than previous treatment with VAD or VAMP chemotherapy.

(G-CSF or GM-CSF) has been clearly shown to improve the rate of neutrophil but not platelet recovery following autologous BMT, and a number of studies of G-CSF or GM-CSF following PBSC transplant have been carried out (see Table 31.4). These studies have generally shown a reduction in the time taken to reach a neutrophil count of $>0.5\times10^9$/litre by some two to three days and generally a reduction in the duration of the post-transplant in-patient stay. However, the cost of post-transplant growth factors has raised questions concerning the economic viability of their use. In a non-randomized study, Shimazaki *et al.* [25] suggested that a lower dose of G-CSF (50 μg/m^2) may be as effective as conventional doses (150 μg/m^2 or 5 μg/kg/day). These results have been confirmed in a randomized study of low-dose G-CSF in patients undergoing PBSC transplant for lymphoproliferative diseases [26]. In this study, the G-CSF group reached an ANC of $>0.5\times10^9$/litre at day +10 compared to day +14 for the placebo group, and there was an associated reduction in post-transplant in-patient stay from 16 to 13 days with significant economic savings which were not compromised by the cost of post-transplant G-CSF.

Contamination of PBSC harvests by tumour cells

One of the initial rationales for using PBSC instead of bone marrow for autografting was that the technique could be used in patients when their bone marrow was infiltrated with disease. However, although PBSC harvests may contain less tumour cells than bone marrow, a number of studies over the last four years have shown that leukaemia, myeloma, non-Hodgkin's lymphoma, breast cancer and neuroblastoma cells can be detected in collections from a significant proportion of patients. Furthermore, gene-marking studies have shown that haemopoietic stem cells infused at the time of bone marrow or PBSC rescue can contribute to relapse in acute myeloid leukaemia, neuroblastoma and chronic myeloid leukemia [27–29]. Also, clinical studies using autologous bone marrow have reported that the elimination of contaminating lymphoma cells from the graft improves the outcome following high-dose chemotherapy [30]. These observations have led to attempts to define patients at risk of contaminated PBSC collections, to minimize tumour-cell contamination at the time of harvest and to develop techniques for the purification of $CD34^+$ cells from harvests in order to deplete tumour-cell contamination.

Myeloma

Using sensitive allele-specific polymerase chain-reaction (PCR), circulating clonal plasma cells can be demonstrated in almost all myeloma patients at diagnosis [31–33];

however, following successful initial cytoreduction with infusional VAD-based regimens, clonal plasma cells cannot be detected in steady-state blood samples [33]. Despite this, PBSC harvests from >50% of patients contain clonally rearranged cells following stem-cell mobilization which can be detected by immunoglobulin gene finger-printing techniques with a sensitivity of 1 in 10^4 [33,34]. Using more sensitive allele-specific PCR techniques, it has been suggested that all PBSC harvests may be contaminated [35]. Owen *et al.* [33] reported that the likelihood of detecting clonal plasma cells in PBSC harvests was

Table 31.4 Results of randomized trials of growth factors following autologous PBSC transplantation

Study	Growth factor (dose)	ANC>0.5 (days)		Post-transplant in-patient stay (days)	
		Growth factor	Placebo	Growth factor	Placebo
McQuaker *et al.* [26] (n=38)	G-CSF 50 μg/m^2/day	10	14	13	16
Linch *et al.* [78] (n=90)	G-CSF (263 μg/kg/day)	9	12	13	16
Spitzer *et al.** [79] (n=40)	G-CSF (7.5 μg/kg/day) +GM-CSF (2.5 μg/kg/day)	10	16	19	21
Klumpp *et al.*** [80] (n=41)	G-CSF (5 μg/kg/day)	10.5	16	18	24
Advani *et al.**** [81] (n=39)	GM-CSF (10 μg/kg/day)	12	10	Data not given	

*All patients received marrow in addition to peripheral blood stem cells.
**20 received marrow in addition to peripheral blood stem cells.
***26 received marrow in addition to blood stem cells.

higher in patients with significant residual bone-marrow disease, particularly those patients with >10% plasma cells in the bone-marrow aspirate immediately prior to mobilization. Gazitt *et al.* [36] have suggested that the kinetics of mobilization of plasma cells in myeloma patients may be different to normal progenitors. Using an immunofluorescent technique for plasma-cell detection, they found that in contrast to $CD34^+$ cells, the highest concentration of myeloma cells was collected during days 5 and 6 of leukapheresis when the percentage and proportion of $CD34^+$ cells was rapidly declining. They suggested that a window of opportunity exists for PBSC collection, with the highest progenitor-cell content and minimum myeloma-cell contamination in the early phase of white-cell recovery following cyclophosphamide-induced neutropenia. In

this study, the trigger for initiation of leukapheresis was a white-cell count of 0.5×10^9/litre.

In another study, Goldschmidt *et al.* [18] found a higher proportion of $CD19^+$ cells in PBSC harvests from myeloma patients mobilized with 7 g/m^2 of cyclophosphamide compared to 4 g/m^2. In this study, the patients receiving 7g/m^2 of cyclophosphamide also had statistically significant higher peak values for $CD34^+$ cells in the peripheral blood although haematological toxicity was more severe and prolonged in patients receiving the higher dose of cyclophosphamide.

The role of positive selection of CD34 cells for engrafting in myeloma is being currently explored in randomized trials. However, these results suggest that myeloma-cell contamination of PBSC harvests can be potentially minimized firstly by maximizing disease response to conventional therapy prior to mobilization and secondly by initiating stem-cell leukapheresis during the early recovery phase of the white-cell counts following chemotherapy.

Non-Hodgkin's lymphoma

High-dose chemotherapy followed by autologous bone marrow or PBSC support is increasingly used for the treatment of patients with relapsed non-Hodgkin's lymphoma. It is well characterized that the infusion of stem cells from either the bone marrow or blood is associated with a risk of infusing contaminating lymphoma cells; 75% of patients with follicular lymphoma have circulating lymphoma cells as detected by PCR for t(14;18), including patients in complete remission after conventional chemotherapy [30,37,38]. $CD34^+$ cell selection of autologous bone marrow was found to successfully purge 8 out of 9 patients of PCR-positive Bc1-2-1gH rearranged cells [39]. However, the use of $CD34^+$ selection in follicular lymphoma is complicated by the demonstration that primitive lymphoid progenitors may be dual CD34/CD19-positive, and recent evidence suggests that such cells may contain the t(14–18) rearrangement [7], raising the possibility that lymphoma progenitor cells may be reinfused following CD34-selection procedures. Of relevance here is the demonstration that the proportion of $CD34^+$ cells co-expressing CD19 is significantly lower in PBSC than in bone-marrow harvests with the number in PBSC harvests being <1% of the $CD34^+$ population [5,20,40] (Table 31.1). Furthermore, in 9 patients known to have t(14;18) PCR-positive stem-cell harvests, 6 were converted to PCR negativity following CD34 selection. These data would suggest that PBSC are likely to be less heavily contaminated with lymphoma cells than bone marrow and are the material of choice for any $CD34^+$ selection procedure in lymphoma.

Isolation and transplantation of $CD34^+$ haemopoietic cells

A number of studies using different purging procedures such as chemicals or monoclonal antibodies have suggested that the elimination of contaminating tumour cells from a bone-marrow graft improves outcome following high-dose chemotherapy. However, purging procedures run the risk of damaging the haemopoietic repopulating cell compartment with the subsequent risk of delayed engraftment and poor graft function.

The revelation that the $CD34^+$ subset of haemopoietic cells contains both progenitor cells (CFU-GM, BFUe) and more primitive cells including LTC-IC gave impetus to the development of techniques of $CD34^+$-positive selection of stem cells. The most frequently used technique is based upon binding between biotinylated monoclonal anti-CD34 antibodies and avidin-coated particles [41]. Engraftment kinetics following $CD34^+$-selected transplants appear comparable to those achieved with unmanipulated grafts. In a series of 7 patients with breast cancer who received a mean of 1.6×10^6/kg $CD34^+$ cells, engraftment times (supported by G-CSF) were comparable, with an ANC of $>0.5\times10^9$/litre achieved after 11 days, and $>20\times10^9$/litre platelets after 17 days. A number of studies of $CD34^+$-selected PBSCT have since been reported and these engraftment kinetics have been confirmed (Table 31.5). All positive selection procedures are associated with significant losses of $CD34^+$ cells ranging from 30 to 50%, resulting in the necessity for collecting greater numbers of cells to offset these losses and to permit cryopreservation of unprocessed leukapheresis products as a back-up. The purity of the positively selected fraction ranges in most series from 40 to 80%. The optimum number of $CD34^+$ cells for rapid engraftment is probably similar to that required for unselected transplants; indeed, Schiller *et al.* [42] noted delayed platelet engraftment in those patients receiving $<2.0\times10^6$/kg purified $CD34^+$ cells (Table 31.5).

The evidence from a number of studies in breast cancer, myeloma and lymphoma is that $CD34^+$ selection can result in significant depletion of contaminating tumour cells [40,42]. Schiller *et al.* [42] treated 37 patients with advanced myeloma with high-dose busulphan and cyclophosphamide followed by $CD34^+$-selected PBSC transplantation. They achieved a

Table 31.5 Engraftment following infusion $CD34^+$-selected PBSC: results of selected studies

Study	Number of $CD34^+$ cells infused ($\times10^6$/kg)	ANC $>0.5\times10^9$/ litre (days)	Platelets $>20\times10^9$/ litre (or platelet independence) (days)
Schiller *et al.* [42] (*n*=37)	4.65 (1.2–20.3)	12 (11–16)	12 (9–52)
Shpall *et al.* [82] (*n*=7)	1.60 (0.4–3.9)	11 (10–12)	17 (11–156)
McQuaker *et al.* [40] (*n*=13)	1.54 (0.88–76)	13 (11–21)	14 (10–44)

2.7 to >4.5 log depletion in the number of contaminating myeloma cells following the selection procedure, and residual tumour-cell contamination was detected only in patients with heavily contaminated harvests. Finally, fluorescent activated cell sorting (FACS) offers a powerful system for purifying populations of haemopoietic stem cells ($CD34^+$, $Thy1^+$, Lin^-). High-speed FACS devices have become available which may provide a clinical-scale system for the purification of immature $CD34^+$ cells with efficient reduction of contaminating tumour cells [43].

Allogeneic PBSC transplants

Introduction

The established advantages of PBSC over bone marrow in autologous transplantation, in particular in terms of faster haemopoietic engraftment, earlier hospital discharge and decreased associated costs [10,44], coupled with the knowledge that haemopoietic growth factors alone can be used to mobilize PBSC, have resulted in the evaluation of PBSC for allogeneic transplantation. The first allogeneic PBSC transplant was performed in 1989 without the use of growth factors [45] and it was not until 1993 that the first allogeneic transplants using G-CSF-mobilized stem cells were reported [19,46]. These studies established that PBSC could be safely mobilized from normal donors and that transplantation resulted in durable trilineage engraftment in the recipients without severe graft-versus-host disease (GvHD). This was subsequently confirmed in several reports mainly of patients with advanced leukaemia and lymphoma [47–50]. These reports also suggested that there may be advantages over allogeneic BMT in terms of faster haemopoietic engraftment. As a consequence of these perceived advantages, the number of allogeneic PBSC transplants being reported to the EBMT are increasing rapidly with each year [1]. It is also emerging that there are likely to be other advantages in terms of decreased procedural costs due to a decrease in antibiotic and transfusion requirements post-transplant [51–53] (Table 31.6). However, there are still many issues concerning allogeneic PBSC transplant that remain to be clarified. The most important of these include the optimum method of stem-cell mobilization in normal donors, the incidence of GvHD in the recipients and whether or not allogeneic PBSC transplant confers any benefit in terms of graft-versus-leukaemia (GvL) effects, faster immune reconstitution and overall disease-free survival.

Mobilization and collection of stem cells from normal donors

Although it has been reported that sufficient PBSC can be collected from the steady state to use for transplantation [45] this required the donor to undergo ten leukapheresis procedures, which is clearly not practical. The major advance in this area has come from the demonstration that haemopoietic growth

Table 31.6 Comparison of allogenic PBSCT transplant and BMT for antibiotic and blood product support requirements

Study	Transplant	Antibiotic use (days)	Amphotericin use (days)	Red-blood cell transfusions	Platelet transfusions
Azevedo	PBSC	16.5 (9–929)	0 (0–20)	9.5 (1–30)	5.5 (2–23)

et al. [53] (n=22)	BMT	24* (10–36)	1 (0–25)	9* (4–25)	9 (4–39)
Russell *et al.* [51] (n=26)	PBSC	N/A	N/A	2 (2–15)	3 (1–21)
	BMT			4 (0–16)	5* (2–15)
Bensinger *et al.* [52] (n=37)	PBSC	N/A	N/A	8 (0–116)	24 (6–246)
	BMT			17* (2–105)	118* (7–988)

*Statistically significant difference. N/A=not available.

factors alone can mobilize sufficient stem cells into the peripheral blood for harvest and subsequent allogeneic transplantation [46]. The growth factor of choice for mobilization of normal donors is G-CSF, due to its excellent safety record and minimal side-effects. GM-CSF has more side-effects but has been used in combination with G-CSF in one study in an attempt to decrease the number of leukapheresis procedures required [54]. Preliminary evidence with this approach suggested that the combination of G-CSF and GM-CSF is able to mobilize similar numbers of $CD34^+$ cells but may also yield a higher proportion of primitive $CD34^+$ subsets than G-CSF alone [55]. However, it is by no means clear whether this would have any clinical advantages.

The optimum mobilization and harvesting schedule in normal donors is still unclear. Doses of G-CSF, ranging from 2.5 to 16 μg/kg/day [47,56] have been suggested. The aim, clearly, is to use a sufficiently large dose of G-CSF to permit collection of enough $CD34^+$ cells in one or two leukapheresis procedures, whilst at the same time minimizing donor side-effects and drug costs. Grigg *et al.* [14] showed that cohorts of donors who received G-CSF at 10 μg/kg/day had significantly higher numbers of $CD34^+$ cells in the graft than did donors who received either 5 or 3 μg/kg/day. Lower doses of G-CSF, while more economical and associated with a lower incidence of donor side-effects, may be disadvantageous in that a greater number of leukapheresis procedures are necessary.

Other groups have used higher doses of G-CSF, from 12 to 16 μg/kg/day, and as shown in Table 31.7, there may be some evidence that donors who receive higher doses of G-CSF mobilize greater numbers of $CD34^+$ cells.

The optimum time to perform the first leukapheresis in terms of yield and graft constitution has been clarified by a number of studies [57]. Dreger *et al.* [19] showed that peak circulating levels of $CD34^+$ cells consistently occurred 24 hours after the fourth dose of G-CSF when this was used at a dose of 10 μg/kg/day. Grigg *et al.* [14] showed similar results; circulating levels of CFU-GM were maximal on day 5 of G-CSF treatment and peak $CD34^+$ levels occurred between days 4 and 6. Another group analyzed leukapheresis products from two cohorts of donors all mobilized with G-CSF at 12 μg/kg/day, with the first leukapheresis performed either on day 4 or day 5 of G-CSF administration. This showed that collection on day 5 resulted in more consistent achievement of a target cell dose of 4×10^6 $CD34^+$ cells/kg in one leukapheresis procedure, with significantly higher numbers of $CD34^+$ cells in the day 5 collection compared to the day 4 collection. There was no significant difference in lymphoid subsets or natural killer cell populations between the two groups.

Table 31.7 Comparison of G-CSF dose and $CD34^+$ yield in normal donors

Study	Dose of G-CSF (μg/kg/day)	$CD34^+$ cell yield ($\times 10^6$/kg recipient)
Bensinger *et al.* [47] (n=8)	16	13.1 (6.9–21.6)
Korbling *et al.* [48] (n=9)	12	10.7 (7.5–22.5)
Miflin *et al.* [59] (n=17)	10	6.8 (2.4–15.6)

The current recommendation by the EBMT, and one which we have utilized, is to mobilize stem cells using G-CSF at a dose of 10 μg/kg/day, starting the G-CSF five days prior to the scheduled transplant date and performing the first leukapheresis on the day following the fourth dose of G-CSF [58]. We have mobilized stem cells in 17 normal donors with this protocol and collected a median of 6.8×10^6 $CD34^+$ cells/kg recipient weight (range $2.4–15.6\times10^6$) with a median of two leukaphereses [59]. In our unit, we analyze the harvest from the first leukapheresis (collected on day −1 of conditioning) for $CD34^+$ cell content and keep it at 4°C overnight. If further $CD34^+$ cells are required, i.e. the first leukapheresis product contains $<4\times10^6$ CD34+ cells/kg recipient weight, then a second leukapheresis is performed the following day and both collections are infused together on day 0.

As in autologous PBSC transplant, measurement of circulating peripheral blood levels of $CD34^+$ cells on the day of leukapheresis can be used to accurately predict the total yield of PBSC in the leukapheresis product [14], although steady-state levels, prior to the initiation of G-CSF therapy, have not been shown to be useful in this respect [60]. There is broad interindividual variation in efficacy of stem-cell mobilization [61], and it is difficult to predict how well an individual donor will mobilize. We have found evidence that male donors are more efficient at mobilizing PBSC than females [59]. In an analysis of 17 donors undergoing PBSC mobilization with a dose of 10 μg/kg/day of G-CSF, we were able to collect significantly more $CD34^+$ cells/kg donor weight in a standard 12-litre leukapheresis in male donors than in female donors. Thus, for male donors, we collected a median of 4.96×10^6 $CD34^+$ cells/kg donor weight, compared with a median of 2.79×10^6 $CD34^+$/kg donor weight in female donors, which was statistically significant ($P<0.05$). Some studies have also shown that younger donors are superior in their ability to mobilize PBSC [19], although this has not been confirmed by other groups [14].

The optimum number of $CD34^+$ cells required for rapid engraftment is still not clear. However, this may not be quite so easily defined as in the autologous situation, reflecting the presence of other factors which can affect engraftment in allogeneic transplantation. These include the development of GvHD, and post-transplant infections, particularly viral infections. Most groups have set a target of $>4\times10^6$ $CD34^+$ cells/kg [62,63], and there is

evidence from a multicentre analysis that this dose of $CD34^+$ cells may be associated with more rapid engraftment [63]. Durable engraftment, however, has certainly occurred with the infusion of as few as 1×10^6 $CD34^+$ cells/kg, although delayed recovery, particularly of platelets, is likely.

Recently, there has also been a report of children being used as PBSC donors for allogeneic transplants to both siblings and parents. Korbling *et al.* [64] reported 5 paediatric donors, aged between 4 and 13 years, who were used as donors for sibling transplants in 3 cases and for haplo-identical transplants to a parent in 3 cases. PBSC were mobilized with G-CSF at a dose of 6 μg/kg/day twice daily, sufficient numbers of stem cells (range $3.2–9.7\times10^6$ $CD34^+$ cells/kg recipient weight) were collected in one or two leukaphereses in all cases. This confirmed that paediatric donors can yield sufficient progenitor cells for prompt engraftment with clinical outcomes similar to adult PBSC transplants.

Composition of PBSC harvests from normal donors

The leukapheresis product not only contains large numbers of $CD34^+$ cells, with numbers up to fivefold more than those found in bone-marrow harvests, but also significantly greater numbers of $CD3^+$ cells, T-cell subsets and NK cells. The total numbers of $CD3^+$, $CD4^+$ and $CD8^+$ cells represent up to 1 log greater numbers of cells than are present in bone-marrow harvests and most recipients will receive more than 2×10^8 $CD3^+$ cells [19,47,50] The composition of 21 consecutive harvests from G-CSF mobilized donors analysed by us is shown in Table 31.8. Obviously, the total number of T-cells and NK cells infused will depend upon the number of leukaphereses performed, but the overall composition of the graft is significantly different from that of a bone-marrow harvest [62].

Side-effects of PBSC mobilization in normal donors

A number of issues are raised by the use of PBSC for allogeneic transplantation. The most important of these are the short-term and possible long-term effects of G-CSF, and also the use of central venous catheters and leukapheresis in healthy donors.

Short-term side-effects of G-CSF, including bone pain, fatigue, headache and myalgia, as experienced in patients undergoing G-CSF mobilization of stem cells, also occur in normal donors. The incidence of side-effects does not appear to correlate with the dose of G-CSF [14,57]. However, at a dose of 10 μg/kg/day, Grigg found the incidence of side-effects to be bone pain 86%, fatigue 46%, headache 33% and myalgia 26% [14]. Most of these side-effects can successfully be treated with mild analgesics. The most common biochemical abnormality is a raised alkaline phosphatase which occurs in around 80% of donors, but reverts to normal values after the G-CSF is stopped.

Concerns over the side-effects of G-CSF, especially in the long term, have been a major factor in delaying the establishment of allogeneic PBSC transplant, and there are also implications concerning the use of G-CSF for unrelated donors. Without G-CSF, the collection of sufficient PBSC for allografting is not feasible on a regular basis. However it is conceivable that stimulating bone marrow with supraphysiological

Table 31.8 Composition of PBSC harvests from normal donors

Component	Korbling *et al.* [62] (*n*=41)	Russell and Miflin (unpublished data) [49] (*n*=21)
Total nucleated cells ($\times 10^8$/kg)	13.36 (5.63–31.13)	10 (4.68–19.15)
$CD34^+$ ($\times 10^6$/kg)	11.7 (4.2–40.1)	5.84 (2.86–15.6)
$CD3^+$ ($\times 10^6$/kg)	393.2(104.6–1039.9)	360 (142–541)
$CD4^+$ ($\times 10^6$/kg)	257.4 (91.9–663.4)	148 (338–2951)
$CD8^+$ ($\times 10^6$/kg)	153.6 (21.5–402.3)	75 (37–170)
$CD19^+$ ($\times 10^6$/kg)	79 (25.5–189)	68 (223–1262)
$CD3–56^+$ ($\times 10^6$/kg)	54.4 (13.7–193.1)	23.6 (11.9–114.1)
N/A=not available.		

doses of G-CSF may have some long-term sequelae, although to date there is no evidence to suggest that this is true [65]. The longest reported follow-up in healthy subjects who had received G-CSF to date is five years [66]. This group studied 5 volunteers who had received G-CSF five years previously. They looked at haematological and biochemical parameters of peripheral blood and also at morphological and cytogenetic characteristics of the bone marrow. Encouragingly, no abnormalities were found. However, to detect any significant increase in adverse events following G-CSF, long-term follow-up on an international scale will be required. Even supposing that a short course of G-CSF was leukemogenic (for which there is absolutely no evidence), Hasenclever and Sexton [67] have estimated that to detect a 0.5% increase in leukaemia incidence over a ten-year period would require a ten-year follow-up on over 2000 donors.

Besides the side-effects of G-CSF, other effects of the mobilization procedure are related to leukapheresis and include a fall in haemoglobin which is usually equivalent to 1 g/dl per leukapheresis, and a fall in platelets by as much as a 60%. In some donors we have seen the platelet count fall below 50×10^9/litre. However, this has not been associated with any adverse clinical events [57,59].

While there have been reports of unrelated donor PBSC [68,69], this has not yet become routinely available, as donor panels have, understandably, been cautious in permitting volunteer donors to undergo mobilization. Some donor panels are, however, now permitting stem-cell collections, particularly as an alternative to a second bone-marrow harvest for rejection.

The use of central venous catheters in healthy volunteers is another contentious subject. Efficient leukapheresis depends upon adequate venous access, and as a consequence, some centres have preferred to insert central venous catheters into their donors when venous access is difficult. However, others have deemed this unacceptable because of the attendant risks. We feel that the majority of donors can undergo successful leukapheresis via peripheral veins and that the use of a central venous catheter is rarely, if

ever, indicated [58]. If difficulties are encountered during leukapheresis, a femoral vein catheter is possibly more acceptable, but in our unit this has not been necessary in 20 consecutive donors.

Bone-marrow harvesting under general anaesthesia is not, of course, a risk-free procedure, Buckner *et al.* [70] described a 0.4% incidence of life-threatening complications associated with bone-marrow donation, and related morbidity secondary to multiple pelvic punctures and bruising should also be taken into account. Whether leukapheresis is ultimately safer than a general anaesthetic and bone-marrow harvest has not been evaluated and will require longer follow-up of donors who have received G-CSF. Currently, it seems that for an individual donor the possible risks of G-CSF mobilization and leukapheresis are certainly not greater than those of anaesthesia and bone-marrow harvesting. However, donor preference should be considered and there are undoubtably some donors who will prefer bone-marrow donation.

Clinical results

Engraftment kinetics

In comparison to allogeneic BMT, there is considerable evidence from initial studies that both neutrophil and platelet engraftment following allogeneic PBSC transplant are rapid (Table 31.9). However, the kinetics of engraftment are affected by the choice of post-transplant GvHD prophylaxis. In a study of 9 patients receiving allogeneic PBSC for advanced leukaemia, Schmitz *et al.* [50] found that the use of methotrexate in combination with cyclosporin to prevent GvHD delayed engraftment when compared to patients receiving cyclosporin alone. In a study of 41 patients collected by Miflin *et al.* [63] from nine transplant centres, the median time to a neutrophil count $>0.5\times10^9$/litre was 14 days and to a platelet count $>20\times10^9$/litre was also 14 days where all but 3 patients had received cyclosporin and methotrexate post-transplant.

The kinetics of engraftment were particularly rapid in a study reported by Korbling *et al.* where cyclosporin and prednisolone were used in combination with post-transplant G-CSF (Table 31.9) [48]. Several comparisons of allogeneic PBSC transplant with historical cohorts of patients undergoing BMT have been published [51–53] and these have shown significantly faster engraftment times in the PBSC transplant groups for both neutrophil and platelets (Table 31.10). Overall, these results appear to indicate that haemopoietic recovery is more rapid with PBSC which has the potential to reduce the morbidity and early mortality associated with allografting. Furthermore, it is likely that the more rapid engraftment kinetics will lead to a reduction in antibiotic and blood product support requirements as well as reducing in-patient stay and procedural costs [51,52,53].

The question of long-term engraftment has also been addressed with evidence from source of continuing donor haemopoiesis persisting for greater than two years post-transplant with no reports of late graft failure [63,71,72].

Immune reconstitution

In a prospective study using case-matched BMT recipients as controls, one report has shown evidence for more rapid immune reconstitution post-allogeneic PBSC transplant [73], in particular a significantly faster reconstitution of $CD4^+$ and $CD19^+$ cells. No

Table 31.9 Engraftment kinetics following allogeneic PBSC transplant

Study	GvHD prophylaxis*	Median $CD34^+$ cells ($\times 10^6$/kg)	ANC >0.5 $\times 10^9$/litre	ANC >1.0 $\times 10^9$/litre	>20 $\times 10^9$/litre	Platelets >50 $\times 10^9$/litre
Schmitz *et al.* [72] (*n*=59)	CSA/MTX	6.47 (1.44–68.8)	15 (9–27)	17 (10–28)	16 (9–76)	18 (12–100)
Miflin *et al.* [63] (*n*=44)	CSA/MTX	5.75 (0.94–35)	14 (10–25)	15 (10–30)	14 (9–130)	17 (10–150)
Russell *et al.* [51] (*n*=26)	CSA/MTX	5.1 (2.7–12.3)	16 (11–28)	Not available	14 (8–32)	Not available
Korbling *et at.* [48] (*n*=9)	CSA/Pred	10.7 (7.5–22.5)	9 (8–10)	9 (8–10)	12 (9–51)	15 (9–59)

*CSA=cyclosporin; MTX=methotrexate; Pred=prednisolone.

Table 31.10 A comparison of engraftment kinetics following allogeneic PBSC transplant and historical BMT controls

Study	Transplant	ANC >0.5 $\times 10^9$/litre	ANC >1.0 $\times 10^9$/litre	Platelets >20 $\times 10^9$/litre	Platelets >50 $\times 10^9$/litre
Azevedo *et al.* [53] (*n*=22)	PBSC	14 (10–20)	N/A	13* (9–88)	N/A
	BMT	20 (10–30)	N/A	22* (16–33)	N/A
Russell *et al.* [51] (*n*=26)	PBSC	16 (11–28)	N/A	14 (8–32)	N/A
	BMT	21.5 (16–33)	N/A	20.5 (16–33)	N/A

Bensinger *et al.* [52] (*n*=37)	PBSC	14 (9–33)	N/A	11 (1–46)	N/A
	BMT	16 (8–33)	N/A	15 (3–190)	N/A
Russell and Miflin (unpublished data) (*n*=13)	PBSC	15 (12–23)	19 (14–26)	14 (9–28)	16 (13–57)
	BMT	23 (15–32)	29 (17–147)	N/A	29 (17–24)

*Platelets $>35\times10^9$/litre.
N/A=not available.

difference was seen in reconstitution of T-suppressor cells, or NK cells which reached normal levels within two months of transplant. Monocyte counts rapidly reached normal levels in both types of transplant. The authors also showed significantly improved cellular *in vitro* responses to phytohaemagglutinin, pokeweed mitogen, tetanus toxoid and *Candida albicans* in PBSC- transplant recipients, which could be partially attributed to faster $CD4^+$ recovery, but which may also be in part due to an adoptive immune transfer. Whether faster immune recovery results in a decreased incidence of post-transplant infections remains to be seen.

Graft-versus-host disease

As previously discussed, the number of immunocompetent T-cells infused in a PBSC graft is approximately tenfold higher than in bone-marrow grafts [19,47,50]. Schmitz *et al.* [50] reported that, in 8 patients, the median number of $CD3^+$ cells was 3.4×10^8/kg (range 1.37–5.41), which is significantly higher than the $>10^7$/kg usual in bone-marrow harvests. Because of this, there were initial concerns that there would be an unacceptably high level of GvHD. This however, does not seem to be the case with several groups reporting the incidence of acute GvHD to be similar to that reported for BMT [51–53]. The incidence of acute GvHD using allogeneic PBSC for several reported series is summarized in Table 31.11. Of the patients transplanted in the UK (*n*=44) 72 % had no or minor (grade 1) GvHD, and only 16% had severe disease (grade 3 or 4 acute GvHD). These patients received standard cyclosporin and short-course methotrexate for GvHD prophylaxis [63], suggesting that, provided optimal GvHD prophylaxis is used, the T-cell load infused is not a problem. Furthermore, there does not appear to be any correlation between the number of T-cells infused and GvHD severity [74]. Why the increased number of T-cells infused has not translated into increased rates of GvHD is unclear. Several theories have been suggested; GvHD may be more dependent upon genetic disparities between donor and host than on T-cell number [65]. Alternatively, G-CSF treatment may itself in some way modify T-cell responses to host antigens [47], or the fact that stem-cell harvests contain populations of T-cells with the immunophenotype of natural suppressor cells ($CD3^+$, $CD4^-$, $CD8^-$) which are suppressive to GvHD reactions, may also be important.

Table 31.11 Incidence of graft-versus-host disease

Study	GvHD prophylaxis*	Acute GvHD Grade 0–1 (%)	Grade 2–4 (%)	Chronic GvHD (%)
Schmitz (n=59)	CSA/MTX (75%)	34	66**	52
Miflin *et al.* [63] (n=44)	CSA/MTX	72	28	47
Bensinger *et al.* [52] (n=37)	CSA/MTX (50%) (CSA/Pred (50%))	63	37	59
Russell *et al.* [51] (n=26)	CSA/MTX	63	37	53

*CSA=cyclosporin; MTX=methotrexate; Pred=prednisolone.
**Probability of developing acute GvHD.

Data concerning chronic GvHD are still relatively immature, with varying rates reported in several series. Most studies have not shown there to be an increased incidence of chronic GvHD. Schmitz *et al.* [72] reported rates of 52% in evaluable patients having chronic GvHD, which was similar to data from a UK series which showed an incidence of 47% [63]. However, a study by Majolino *et al.* [71] reported that 6 out of 6 evaluable patients suffered extensive GvHD, despite GvHD prophylaxis with cyclosporin and methotrexate. Russell *et al.* also found no significant difference in rates of chronic GvHD between patients undergoing allogeneic PBSC transplant and a cohort of case-matched controls undergoing BMT, with an incidence of 50 % [51].

$CD34^+$ allogeneic PBSC

A number of groups have explored the use of $CD34^+$ selection as a means of removing T-cells from G-CSF-mobilized PBSC and hence reducing GvHD while still maintaining sufficient T-cells to retain a GvL effect and to prevent rejection [75]. $CD34^+$ selection procedures inevitably result in the loss of $CD34^+$ cells, which require a greater number of leukapheresis procedures to provide sufficient cells to overcome the losses. Although $CD34^+$ selection can reduce the number of T-cells infused by 3–4 logs, preliminary evidence suggests that the incidence of severe (grade 3 or 4) acute GvHD is not diminished unless adequate post-transplant immunosuppression with cyclosporin and metho-trexate is used [76]. One reason for this failure may be that the number of T-cells infused (approximately 1×10^6/kg) is above the reported threshold for GvHD using T-cell-depleted BMT [77]. An alternative explanation is that the removal of T-cell subsets suppressive to GvHD may occur during the selection process, thus resulting in higher rates of acute GvHD than expected.

Overall, these results suggest that current methods of $CD34^+$ cell selection need to be improved to abolish GvHD, although whether this may have a negative impact on relapse rates remains to be seen.

Conclusions

In the next few years, the use of high-dose chemotherapy supported by autologous or allogeneic blood stem cells will continue to grow and we are likely to see the development of new therapeutic strategies based upon stem-cell therapy. These will include the use of multiple cycles of high-dose therapy, the use of more purified stem-cell products and greater use of graft engineering to modify the composition of the graft in allogeneic PBSC transplant. These approaches are likely to contribute to improvements in the clinical outcome of patients receiving high-dose chemotherapy.

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