Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Mark Levis (1), Johns Hopkins University School of Medicine, Department of Oncology, Baltimore, Maryland

Donald Small (1), Johns Hopkins University School of Medicine, Departments of Oncology and Pediatrics, Baltimore, Maryland

Steven Soignet (35), The Arcus Group, LLC, New York, New York 10012, and Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Peter Maslak (35), Hematology Laboratory Service, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Farhad Ravandi (59), Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Jorge Cortes (59), Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Elihu Estey (99), Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Nicole Lamanna (107), Instructor, Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Mark Weiss (107), Associate Attending, Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Thomas S. Lin (127), Division of Hematology and Oncology, The Ohio State University, The Arthur James Comprehensive Cancer Center, Columbus, Ohio 43210

John C. Byrd (127), Division of Hematology and Oncology, The Ohio State University, The Arthur James Comprehensive Cancer Center, Columbus, Ohio 43210

Eric L. Sievers (169), Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109; and Department of Pediatrics, University of Washington, Seattle, Washington 98105

John M. Burke (185), Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Joseph G. Jurcic (185), Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Michael Rosenblum (209), Immunopharmacology and Targeted Therapy Section, Department of Bioimmunotherapy, M. D. Anderson Cancer Center, Houston, Texas 77030

George J. Weiner (229), Holden Comprehensive Cancer Center, Department of Internal Medicine, University of Iowa, Iowa City, Iowa 52242

Brian K. Link (229), Holden Comprehensive Cancer Center, Department of Internal Medicine, University of Iowa, Iowa City, Iowa 52242

Sijie Lu (255), The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Eric Wieder (255), The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Krishna Komanduri (255), The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Qing Ma (255), The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Jeffrey J. Molldrem (255), The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

John M. Timmerman (271), Division of Hematology/Oncology, University of California, Los Angeles, Center for Health Sciences 42-121, Los Angeles, California 90095-1678

Sherif S. Farag (295), Division of Hematology and Oncology, Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210

Michael A. Caligiuri (295), Division of Hematology and Oncology, Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210

Vincent T. Ho (319), Bone Marrow Transplant Program, Dana-Farber Cancer Institute, Boston, Massachusetts

Edwin P. Alyea (319), Bone Marrow Transplant Program, Dana-Farber Cancer Institute, Boston, Massachusetts

Renier J. Brentjens (347), Department of Medicine and Clinical Laboratories, Leukemia Service, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Michel Sadelain (347), Department of Medicine and Clinical Laboratories, Leukemia Service, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Preface

In recent years, we have witnessed a paradigm shift in cancer treatment. Greater understanding of signaling pathways that regulate cell growth, cell cycle progression, and programmed cell death has provided new insights into the molecular mechanisms of disease. While traditional cytotoxic agents still form the backbone of cancer therapy, advances in molecular biology and immunology have led to the identification of novel therapeutic targets and treatment strategies. This volume highlights many of the major developments in biologically targeted, immunologic, and chemotherapeutic approaches to the treatment of leukemia and lymphoma over the past decade.

The remarkable activity of imatinib mesylate, an inhibitor of the ABL kinase of the BCR/ABL fusion protein that causes chronic myeloid leukemia (CML), provides "proof-of-concept" that molecularly targeted therapies will become an important new class of cancer therapeutics. In addition to describing the development and use of imatinib, Levis and Small (Chapter 1) examine other kinases, such as flt-3, that may be clinically useful targets. The success of imatinib in CML, however, has been difficult to translate to other malignancies. Unlike CML, where BCR/ABL is the causative molecular abnormality and may be the sole leukemogenic event early in the disease, single pathogenetic abnormalities do not exist for the vast majority of malignancies. For small-molecule inhibitors to achieve broader success, it is likely that the most useful agents will target early oncogenic events and that blocking multiple pathways critical for cell survival will be required for clinically meaningful responses. Estey (Chapter 4) provides a framework for investigating these new agents by addressing issues regarding patient selection, efficacy endpoints, and the need for comparative studies.

Like CML, acute promyelocytic leukemia (APL) has become an ideal model for the study of molecularly targeted therapies. In contrast to the development of imatinib, however, which represents a rationally designed compound targeting specific molecular lesions, the success of empiric therapies in APL has led to a new biological understanding of the disease. Differentiation therapy with all-trans retinoic acid that directly targets PML-RARα, the underlying molecular abnormality in APL, has produced complete remissions in up to 90% of patients. Similarly, arsenic trioxide, which degrades PML-RARα and leads to non-terminal differentiation and apoptosis, has shown significant activity. Soignet and Maslak (Chapter 2) outline the current role of these agents in the treatment of APL.

Until the widespread application of molecularly targeted therapies proves more clinically useful, new chemotherapeutic agents will be required. An expanding knowledge of cancer biology has led to the development of a large number of novel drugs, including inhibitors of multi-drug resistance, angiogenesis, farnesyltransferase, and proteosomes, all comprehensively reviewed by Cortes (Chapter 3). Among the most promising therapeutic approaches, hypomethylating agents such as 5-azacitidine and decitabine, capable of activating silenced genes, have shown significant activity in myelodysplastic syndromes and acute myeloid leukemia (AML). Additionally, inhibition of histone deacetylases by phenylbutyrate, SAHA, and depsipeptide, among others, may reverse transcriptional repression caused by histone binding to DNA. In combination, hypomethylation and histone deacetylase inhibition provide an attractive therapeutic strategy for APL and other core binding-factor leukemias, where transcriptional block may play a particularly important role in leukemogenesis. Lamanna and Weiss (Chapter 5) focus on the purine analogs that have shown activity in lymphoid malignancies and the more recent clinical evidence leading to their use in a variety of applications, including non-myeloablative stem cell transplantation and treatment of graft-versus-host disease.

Monoclonal antibodies have now become an important therapeutic modality for cancer, but the overly optimistic view of the early 1980s that they were "magic bullets" has now been replaced by a more realistic understanding of their therapeutic potential. The intrinsic immunologic activity seen with the anti-CD20 antibody rituximab against low-grade lymphoma has provided a foundation for further development of native antibody therapy. Lin and Byrd (Chapter 6) outline recent advances using this approach for the treatment of chronic lymphocytic leukemia (CLL), including new therapeutic targets and chemoimmunotherapy combinations; Weiner and Link (Chapter 10) discuss similar applications for lymphoma.

In an effort to enhance potency, antibodies may be used as vehicles to deliver radioisotopes, drugs, and toxins directly to tumor cells. Weiner and Link discuss radioimmunotherapeutic approaches for lymphoma, including iodine-131-tositumomab and yttrium-90-ibritumomab tiuxetan. In

examining the radioimmunotherapy of leukemia, Burke and Jurcic (Chapter 8) highlight the use of α particle-emitting isotopes, which may allow for more specific tumor cell killing compared to β-emitters. Sievers (Chapter 7) details the development of the anti-CD33-calicheamicin construct gemtuzumab ozogamicin for AML, while Rosenblum (Chapter 9) reviews various strategies for targeting toxins to tumor cells, including cytokines and growth factors, in addition to monoclonal antibodies.

While passive treatment with antibody-based therapies has shown potent anti-tumor effects, it represents only one immunotherapeutic approach. In addition to eliciting antibody responses, vaccine strategies may also produce T-cell responses that allow ongoing surveillance against tumor cells. Lu and colleagues (Chapter 11) review the biological basis of antileukemia immunity and highlight potential leukemia-associated target antigens. Timmerman (Chapter 12) focuses on therapeutic vaccines targeting lymphoma "idiotype." Farag and Caligiuri (Chapter 13) examine the use of cytokines to harness effector cells, including natural killer (NK) cells and monocytes, against autologous leukemia and lymphoma.

Passive cellular therapy has also gained a role in the management of hematologic malignancies, as demonstrated by the ability of donor lymphocyte infusions (DLIs) to induce durable complete remissions in CML. Ho and Alyea (Chapter 14) discuss the biological basis for a graft-versus-tumor effect, current clinical applications of DLI, and the development of nonmyeloablative approaches for allogeneic stem cell transplantation. Finally, Brentjens and Sadelain (Chapter 15) explore the use of gene transfer techniques to engineer tumor cells capable of activating host immune cells, to modify dendritic cells to express tumor antigens, and to alter patient T-cell specificity to recognize antigens present on tumor cells.

The comprehensive reviews in this volume reflect our rapidly expanding knowledge of hematologic malignancies and should provide an exceptional resource for clinicians caring for patients with leukemia and lymphoma as well as clinical or laboratory researchers. In closing, we would like to thank all those who contributed to this collection and Tom August for the invitation to edit this volume.

Joseph G. Jurcic
David A. Scheinberg
April 26, 2004

Mark Levis* and Donald Small*,†

Johns Hopkins University School of Medicine
Departments of *Oncology and †Pediatrics
Baltimore, Maryland

Kinase Inhibitors in Leukemia

I. Chapter Overview

Constitutively activated kinases appear to play a role in the development and maintenance of a significant number of leukemias. The remarkable early clinical successes of imatinib mesylate, an orally available tyrosine kinase inhibitor, in the treatment of BCR/ABL-expressing leukemias imply that small-molecule kinase inhibitors will likely become an important new class of agents used to treat these diseases. The introduction of these drugs has broadened our understanding of the molecular basis of leukemia, as well as increased the treatment options available to patients. In this chapter, we review the mechanism of action of these compounds and discuss the potential range of disorders to which they can be applied. A large number of kinase inhibitors are in clinical development, but imatinib is thus far the only such drug approved for use in patients with leukemia. Therefore, in addition to carefully examining the development and clinical use of imatinib, we

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

focus on those kinases that appear to be promising targets in hematologic disorders, hoping that the introduction of clinically useful inhibitors for these kinases will soon follow.

II. Introduction

In 1980, the transforming protein of Rous sarcoma virus was discovered to be a tyrosine kinase (Sefton *et al*., 1980). Following this seminal finding, researchers have struggled to unravel the complex roles of the enzyme in the normal functions of a cell and have sought to understand how the dysregulation of protein kinases leads to malignant transformation. The intent of such research is to better understand the molecular basis of human cancer and to exploit this knowledge in the development of new therapies. Protein kinases seem to be particularly important in the pathogenesis of hematologic malignancies, especially myeloid leukemias. Beginning with the discovery of the Philadelphia chromosome (Ph+) product, BCR/ABL, and proceeding through to the recent characterization of a PDGFRα fusion protein in hypereosinophilic syndrome (HES) and eosinophilic leukemia, mutation-activated tyrosine kinases continue to be identified as causative factors in hematopoietic disorders (Ben-Neriah *et al*., 1986; Cools *et al*., 2003). Indeed, the first successful clinical use of a small-molecule kinase inhibitor was in the treatment of chronic myeloid leukemia (CML) (Druker *et al*., 2001b).

At present, with the cloning of the human genome and the advent of technologies such as proteomics and microarray analysis, potential new oncogenic kinases are being identified at an increasing rate. The fields of structural biology and pharmaceutical chemistry have kept pace with these advances in our understanding of carcinogenesis. There is no shortage of candidate small molecules designed to inhibit the kinase activity of these potential therapeutic targets (at least *in vitro*), and it seems safe to assume that for virtually any kinase found, a relatively selective inhibitor suitable for clinical testing can be produced.

III. Factors in Identifying an Ideal Therapeutic Target

Which kinases should be targeted for leukemia therapy? Presumably, the focus should be on kinases that are essential in generating or maintaining the transformed state. BCR/ABL, the *sine qua non* of CML, is an example of such a kinase. Other choices are less obvious, however, given that malignant transformation is probably a multistep process, with many aberrant proteins playing a role (Vogelstein and Kinzler, 1993). Nonetheless, some guiding

principles can be used to establish the credentials of a kinase as a suitable target for therapy.

A. Activating Mutations

The highest-priority targets should probably be kinases that have increased activity through some form of mutation. The most easily recognizable of these are the kinases activated by chromosomal translocation, such as BCR/ABL (a product of the 9;21 translocation in CML) or TEL-PDGFR (caused by the 5;12 translocation occasionally seen in chronic myelomonocytic leukemia) (Golub *et al*., 1994; Nowell and Hungerford, 1960; Rowley, 1973). Cytogenetic analysis, performed on virtually all clinical cases of leukemia, facilitated the relatively early discovery of these fusion oncoproteins. However, translocations (at least those apparent with clinical cytogenetic analysis) cause only a fraction of the known kinase-activating mutations associated with cancer. Other types of constitutively activating mutations are less easily identified, as there are remarkably diverse means of abnormally upregulating the activity of a kinase. Receptor tyrosine kinases illustrate this point well.

A receptor tyrosine kinase (Fig. 1) typically consists of an extracellular ligand-binding domain, a transmembrane domain, and, intracellularly, a juxtamembrane domain and a kinase domain containing the adenosine triphosphate (ATP)-binding pocket (van der Geer *et al*., 1994). On binding of ligand, the receptors dimerize and undergo autophosphorylation, a covalent modification that stimulates the activity of the kinase domain. The activated receptor then transduces its signals via transfer of phosphate from ATP to tyrosine residues on various downstream proteins. For many cell surface receptors, the juxtamembrane domain serves as a negative regulatory domain, inhibiting the activity of the kinase domain until ligand binding occurs (Hubbard, 2001).

Abnormal activation of these receptors can occur in a variety of ways. Perhaps the simplest is through overexpression of the wild-type receptor. This can occur by gene amplification, or, presumably, through epigenetic alterations affecting transcription, translation, and perhaps even receptor turnover. A classic example of this type of gene amplification is found in the epidermal growth factor family of receptors in solid tumors, most notably c-erbB in breast cancer (Slamon *et al*., 1987).

Point mutations localized to the so-called activation loop of the kinase domain can constitutively activate a receptor by shifting the ATP-binding pocket to a more open, accessible conformation. Such mutations in the kinase domain have been identified in KIT, MET, FLT3, and PDGFRα, all associated with malignancy (Furitsu *et al*., 1993; Hirota *et al*., 2003; Jeffers *et al*., 1997; Yamamoto *et al*., 2001).

Crystal structure analysis of the EphB2 receptor suggests that mutations within the juxtamembrane region disrupt the inhibitory influence this

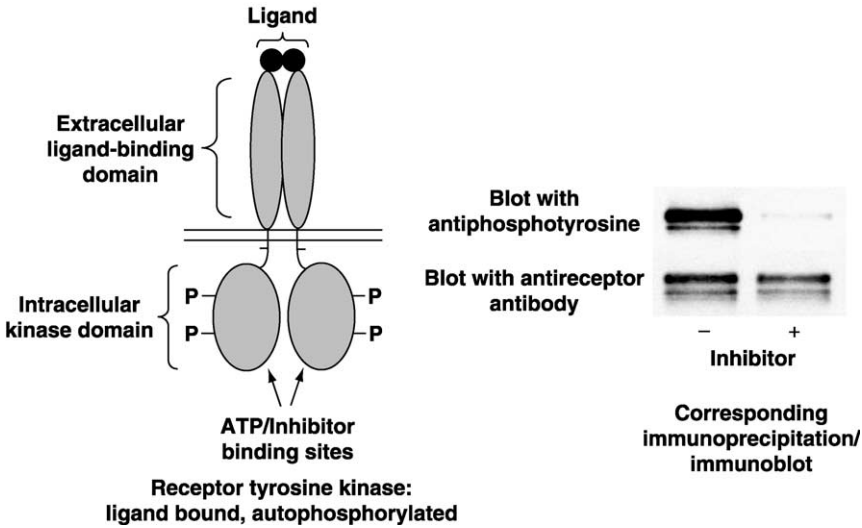

FIGURE 1 Left: A stylized receptor tyrosine kinase with cognate ligand bound. The receptor has dimerized and undergone autophosphorylation. Right: The assay commonly used to determine *in vitro* efficacy of a small molecule inhibitor. Cells expressing the activated receptor are exposed to the inhibitor in question, and then the cells are lysed and subject to immunoprecipitation, using antireceptor antibodies. The immunoprecipitate is resolved by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to a membrane. The membrane can be probed with either an antiphosphotyrosine antibody (upper row) or the antireceptor antibody (lower row).

domain exerts over the kinase domain (Wybenga-Groot *et al.*, 2001). Point mutations, deletions, insertions, and internal tandem duplications have been found in the juxtamembrane domain of the KIT receptor in gastrointestinal stromal tumors (GISTs), all associated with a constitutively phosphorylated receptor (Antonescu *et al.*, 2003). Likewise, internal tandem duplications of the Fms-like tyrosine kinase-3 (FLT3) gene (FLT3/ITD mutations) constitutively activate FLT3 and are the most common molecular abnormality found in acute myeloid leukemia (AML) (Gilliland and Griffin, 2002; Levis and Small, 2003).

Activating mutations can likewise be found within the extracellular domain. Some of these mutations in the KIT receptor in GIST appear to activate the receptor by promoting dimerization through an abnormal disulfide bond (Santoro *et al.*, 1995). For other mutations in the extracellular domain, the mechanism of constitutive activation is unclear (Lux *et al.*, 2000).

Adding to the complexity of this situation is the likelihood that a given kinase inhibitor will be effective against only a subset of the mutations affecting a particular kinase. In particular, a number of small-molecule kinase inhibitors appear to be ineffective against mutations in the kinase

domain. For example, imatinib has little effect on the activity of some of the less frequent KIT kinase domain mutations that occur in GIST, and AG1296, a tyrphostin, is ineffective against analogous mutations in the FLT3 receptor (Frost *et al.*, 2002; Grundler *et al.*, 2003).

B. Prognosis

A kinase becomes a more inviting target for therapy if activating mutations of the kinase are associated with a worse prognosis. In breast cancer, patients whose tumors have an amplification of the Her2 (c-erbB) gene have a worse overall survival compared with patients lacking Her2 overexpression. In acute lymphoblastic leukemia (ALL), patients whose blasts contain the BCR/ABL translocation product are usually incurable without allogeneic stem cell transplantation. It remains to be seen whether imatinib will impact this grim reality in any way.

C. Is the Target Expressed by the Stem Cell?

A final issue in selecting an appropriate kinase to target relates to the origins of a malignancy. Cancer cells appear to be derived from progenitor cells that in many ways resemble the stem cells from which nontransformed cells arise (Reya *et al.*, 2001). These cancer stem cells might not have the phenotypic properties identical to those of the bulk tumor (or in the case of leukemia, the malignant cells circulating in peripheral blood), and a therapy directed against the bulk of the tumor might have no impact on the stem cell. It is possible that a kinase-activating mutation could occur as a late hit in a subpopulation of transformed cells, conferring a growth advantage to cells with the mutation. A kinase inhibitor might effectively eliminate this subpopulation (which could make up most of the visible tumor), but have no effect on the stem cell. This might render the kinase inhibitor useful as a palliative measure but ultimately ineffective in helping to cure the disease. Stem cells, malignant or normal, are difficult to isolate and characterize, but an effort should always be made to identify kinase targets within these crucial cells.

IV. Kinase Targets in Leukemia: Current Treatment Options

At present, imatinib and gefitinib (Fig. 2) are the only small-molecule kinase inhibitors approved for use in patients (Cohen *et al.*, 2003; Johnson *et al.*, 2003). Gefitinib is an EGFR (c-erbB1) inhibitor, whereas imatinib was introduced as an inhibitor of BCR/ABL. Imatinib, however, has quickly demonstrated clinical usefulness as a KIT and PDGFRα inhibitor, which

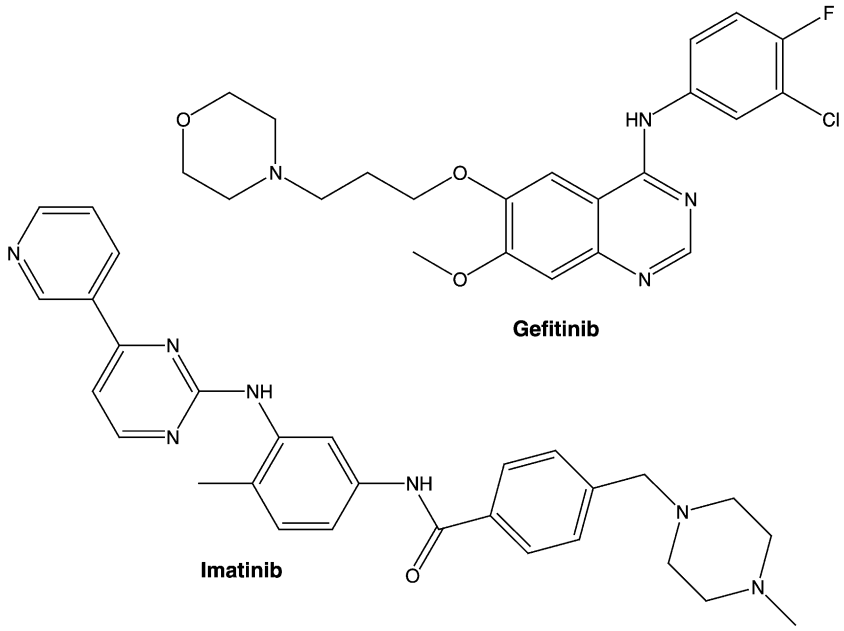

FIGURE 2 Two small-molecule tyrosine kinase inhibitors currently in clinical use.

illustrates an important point about these molecules: none of them is truly specific for a single kinase. Each new kinase inhibitor entering clinical use will have a number of defined targets and possibly a greater number of targets that are either unknown or whose role in normal or malignant cell function is unclear. Therefore, in analyzing the potential role of kinase inhibitors in leukemia therapy, it is of greater utility to examine the potential targets that have thus far been uncovered rather than focus on what drugs are available.

A. BCR/ABL

Ph+, a hallmark of CML, is the result of a balanced translocation between chromosomes 9 and 22 and leads to the generation of a fusion gene, BCR/ABL (Nowell and Hungerford, 1960; Rowley, 1973). This translocation, when it occurs within a hematopoietic stem cell, appears to be of fundamental importance for the development of CML. The wild-type ABL gene encodes a tyrosine kinase that normally shuttles between the cytoplasm and the nucleus and is a key regulator of apoptosis in response to DNA damage (Wang, 2000). The BCR gene (breakpoint cluster region) encodes a multipurpose protein with an oligomerization domain. When the N-terminus of BCR is fused to the C-terminus of ABL, the fusion product

is localized exclusively to the cytoplasm and its tyrosine kinase domain is constitutively activated through homooligomerization (McWhirter *et al.*, 1993; Van Etten *et al.*, 1989). The t(9;22) translocation gives rise to three major variants of BCR/ABL, depending on where the breakpoints occur (Fig. 3). In the vast majority of CML cases, the breakpoints occur within the major breakpoint cluster region (M-BCR) of BCR, between exons 13 and 14 (b2) or exons 14 and 15 (b3) and within the first or second intron of ABL (just prior to exon a2) (Ben-Neriah *et al.*, 1986). The resultant mRNA is designated b2a2 or b3a2, and the final protein product is the 210-kDa fusion protein $p210^{BCR/ABL}$. In adult ALL, 17–30% of patients are Ph+, but the breakpoint in BCR occurs between the e1 and e2 exons (Bloomfield *et al.*, 1986; Clark *et al.*, 1988; Westbrook *et al.*, 1992). This leads to the shorter e1a2 transcript and the smaller protein $p190^{BCR/ABL}$. These patients have a much worse prognosis than do BCR/ABL-negative ALL patients. Finally, an entirely different breakpoint in BCR can result in the e19a2 transcript, encoding for the larger $p230^{BCR/ABL}$, which is associated with chronic neutrophilic leukemia (CNL) (Pane *et al.*, 1996). The transforming activity of the BCR/ABL oncoproteins has been demonstrated *in vitro* through diverse assays, including transformation of fibroblasts, induction of long-term survival of bone marrow progenitors, and conferring IL-3 independence to Ba/F3 cells (Daley and Baltimore, 1988; Lugo and Witte, 1989; McLaughlin *et al.*, 1987). All three BCR/ABL variants activate downstream signaling pathways such as JAK/STAT, RAS/MAPK, CRK-L, and PI-3 kinase, but differences in the BCR sequence content lead to very different cellular, and therefore clinical, consequences. For example, in studies that used mouse bone marrow, $p210^{BCR/ABL}$ and $p230^{BCR/ABL}$ expression induced myeloid

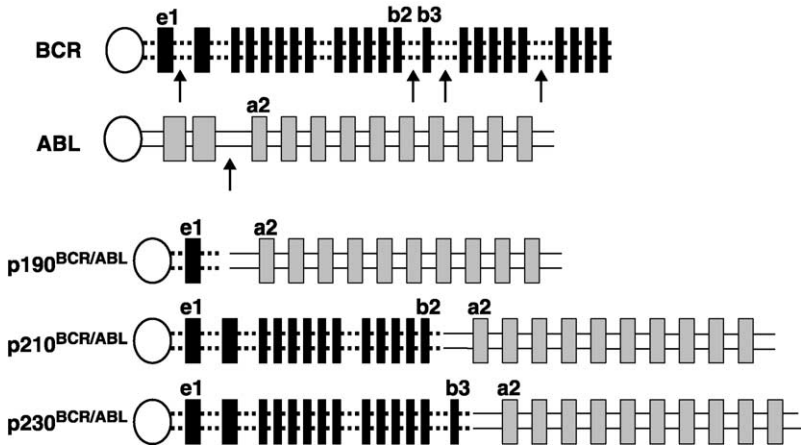

FIGURE 3 The three major splice variants of BCR/ABL. Arrows denote the breakpoints generating the fusion proteins.

differentiation, whereas $p190^{BCR/ABL}$ led to lymphoid differentiation (Quackenbush *et al*., 2000). BCR/ABL, then, is an example of an ideal target for a kinase inhibitor. It is a kinase activated by mutation, is present in the stem cell that gives rise to CML, and confers a poor prognosis in ALL.

Imatinib mesylate, originally known as CGP 57148 and later as STI-571, is a 2-phenylaminopyrimidine derivative that is a relatively potent inhibitor of the tyrosine kinase activity of ABL, the BCR/ABL variants, PDGFRα and β, and KIT (Table I; Buchdunger *et al*., 1996, 2000; Carroll *et al*., 1997; Druker *et al*., 1996). In preclinical studies, imatinib inhibited proliferation and induced apoptosis in a variety of BCR/ABL-expressing myeloid and lymphoid cell lines *in vitro* and preferentially inhibited the growth of CML progenitor cells in colony-forming assays of bone marrow from CML patients (Beran *et al*., 1998; Carroll *et al*., 1997; Deininger *et al*., 1997; Druker *et al*., 1996). In murine models of CML, the drug inhibited BCR/ABL phosphorylation *in vivo* and prolonged survival (le Coutre *et al*., 1999; Wolff and Ilaria, 2001).

As might be predicted from the preclinical data, imatinib has met with initial success in human patients. It has a favorable pharmacokinetic profile, allowing a once-daily oral dosing with a minimum of side effects. Imatinib inhibits BCR/ABL activity *in vivo* as evidenced by the suppression of downstream signaling in the circulating leukemia cells of treated patients (Druker *et al*., 2001b). It induces hematologic and cytogenetic responses in the majority of newly diagnosed CML cases, as well as in a significant number of CML patients who have failed α-interferon therapy. In a Phase I trial, the drug induced hematologic responses in 55% of patients with CML in myeloid blast crisis and in 70% of patients with CML in lymphoid blast crisis or with Ph+ ALL. The responses in the blast crisis or ALL patients were typically of short duration, but in the chronic-phase patients, hematologic and cytogenetic responses appeared to be sustained (Kantarjian *et al*.,

TABLE I Spectrum of Kinases Inhibited by Imatinib Mesylate

Kinase	*IC_{50} (nM)*	*Reference*
v-ABL	300	Buchdunger *et al*., 1996
c-ABL	250	Carroll *et al*., 1997
p210	250	Druker *et al*., 1996
Wild-type KIT	100	Buchdunger *et al*., 2000
D815 KIT	>1000	Frost *et al*., 2002
Other KIT	<100	Frost *et al*., 2002
Wild-type PDGFRα	100	Buchdunger *et al*., 2000
Wild-type PDGFRβ	100	Buchdunger *et al*., 2000
Fip1L1-PDGFRα	5	Cools *et al*., 2003
Tel-PDGFRβ	150	Carroll *et al*., 1997

2002). A small number of patients have actually become RT-PCR negative for detection of BCR/ABL transcripts. On the basis of this data, the U.S. Food and Drug Administration (FDA) granted accelerated approval of imatinib for the treatment of CML in blast crisis. In a randomized comparison of imatinib and interferon/cytarabine for newly diagnosed CML, the imatinib-treated group had superior rates of hematologic and cytogenetic responses, had a lower rate of clinical progression, and the inhibitor was better tolerated (O'Brien *et al*., 2003). The FDA thereupon approved the use of imatinib for newly diagnosed CML, contingent on the completion of Phase IV studies confirming an improvement in overall long-term survival (Johnson *et al*., 2003).

The FDA stipulation that Phase IV studies be completed and that the survival benefit be established was an important one. CML is, by definition, a chronic disease, with a median survival of 5–6 years (Sawyers, 1999). Any new treatment for this disease cannot be truly assessed until it has been used for at least this amount of time. Allogeneic stem cell transplantation remains a curative option, but is available to only roughly 30% of patients (Savage and Goldman, 1997). α-Interferon can induce hematologic and cytogenetic responses and in a minority of patients can cure the disease, but it causes significant side effects and is often poorly tolerated (Bonifazi *et al*., 2001). Prior to the development of imatinib, most patients were relegated to maintenance therapy with nonspecific cytotoxic agents such as hydroxyurea or busulfan, and death from blast crisis would occur after 5–6 years (Silver *et al*., 1999). Imatinib has moved quickly to fill the void in treatment for many CML patients, but it remains to be seen whether survival is truly improved by this drug.

Resistance to imatinib was seen with the relatively short responses in patients with CML blast crisis and Ph+-ALL, but it was subsequently observed in accelerated phase and then in chronic-phase CML patients (Druker *et al*., 2001a,b; Gorre *et al*., 2001; Hochhaus *et al*., 2002). Clinical resistance to imatinib typically occurs coincident with reactivation of the kinase activity of BCR/ABL, and the molecular mechanisms of this reactivation can be divided into two major categories. The first consists of point mutations within the kinase domain. Crystal structure studies of ABL in complex with imatinib confirm that many of these point mutations involve amino acid residues clustered around the ATP-binding pocket, especially the A-loop, P-loop, and Thr-315 (Gorre *et al*., 2001; Hochhaus *et al*., 2002; Nagar *et al*., 2002; Schindler *et al*., 2000). These residues interact directly with imatinib through hydrophobic interaction or hydrogen bonding. Thus, the mutations are felt to interfere with the ability of imatinib to compete with ATP for binding to the active site. When the mutations were studied *in vitro* in cell-based assays, virtually all of them were found to increase the IC_{50} of imatinib for inhibition of BCR/ABL kinase activity, some dramatically so (Corbin *et al*., 2003; Hochhaus *et al*., 2002; La Rosee *et al*., 2002).

The increased IC_{50} is accompanied by a resultant decrease in cytotoxic effect *in vitro*. A fascinating feature of these point mutations is that in most, if not all, cases, they are probably present *prior* to the initiation of imatinib treatment (Roche-Lestienne *et al*., 2002; Shah *et al*., 2002). The imatinib treatment then acts as a selective pressure, increasing the prevalence of these resistant cells.

The second major category of molecular resistance is the overexpression of $p210^{BCR/ABL}$. Gene amplification of BCR/ABL was initially seen in resistant cell lines, typically generated by continuous culture in imatinib (le Coutre *et al*., 2000; Mahon *et al*., 2000; Weisberg and Griffin, 2000). Analysis of leukemia specimens from resistant patients in the early trials quickly confirmed that the phenomenon occurs with a significant frequency *in vivo*. The increased levels of $p210^{BCR/ABL}$ expression are due to a variety of mechanisms, including gene amplification, increased levels of mRNA transcripts, and acquisition of additional copies of Ph+ (Gorre *et al*., 2001; Hochhaus *et al*., 2002).

Imatinib-resistant BCR/ABL activity represents the most common mechanism of treatment failure in CML at present, but at least this appears to be an obstacle that can be surmounted. Novel BCR/ABL inhibitors are currently in development, and their combination with imatinib could allow for more effective, sustained inhibition of BCR/ABL within the leukemic clone (La Rosee *et al*., 2002; Von Bubnoff *et al*., 2003). The use of multiple inhibitors of BCR/ABL, each binding to the ATP pocket in slightly different ways, would likely overcome the problem of selection of resistant CML clones expressing BCR/ABL mutants resistant to imatinib. This might enable CML to be treated as a chronic disease, albeit one that requires therapy throughout the lifetime of an individual.

Additional, possibly clinically relevant mechanisms of resistance to imatinib that are unrelated to BCR/ABL have been uncovered as well. In a study of CML patients in myeloid blast crisis, imatinib response correlated with these patients showing only low-level expression of the transmembrane transporter protein mrp-1 (Lange *et al*., 2003). This suggests that drug transporters might affect intracellular levels of imatinib. Plasma protein binding might also play a role in a patient's response to the drug, as the levels of α-1-acid glycoprotein, an important drug-binding protein in human plasma, fluctuate sufficiently to affect free imatinib levels, thereby affecting the clinical response (Gambacorti-Passerini *et al*., 2000; Sausville, 2000).

Perhaps the most important obstacle that stands between imatinib and the cure of CML is the leukemic stem cell. CML is a disease of the hematopoietic stem cell, a cell type with properties that are probably very different from those of the bulk of leukemia cells. These cells have proven resistant to chemotherapy and might very well prove resistant to imatinib therapy. Newly diagnosed chronic-phase CML patients achieve a complete

cytogenetic response to imatinib at a relatively high rate. However, cytogenetic analysis using standard karyotype analysis is relatively insensitive at detecting minimal residual disease, and even RT-PCR has its limits. Most RT-PCR assays for BCR/ABL can detect 1 positive cell in 10^6 (Bose *et al.*, 1998). If a patient's tumor burden at diagnosis is 10^{12} cells, this potentially leaves 10^6 Ph+ cells as residual disease. As might therefore be predicted, even in CML patients achieving a complete cytogenetic remission, CD34+ bone marrow cells and long-term culture-initiating cells were found to still be BCR/ABL positive, indicating the persistence of the leukemic clone (Bhatia *et al.*, 2003). More recently, the majority of a small cohort of patients who had achieved a complete molecular remission by imatinib treatment (as defined by RT-PCR negativity) were found to be in either molecular or frank clinical relapse (Mauro *et al.*, 2003). Possibly explaining this is the finding of a population of primitive stem cells in CML patients that are maintained in a quiescent but viable state despite imatinib therapy (Graham *et al.*, 2002). It might be that inhibition of $p210^{BCR/ABL}$ merely prevents leukemic stem cells from entering the cell cycle, but is insufficient to induce them to undergo apoptosis.

Thus, a significant body of data has now emerged suggesting that although imatinib might limit progression of CML in the short term, it is unlikely to ever cure the disease by itself. Nonetheless, this drug represents a tremendous advance in the field of cancer medicine in general and leukemia therapy in particular. With regard to CML, the combination of imatinib with additional BCR/ABL inhibitors could be used to maintain the disease in a quiescent state for years. The paradigm would be similar to the one established in the treatment of HIV, in which several inhibitors of viral replication are used to overcome clinical resistance but never actually cure the disease (Yeni *et al.*, 2002).

Blast crisis CML and Ph+ ALL have proven much less responsive to imatinib therapy. These are acute leukemias by nature and, as such, are much more aggressive diseases than chronic-phase CML. Unlike Ph− ALL, Ph+ ALL is not considered curable with chemotherapy, and therefore allogeneic stem cell transplant remains the only curative option. Hematologic responses to imatinib are generally fair (50–70%) but of short duration, although occasional patients achieve a durable cytogenetic response (Ottmann *et al.*, 2002). Ph+ ALL is an example of a malignancy in which the presence of a kinase activated by mutation confers a prognosis worse than that of patients lacking the mutation. Targeting the mutated kinase has improved survival for a few patients, but only marginally. Likewise, although many CML blast crisis patients respond hematologically to imatinib, most eventually lose responsiveness and die (Druker *et al.*, 2001a). These results are likely a reflection of a disease characterized by numerous mutations rather than one. Chronic-phase CML might be characterized as having relatively few genetic hits, whereas the acute BCR/ABL-positive

leukemias are known to have additional alterations that would be unaffected by imatinib therapy (Mahon *et al*., 2000). Thus, the limited effectiveness of the single targeted therapy in acute BCR/ABL leukemias is not unexpected.

B. ETV6–PDGFRβ

Chronic myelomonocytic leukemia (CMMoL) is an unusual leukemia with features of both a myeloproliferative and myelodysplastic disorder (Cortes, 2003). An uncommon variant of CMMoL is characterized by eosinophilia and the presence of a 5;12 translocation, involving the PDGFRβ gene, and, most commonly, the transcription factor ETV6 (Berkowicz *et al*., 1991; Golub *et al*., 1994). The resultant fusion product, ETV6–PDGFRβ (also known as TEL–PDGFRβ), is an oncoprotein that, like BCR/ABL, spontaneously associates, leading to constitutive activation of the tyrosine kinase domain (Carroll *et al*., 1996; Golub *et al*., 1994). ETV6–PDGFRβ confers factor independence when transfected into murine cell lines and can induce a myeloproliferative disease in transgenic mice (Tomasson *et al*., 1999). Imatinib was shown to inhibit autophosphorylation of ETV6–PDGFRβ and prolonged survival in the transgenic model system (Carroll *et al*., 1997). Accordingly, case reports are now accumulating in the literature wherein imatinib induces complete remissions in patients with CMMoL harboring t(5;12) (Apperley *et al*., 2002; Gunby *et al*., 2003; Magnusson *et al*., 2002; Pitini *et al*., 2003). The remissions have generally extended to the molecular level and appear durable. Unfortunately (and not surprisingly), imatinib has had little effect on CMMoL lacking a PDGFRβ rearrangement (Cortes *et al*., 2003).

C. FIP1-L1-PDGFRα

Idiopathic HES is a rare disorder characterized by persistent eosinophilia with organ dysfunction from infiltration of eosinophils (Chusid *et al*., 1975). When there is a clonal cytogenetic abnormality, or increased marrow blasts, the disorder is labeled chronic eosinophilic leukemia (CEL). Like CML, these diseases occasionally respond to interferon-α, although neither HES nor CEL carries a BCR/ABL abnormality. When imatinib was approved for use in CML, clinicians, following a rather curious logic, used it to treat HES patients. They reasoned that because both CML and HES responded to interferon-α, perhaps HES, like CML, would respond to imatinib. Anecdotal reports began to surface describing HES patients responding in dramatic fashion to imatinib (often at a dose lower than that required for treating CML) (Ault *et al*., 2002; Gleich *et al*., 2002;

Pardanani *et al*., 2003b; Schaller and Burkland, 2001). Following up on a t(1;4) translocation present in a responding patient, investigators identified a new fusion gene, FIP1-L1-PDGFRα, that was the result of an interstitial deletion on chromosome 4 (Cools *et al*., 2003). This mutation was subsequently found in EOL-1 cells, which are derived from a patient with CEL (Griffin *et al*., 2003; Saito *et al*., 1985). The protein product of the fusion gene, containing the kinase domain of PDGFRα, was constitutively phosphorylated and conferred factor independence in Ba/F3 cells. As expected, imatinib was a potent inhibitor of the mutated kinase and was cytotoxic to EOL-1 cells. Interestingly, in keeping with what had been observed clinically, the IC_{50} of imatinib for inhibiting FIP1-L1-PDGFRα was 3.2 nM, much lower than the IC_{50} for inhibiting BCR/ABL. This again illustrates that the type of activating mutation can greatly influence the potency of a given inhibitor for a given kinase.

In the study that first reported the discovery of FIP1-L1-PDGFRα, 15 patients with HES and one with CEL were screened for the presence of the fusion protein (Cools *et al*., 2003). Nine patients (including the CEL patient) harbored the mutation, and five of these had a clinically significant, durable response to imatinib. One of the responders subsequently relapsed; DNA sequence analysis of the imatinib-resistant cells revealed the presence of a point mutation in the ATP-binding pocket of FIP1-L1-PDGFRα that was analogous to the imatinib-resistant Thr-315 mutation of BCR/ABL seen commonly in CML.

This story is far from complete. Reports of the success of imatinib in treating HES continue to surface, but the original investigators noted that four patients harboring FIP1-L1-PDGFRα failed to respond, whereas four of five patients lacking the abnormality did respond to the drug. This implies the existence of additional imatinib-responsive alterations in this disorder that have yet to be characterized. The tantalizing association of PDGFR (α or β) abnormalities with elevated eosinophil counts potentially justifies an empiric trial of imatinib in any patient with a myeloproliferative disorder and unexplained eosinophilia.

Perhaps to justify this, another disease associated with eosinophilia has turned up that might respond to imatinib. Systemic mast cell disease (SMCD) is an unusual disorder in which mast cells proliferate beyond the skin, with resultant organ infiltration and dysfunction (Valent *et al*., 2001). SMCD is normally associated with activating mutations of KIT (see later), and occasionally patients are noted to have peripheral eosinophilia. Recently, investigators empirically treated five patients with SMCD and eosinophilia with imatinib (Pardanani *et al*., 2003a). Three of the five so treated achieved clinical remission, and all three harbored the FIP1-L1-PDGFRα rearrangement. The two patients who failed to respond harbored KIT mutations.

V. Kinase Targets in Leukemia: On the Horizon

A. FLT3

FLT3 is a member of the class III or so-called split kinase subfamily of receptor tyrosine kinases (van der Geer *et al*., 1994). This group includes the structurally related receptors KIT, FMS, and PDGFRα and β. FLT3, cloned out of a human hematopoietic stem cell cDNA library, was recognized early on as a receptor that had an important role in hematopoiesis. In human hematopoietic cells, FLT3 expression is restricted to the CD34+ fraction, along with a smaller fraction of CD34− dendritic precursors (Gotze *et al*., 1998; Small *et al*., 1994). Targeted disruption of either the FLT3 receptor or its ligand (FL) in mice is not lethal, but leads to reduced numbers of bone marrow hematopoietic precursors in general and a reduction of lymphoid precursors in particular (Mackarehtschian *et al*., 1995; McKenna *et al*., 2000). FLT3 appears to act in synergy with other cytokine receptors to promote expansion of hematopoietic precursors (Broxmeyer *et al*., 1995; Hirayama *et al*., 1995; Nicholls *et al*., 1999; Ray *et al*., 1996; Veiby *et al*., 1996). Studies in murine and human systems suggest that FLT3 expression and function is mainly associated with the so-called short-term reconstituting hematopoietic stem cells, that is, those capable of multilineage myeloid reconstitution but lacking unlimited self-renewal capacity (Adolfsson *et al*., 2001; Christensen and Weissman, 2001). However, a recent study showed that in human CD34+ cells, the FLT3-positive fraction has the active long-term reconstituting activity (Sitnicka *et al*., 2003).

In common with the other members of its receptor subfamily, FLT3 has an extracellular portion comprising five immunoglobulin-like domains, a single transmembrane domain, and an intracellular portion consisting of a juxtamembrane domain followed by the interrupted kinase domains (Fig. 4; Small *et al*., 1994; van der Geer *et al*., 1994). Following ligand-induced dimerization and autophosphorylation, FLT3 transduces proliferative and antiapoptotic signals through downstream proteins such as Ras-GAP, PLC-β, PI3-kinase, STAT5, and MAP kinase (Dosil *et al*., 1993; Lavagna-Sevenier *et al*., 1998; Rosnet *et al*., 1996a; Zhang *et al*., 1999). The juxtamembrane domain of FLT3 is of particular interest because it apparently exerts a negative regulatory influence on the tyrosine kinase activity of the receptor, the exact nature of which has yet to be elucidated. Another part of FLT3 of particular interest is the activation loop (A-loop), localized to the N-terminal kinase domain (Fig. 4). When the ligand binds to the receptor, the activation loop assumes an open conformation, allowing ATP access to the ATP-binding pocket.

Wild-type FLT3 is expressed by the leukemic blasts in the majority of cases of acute leukemia, and the expression is no longer tightly coupled to CD34 expression (Birg *et al*., 1992; Carow *et al*., 1996; Drexler, 1996;

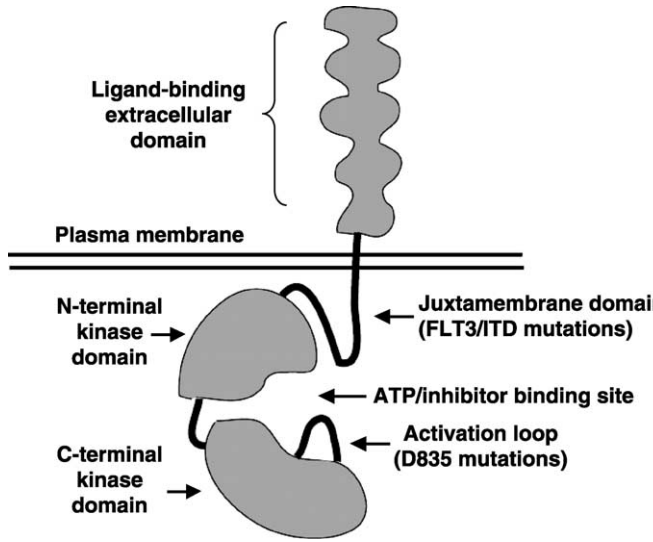

FIGURE 4 A model of the FLT3 receptor showing the location of the major types of activating mutations.

Meierhoff *et al.*, 1995; Rosnet *et al.*, 1996b). In 1996, internal tandem duplication mutations of FLT3 (FLT3/ITD mutations) localized to the juxtamembrane domain were discovered in a significant fraction of AML cases (Nakao *et al.*, 1996). FLT3/ITD mutations range in size from three to more than 400 base pairs (bp) and always occur in multiples of three so that the reading frame is maintained (Schnittger *et al.*, 2002). They are usually contained within exon 14 typically, but not always, near amino acid residues 590 to 600 of the FLT3 sequence within the juxtamembrane domain. Inferring from the crystal structure analysis of the EphB2 receptor, it seems likely that any such insertion (or a deletion) in this domain would be expected to disrupt its influence over the kinase domain, facilitating activation (Wybenga-Groot *et al.*, 2001). In confirmation of this, FLT3/ITD mutations lead to constitutive FLT3 activation and when transfected into Ba/F3 and 32D cells confer factor independence and cause transformation (Hayakawa *et al.*, 2000; Kiyoi *et al.*, 1998, 2002; Mizuki *et al.*, 2000). This influence of the juxtamembrane region appears to be a common feature of this receptor subfamily, as activating mutations of KIT and PDGFRα also occur within this domain (Hirota *et al.*, 1998, 2003; Ma *et al.*, 1999).

The other type of FLT3-activating mutation is located within the activation loop of the kinase domain. The Asp-835 of FLT3, which follows a well-characterized Asp-Phe-Gly motif, is conserved across several subfamilies of receptor tyrosine kinases. Activating point mutations of the corresponding residue in a number of different receptors have been identified and in KIT,

MET, and RET are associated with human malignancies. Shortly after the discovery of FLT3/ITD mutations, two independent groups of investigators reported finding point mutations at this aspartate residue in AML patients (Abu-Duhier *et al*., 2001; Yamamoto *et al*., 2001). Additional activating point mutations at Ile-836, as well as small deletions or insertions at nearby amino acids, have also been reported to occur less frequently (Spiekermann *et al*., 2002; Yamamoto *et al*., 2001). The mechanism by which these types of mutations cause constitutive kinase activity is assumed to be the same as that determined in other receptor tyrosine kinases, namely, that the mutations stabilize the activation loop in the open ATP-binding conformation (Hubbard *et al*., 1994; Till *et al*., 2001).

Following these initial observations, dozens of studies comprising the results of screening more than 5000 adult and pediatric AML samples for FLT3 mutations have been published (Abu-Duhier *et al*., 2000; Boissel *et al*., 2002; Iwai *et al*., 1999; Kiyoi *et al*., 1999; Kondo *et al*., 1999; Kottaridis *et al*., 2001; Meshinchi *et al*., 2001; Rombouts *et al*., 2000; Schnittger *et al*., 2002; Stirewalt *et al*., 2001; Thiede *et al*., 2002; Whitman *et al*., 2001; Xu *et al*., 1999). From these studies, FLT3/ITD mutations can be estimated to occur in 22.9% of *de novo* AML (i.e., AML not arising from preexisting myelodysplasia) and their presence clearly confers a poor prognosis (Levis and Small, 2003). D835 mutations occur in 7% of cases, with a less certain clinical impact (Abu-Duhier *et al*., 2000; Levis and Small, 2003; Yamamoto *et al*., 2001). Finally, the recently described mixed lineage leukemia (MLL) has been shown through microarray analysis to express high levels of FLT3 mRNA (Armstrong *et al*., 2002). Of 30 clinical MLL specimens, 5 were found to harbor FLT3 D835 mutations, and MLL-derived cell lines displayed a cytotoxic response to FLT3 inhibition (Armstrong *et al*., 2003). Overall, FLT3 mutations now represent the most common molecular abnormality in AML, and the receptor appears to be a very important contributor to leukemogenesis in general. The large body of data on the incidence and prognostic impact of FLT3 mutations establishes this receptor as a worthy therapeutic target.

Finally, FLT3 appears to often be constitutively activated in leukemia cells through an autocrine loop. Although the majority of AML blasts express the FLT3 receptor (either wild type or mutant), virtually 100% of them also coexpress FL, with resultant autocrine- or paracrine-mediated FLT3 autophosphorylation (Zheng *et al*., 2004).

In response to the evidence that FLT3 plays an important role in the pathogenesis of a significant proportion of AML cases, various research groups worldwide are investigating more than a dozen small-molecule ATP-competitive FLT3 inhibitors in the hope of developing a new molecularly targeted therapy for this disease (Table II; Gazit *et al*., 2003; Kelly *et al*., 2002; Levis *et al*., 2001, 2002; Murata *et al*., 2003; O'Farrell *et al*., 2003; Teller *et al*., 2002; Tse *et al*., 2001; Weisberg *et al*., 2002; Yee *et al*., 2002).

TABLE II Small-Molecule FLT3 Inhibitors Currently in Clinical Development

Compound	*Class*	*FLT3 IC_{50} (nM)*	*Other receptors inhibited*[a]	*Reference*
CEP-701	Indolocarbazole	3	TrkA	Levis *et al.*, 2002
PKC412	Indolocarbazole	10	PDGFRβ	Weisberg *et al.*, 2002
MLN-518	Quinazoline	30	KIT, PDGFRα, PDGFRβ	Kelly *et al.*, 2002
SU11248	Indolinone	50	KIT, PDGFRβ, VEGFR2	O'Farrell *et al.*, 2002

Note: The listed IC_{50} refers to results obtained from cell-based autophosphorylation assays.
[a] Inhibition occurs with an IC_{50} that is within one order of magnitude of the IC_{50} for FLT3 inhibition.

These FLT3 inhibitors span several different chemical classes, including indolocarbazoles, indolinones, and quinazolines, and they have a relatively broad range of potency and selectivity. Not surprisingly, many of them inhibit KIT and PDGFRα and β with similar potency. In general, these compounds have been found to inhibit FLT3 autophosphorylation in FLT3/ITD-expressing cell lines *in vitro* (with resultant cytotoxic effects) and to improve survival in a variety of mouse models of FLT3/ITD leukemia (Kelly *et al.*, 2002; Levis *et al.*, 2002; O'Farrell *et al.*, 2003; Weisberg *et al.*, 2002).

The indolocarbazoles CEP-701 and PKC412 have been tested in Phase II trials with relapsed/refractory AML patients harboring FLT3-activating mutations (Smith *et al.*, 2002; Stone *et al.*, 2002). In both trials *in vivo* FLT3 autophosphorylation was monitored and correlated with responses, and for both drugs plasma levels that could effectively inhibit FLT3 were achieved without significant toxicity. In the CEP-701 study, 5 of 14 patients treated achieved significant reductions in peripheral blood or marrow blast percentages, although no complete remissions were seen. Patients who showed no response were demonstrated either to have blasts that were resistant *in vitro* to the cytotoxic effects of CEP-701 or to have failed to achieve adequate FLT3 inhibition *in vivo*. In the PKC412 trial, 7 of 20 patients treated achieved a clinically significant reduction in peripheral blast counts, and 1 patient achieved complete remission. The responses in both trials were typically of short duration (i.e., weeks), indicating that resistance to this monotherapy develops relatively quickly. Both drugs are proceeding into trials in which they are combined with standard AML chemotherapy regimens in the hope of attaining more durable responses.

Phase I trials of SU11248 (an indolinone) and of MLN-518 (a quinazoline) have been conducted. Preliminary results suggest that both drugs can successfully inhibit FLT3 *in vivo* (Foran *et al.*, 2002; Heinrich *et al.*, 2002b). However, SU11248, a multitargeted compound, was associated with some degree of toxicity, necessitating intermittent dosing schedules. The relative

selectivity of FLT3 inhibitors, therefore, might play a crucial role in determining which drugs can be safely combined with traditional cytotoxic chemotherapy.

B. KIT

Another member of the class III receptor tyrosine kinase family, this 145-kDa glycoprotein is the product of the c-kit gene, the cellular homologue of the viral oncogene v-kit (Besmer *et al*., 1986; Yarden *et al*., 1987). KIT appears to play an important role in the pathogenesis of a number of malignancies (reviewed in Heinrich *et al*., 2002a) and is expressed by the leukemic blasts in the majority of AML cases (Ikeda *et al*., 1991). Like FLT3, KIT is constitutively activated by juxtamembrane and A-loop mutations, and these mutations, along with overexpression or autocrine activation of the receptor, are associated with specific types of malignancies, including SMCD and AML (Longley *et al*., 2001).

SMCD, as noted previously, is a rare disorder caused by mast cell accumulation in a variety of tissues that can progress to mast cell leukemia or AML (Valent *et al*., 2001). The D816 A-loop mutation of c-kit was originally isolated and characterized from mast cell lines and subsequently from the cells of patients with SMCD (Nagata *et al*., 1995; Tsujimura *et al*., 1994). Although there are possibly rare variants of SMCD that harbor juxtamembrane mutations of KIT, in the vast majority of these cases the KIT-activating mutation resides within the A-loop (Longley *et al*., 2001).

Two independent groups have noted an increased incidence of KIT mutations in AML patients with core binding factor abnormalities t(8;21) and inv(16) (Beghini *et al*., 2000; Care *et al*., 2003). In t(8;21) patients, these have mostly been the D816 A-loop mutations. One group found 15 of 63 patients with inv(16) harbored deletions or insertion in exon 8 of c-kit, and these patients appeared to have a higher probability of relapse (Care *et al*., 2003). It is not known whether these extracellular mutations cause constitutive kinase activity (like the exon 9 mutations seen in GIST), but if so they would likely be imatinib sensitive.

There have been anecdotal reports of responses by AML patients to imatinib (Kindler *et al*., 2003; Schittenhelm *et al*., 2003). Likewise, administration of the indolinone SU5416 led to a complete remission in a patient with AML who expressed high levels of KIT (Mesters *et al*., 2001). However, the receptor targeted in these cases was not clearly established. In general, the KIT mutations found in AML and SMCD or mast cell leukemia are localized to the A-loop; therefore, imatinib will not be a treatment option, as it has essentially no activity against this type of mutation (Frost *et al*., 2002; Zermati *et al*., 2003). Interestingly, the indolocarbazole FLT3 inhibitors are

active against the FLT3 A-loop mutants, so it seems likely that compounds can be developed that will be effective against these KIT mutations (Grundler *et al*., 2003; Levis *et al*., 2002).

C. Other Targets

There are a number of other kinases that are being investigated as potential therapeutic targets in leukemia. Vascular endothelial growth factor (VEGF) appears to be important to hematopoietic stem cell survival, and VEGF receptors are often expressed by leukemic blasts in AML (Fiedler *et al*., 1997a,b; Gerber *et al*., 2002; Verstovsek *et al*., 2002). Moreover, increased levels of soluble VEGF are commonly noted in AML patients, and higher levels seem to correlate with a poor prognosis (Aguayo *et al*., 2002; Padro *et al*., 2002). However, no activating mutations for the VEGF receptors have been described, and the presence of the wild-type receptors on leukemic blasts has no prognostic impact (Meshinchi *et al*., 2003; Verstovsek *et al*., 2002). It is possible that the increased level of plasma VEGF in AML is merely reflective of the increased metabolic activity of the bone marrow, and, like an elevated white blood cell count or lactate dehydrogenase level, is a result rather than a cause of the underlying disease. Angiogenesis inhibitors are currently being widely investigated in a number of solid tumors with some preliminary reports of efficacy. Bevacizumab, an anti-VEGFR monoclonal antibody with efficacy in colorectal and renal cancer, is currently being tested in Phase I/II trials in AML patients (Yang *et al*., 2003).

B-RAF kinase, a serine/threonine kinase that plays a pivotal role in the RAS-MAP kinase pathway, is activated by mutation in a number of solid tumors, notably melanoma (Davies *et al*., 2002; Mercer and Pritchard, 2003). However, hematologic malignancies have not been found to harbor these mutations (Smith *et al*., 2003). BAY 43-9006, a small-molecule RAF kinase inhibitor, is being tested in a variety of malignancies, but this drug at present does not seem to have a clear therapeutic role in leukemia (Lyons *et al*., 2001). On the other hand, the FGF receptor is activated by a translocation in some cases of multiple myeloma and might represent a suitable target when a clinically useful inhibitor can be identified (Chesi *et al*., 2001; Li *et al*., 2001). Likewise, both ARG and TrkA are kinases that have been activated by translocation events in rare cases of AML (Okuda *et al*., 2001; Reuther *et al*., 2000). Both these enzymes are readily amenable to inhibition by agents currently in development (ARG by imatinib and TrkA by CEP-701) (George *et al*., 1999; Okuda *et al*., 2001). Finally, heteroduplex analysis has been used to screen a variety of malignancies for kinase mutations, and this technique will almost certainly uncover new potential kinases to target in leukemia (Bardelli *et al*., 2003).

VI. Conclusions

Kinase inhibitors now have an increasingly prominent role in the treatment of myeloid leukemias. ABL and PDGFRα and β have proven credentials as therapeutic targets, and FLT3 and KIT are likely to be established as targets very soon. The clinical successes that have resulted from the targeting of these kinases illustrate some important points about selecting an appropriate target for the therapy of any kind of cancer. These points relate to the presence and significance of activating mutations. When malignant cells express a kinase that has been activated by mutation, it implies that the function of that kinase is of fundamental importance to those cells. When such a mutation is pathognomonic for the disease (such as BCR/ABL), it becomes an even more inviting target. Whether the mutation is present in, or important to, the cancer stem cell population will always be an important issue. However, even if the genetic defect is found in only those cancer cells that lack the potential for self-renewal, inhibiting the activated product of the gene can still lead to significant clinical benefit: these progeny cells are usually responsible for most of the symptoms of a malignancy.

Only a few mutation-activated kinases have thus far been found in lymphoid malignancies [Ph+ ALL and the NMP/ALK fusion protein of anaplastic lymphoma (Morris *et al.*, 1994)], but it is only a matter of time before more are identified. The kinase-activating genetic alterations in these neoplasms might tend to fall along the lines of gene amplification, which will require screening approaches different from those used for identifying translocations or point mutants.

The enthusiasm for using kinase inhibitors in the treatment of leukemia must be tempered by the fact that none of these agents has thus far led to cures, although some patients with CML have been able to undergo allogeneic stem cell transplantation after achieving disease control with imatinib. In addition, the follow-up duration with these agents is not long enough to definitively establish a survival benefit. Nonetheless, the clinical impact of imatinib in CML, HES, and some cases of CMMoL has been dramatic, and the expectation is that additional agents will have an equally dramatic impact in other hematologic diseases in the near future.

References

Abu-Duhier, F. M., Goodeve, A. C., Wilson, G. A., Care, R. S., Peake, I. R., and Reilly, J. T. (2001). Identification of novel FLT-3 Asp835 mutations in adult acute myeloid leukaemia. *Br. J. Haematol.* **113,** 983–988.

Abu-Duhier, F. M., Goodeve, A. C., Wilson, G. A., Gari, M. A., Peake, I. R., Rees, D. C., Vandenberghe, E. A., Winship, P. R., and Reilly, J. T. (2000). FLT3 internal tandem duplication mutations in adult acute myeloid leukaemia define a high-risk group. *Br. J. Haematol.* **111,** 190–195.

Adolfsson, J., Borge, O. J., Bryder, D., Theilgaard-Monch, K., Astrand-Grundstrom, I., Sitnicka, E., Sasaki, Y., and Jacobsen, S. E. (2001). Upregulation of Flt3 expression within the bone marrow Lin(−)Sca1(+)c- kit(+) stem cell compartment is accompanied by loss of self-renewal capacity. *Immunity* **15,** 659–669.

Aguayo, A., Kantarjian, H. M., Estey, E. H., Giles, F. J., Verstovsek, S., Manshouri, T., Gidel, C., O'Brien, S., Keating, M. J., and Albitar, M. (2002). Plasma vascular endothelial growth factor levels have prognostic significance in patients with acute myeloid leukemia but not in patients with myelodysplastic syndromes. *Cancer* **95,** 1923–1930.

Antonescu, C. R., Sommer, G., Sarran, L., Tschernyavsky, S. J., Riedel, E., Woodruff, J. M., Robson, M., Maki, R., Brennan, M. F., Ladanyi, M., DeMatteo, R. P., and Besmer, P. (2003). Association of KIT Exon 9 Mutations with Nongastric Primary Site and Aggressive Behavior: KIT Mutation Analysis and Clinical Correlates of 120 Gastrointestinal Stromal Tumors. *Clin. Cancer Res.* **9,** 3329–3337.

Apperley, J. F., Gardembas, M., Melo, J. V., Russell-Jones, R., Bain, B. J., Baxter, E. J., Chase, A., Chessells, J. M., Colombat, M., Dearden, C. E., Dimitrijevic, S., Mahon, F. X., Marin, D., Nikolova, Z., Olavarria, E., Silberman, S., Schultheis, B., Cross, N. C., and Goldman, J. M. (2002). Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet- derived growth factor receptor beta. *N. Engl. J. Med.* **347,** 481–487.

Armstrong, S. A., Kung, A. L., Mabon, M. E., Silverman, L. B., Stam, R. W., Den Boer, M. L., Pieters, R., Kersey, J. H., Sallan, S. E., Fletcher, J. A., Golub, T. R., Griffin, J. D., and Korsmeyer, S. J. (2003). Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. *Cancer Cell* **3,** 173–183.

Armstrong, S. A., Staunton, J. E., Silverman, L. B., Pieters, R., den Boer, M. L., Minden, M. D., Sallan, S. E., Lander, E. S., Golub, T. R., and Korsmeyer, S. J. (2002). MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nat. Genet.* **30,** 41–47.

Ault, P., Cortes, J., Koller, C., Kaled, E. S., and Kantarjian, H. (2002). Response of idiopathic hypereosinophilic syndrome to treatment with imatinib mesylate. *Leuk. Res.* **26,** 881–884.

Bardelli, A., Parsons, D. W., Silliman, N., Ptak, J., Szabo, S., Saha, S., Markowitz, S., Willson, J. K., Parmigiani, G., Kinzler, K. W., Vogelstein, B., and Velculescu, V. E. (2003). Mutational analysis of the tyrosine kinome in colorectal cancers. *Science* **300,** 949.

Beghini, A., Peterlongo, P., Ripamonti, C. B., Larizza, L., Cairoli, R., Morra, E., and Mecucci, C. (2000). C-kit mutations in core binding factor leukemias. *Blood* **95,** 726–727.

Ben-Neriah, Y., Daley, G. Q., Mes-Masson, A. M., Witte, O. N., and Baltimore, D. (1986). The chronic myelogenous leukemia-specific P210 protein is the product of the bcr/abl hybrid gene. *Science* **233,** 212–214.

Beran, M., Cao, X., Estrov, Z., Jeha, S., Jin, G., O'Brien, S., Talpaz, M., Arlinghaus, R. B., Lydon, N. B., and Kantarjian, H. (1998). Selective inhibition of cell proliferation and BCR-ABL phosphorylation in acute lymphoblastic leukemia cells expressing Mr 190,000 BCR-ABL protein by a tyrosine kinase inhibitor (CGP- 57148). *Clin. Cancer Res.* **4,** 1661–1672.

Berkowicz, M., Rosner, E., Rechavi, G., Mamon, Z., Neuman, Y., Ben-Bassat, I., and Ramot, B. (1991). Atypical chronic myelomonocytic leukemia with eosinophilia and translocation (5;12). A new association. *Cancer Genet. Cytogenet.* **51,** 277–278.

Besmer, P., Murphy, J. E., George, P. C., Qiu, F. H., Bergold, P. J., Lederman, L., Snyder, H. W., Jr., Brodeur, D., Zuckerman, E. E., and Hardy, W. D. (1986). A new acute transforming feline retrovirus and relationship of its oncogene v-kit with the protein kinase gene family. *Nature* **320,** 415–421.

Bhatia, R., Holtz, M., Niu, N., Gray, R., Snyder, D. S., Sawyers, C. L., Arber, D. A., Slovak, M. L., and Forman, S. J. (2003). Persistence of malignant hematopoietic progenitors in chronic

myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. *Blood* **101,** 4701–4707.

Birg, F., Courcoul, M., Rosnet, O., Bardin, F., Pebusque, M. J., Marchetto, S., Tabilio, A., Mannoni, P., and Birnbaum, D. (1992). Expression of the FMS/KIT-like gene FLT3 in human acute leukemias of the myeloid and lymphoid lineages. *Blood* **80,** 2584–2593.

Bloomfield, C. D., Goldman, A. I., Alimena, G., Berger, R., Borgstrom, G. H., Brandt, L., Catovsky, D., de la Chapelle, A., Dewald, G. W., and Garson, O. M. *et al.* (1986). Chromosomal abnormalities identify high-risk and low-risk patients with acute lymphoblastic leukemia. *Blood* **67,** 415–420.

Boissel, N., Cayuela, J. M., Preudhomme, C., Thomas, X., Grardel, N., Fund, X., Tigaud, I., Raffoux, E., Rousselot, P., Sigaux, F., Degos, L., Castaigne, S., Fenaux, P., and Dombret, H. (2002). Prognostic significance of FLT3 internal tandem repeat in patients with *de novo* acute myeloid leukemia treated with reinforced courses of chemotherapy. *Leukemia* **16,** 1699–1704.

Bonifazi, F., de Vivo, A., Rosti, G., Guilhot, F., Guilhot, J., Trabacchi, E., Hehlmann, R., Hochhaus, A., Shepherd, P. C., Steegmann, J. L., Kluin-Nelemans, H. C., Thaler, J., Simonsson, B., Louwagie, A., Reiffers, J., Mahon, F. X., Montefusco, E., Alimena, G., Hasford, J., Richards, S., Saglio, G., Testoni, N., Martinelli, G., Tura, S., and Baccarani, M. (2001). Chronic myeloid leukemia and interferon-alpha: A study of complete cytogenetic responders. *Blood* **98,** 3074–3081.

Bose, S., Deininger, M., Gora-Tybor, J., Goldman, J. M., and Melo, J. V. (1998). The presence of typical and atypical BCR-ABL fusion genes in leukocytes of normal individuals: Biologic significance and implications for the assessment of minimal residual disease. *Blood* **92,** 3362–3367.

Broxmeyer, H. E., Lu, L., Cooper, S., Ruggieri, L., Li, Z. H., and Lyman, S. D. (1995). Flt3 ligand stimulates/costimulates the growth of myeloid stem/progenitor cells. *Exp. Haematol.* **23,** 1121–1129.

Buchdunger, E., Cioffi, C. L., Law, N., Stover, D., Ohno-Jones, S., Druker, B. J., and Lydon, N. B. (2000). Abl protein-tyrosine kinase inhibitor STI571 inhibits *in vitro* signal transduction mediated by c-kit and platelet-derived growth factor receptors. *J. Pharmacol. Exp. Ther.* **295,** 139–145.

Buchdunger, E., Zimmermann, J., Mett, H., Meyer, T., Muller, M., Druker, B. J., and Lydon, N. B. (1996). Inhibition of the Abl protein-tyrosine kinase *in vitro* and *in vivo* by a 2-phenylaminopyrimidine derivative. *Cancer Res.* **56,** 100–104.

Care, R. S., Valk, P. J., Goodeve, A. C., Abu-Duhier, F. M., Geertsma-Kleinekoort, W. M., Wilson, G. A., Gari, M. A., Peake, I. R., Lowenberg, B., and Reilly, J. T. (2003). Incidence and prognosis of c-KIT and FLT3 mutations in core binding factor (CBF) acute myeloid leukaemias. *Br. J. Haematol.* **121,** 775–777.

Carow, C. E., Levenstein, M., Kaufmann, S. H., Chen, J., Amin, S., Rockwell, P., Witte, L., Borowitz, M. J., Civin, C. I., and Small, D. (1996). Expression of the hematopoietic growth factor receptor FLT3 (STK-1/Flk2) in human leukemias. *Blood* **87,** 1089–1096.

Carroll, M., Ohno-Jones, S., Tamura, S., Buchdunger, E., Zimmermann, J., Lydon, N. B., Gilliland, D. G., and Druker, B. J. (1997). CGP 57148, a tyrosine kinase inhibitor, inhibits the growth of cells expressing BCR-ABL, TEL- ABL, and TEL-PDGFR fusion proteins. *Blood* **90,** 4947–4952.

Carroll, M., Tomasson, M. H., Barker, G. F., Golub, T. R., and Gilliland, D. G. (1996). The TEL/platelet-derived growth factor beta receptor (PDGF beta R) fusion in chronic myelomonocytic leukemia is a transforming protein that self-associates and activates PDGF beta R kinase-dependent signaling pathways. *Proc. Natl. Acad. Sci. USA* **93,** 14845–14850.

Chesi, M., Brents, L. A., Ely, S. A., Bais, C., Robbiani, D. F., Mesri, E. A., Kuehl, W. M., and Bergsagel, P. L. (2001). Activated fibroblast growth factor receptor 3 is an oncogene that contributes to tumor progression in multiple myeloma. *Blood* **97,** 729–736.

Christensen, J. L., and Weissman, I. L. (2001). Flk-2 is a marker in hematopoietic stem cell differentiation: A simple method to isolate long-term stem cells. *Proc. Natl. Acad. Sci. USA* **98,** 14541–14546.

Chusid, M. J., Dale, D. C., West, B. C., and Wolff, S. M. (1975). The hypereosinophilic syndrome: Analysis of fourteen cases with review of the literature. *Medicine (Baltimore)* **54,** 1–27.

Clark, S. S., McLaughlin, J., Timmons, M., Pendergast, A. M., Ben-Neriah, Y., Dow, L. W., Crist, W., Rovera, G., Smith, S. D., and Witte, O. N. (1988). Expression of a distinctive BCR-ABL oncogene in Ph1-positive acute lymphocytic leukemia (ALL). *Science* **239,** 775–777.

Cohen, M. H., Williams, G. A., Sridhara, R., Chen, G., and Pazdur, R. (2003). FDA drug approval summary: Gefitinib (ZD1839) (Iressa) tablets. *Oncologist* **8,** 303–306.

Cools, J., DeAngelo, D. J., Gotlib, J., Stover, E. H., Legare, R. D., Cortes, J., Kutok, J., Clark, J., Galinsky, I., Griffin, J. D., Cross, N. C., Tefferi, A., Malone, J., Alam, R., Schrier, S. L., Schmid, J., Rose, M., Vandenberghe, P., Verhoef, G., Boogaerts, M., Wlodarska, I., Kantarjian, H., Marynen, P., Coutre, S. E., Stone, R., and Gilliland, D. G. (2003). A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N. Engl. J. Med.* **348,** 1201–1214.

Corbin, A. S., La Rosee, P., Stoffregen, E. P., Druker, B. J., and Deininger, M. W. (2003). Several Bcr-Abl kinase domain mutants associated with imatinib mesylate resistance remain sensitive to imatinib. *Blood* **101,** 4611–4614.

Cortes, J. (2003). CMML: A biologically distinct myeloproliferative disease. *Curr. Haematol. Rep.* **2,** 202–208.

Cortes, J., Giles, F., O'Brien, S., Thomas, D., Albitar, M., Rios, M. B., Talpaz, M., Garcia-Manero, G., Faderl, S., Letvak, L., Salvado, A., and Kantarjian, H. (2003). Results of imatinib mesylate therapy in patients with refractory or recurrent acute myeloid leukemia, high-risk myelodysplastic syndrome, and myeloproliferative disorders. *Cancer* **97,** 2760–2766.

Daley, G. Q., and Baltimore, D. (1988). Transformation of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210bcr/abl protein. *Proc. Natl. Acad. Sci. USA* **85,** 9312–9316.

Davies, H., Bignell, G. R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M. J., Bottomley, W., Davis, N., Dicks, E., Ewing, R., Floyd, Y., Gray, K., Hall, S., Hawes, R., Hughes, J., Kosmidou, V., Menzies, A., Mould, C., Parker, A., Stevens, C., Watt, S., Hooper, S., Wilson, R., Jayatilake, H., Gusterson, B. A., Cooper, C., Shipley, J., Hargrave, D., Pritchard-Jones, K., Maitland, N., Chenevix-Trench, G., Riggins, G. J., Bigner, D. D., Palmieri, G., Cossu, A., Flanagan, A., Nicholson, A., Ho, J. W., Leung, S. Y., Yuen, S. T., Weber, B. L., Seigler, H. F., Darrow, T. L., Paterson, H., Marais, R., Marshall, C. J., Wooster, R., Stratton, M. R., and Futreal, P. A. (2002). Mutations of the BRAF gene in human cancer. *Nature* **417,** 949–954.

Deininger, M. W., Goldman, J. M., Lydon, N., and Melo, J. V. (1997). The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL-positive cells. *Blood* **90,** 3691–3698.

Dosil, M., Wang, S., and Lemischka, I. R. (1993). Mitogenic signalling and substrate specificity of the Flk2/Flt3 receptor tyrosine kinase in fibroblasts and interleukin 3-dependent hematopoietic cells. *Mol. Cell. Biol.* **13,** 6572–6585.

Drexler, H. G. (1996). Expression of FLT3 receptor and response to FLT3 ligand by leukemic cells. *Leukemia* **10,** 588–599.

Druker, B. J., Sawyers, C. L., Kantarjian, H., Resta, D. J., Reese, S. F., Ford, J. M., Capdeville, R., and Talpaz, M. (2001a). Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N. Engl. J. Med.* **344,** 1038–1042.

Druker, B. J., Talpaz, M., Resta, D. J., Peng, B., Buchdunger, E., Ford, J. M., Lydon, N. B., Kantarjian, H., Capdeville, R., Ohno-Jones, S., and Sawyers, C. L. (2001b). Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N. Engl. J. Med.* **344,** 1031–1037.

Druker, B. J., Tamura, S., Buchdunger, E., Ohno, S., Segal, G. M., Fanning, S., Zimmermann, J., and Lydon, N. B. (1996). Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat. Med.* **2,** 561–566.

Fiedler, W., Graeven, U., Ergun, S., Verago, S., Kilic, N., Stockschlader, M., and Hossfeld, D. K. (1997). Vascular endothelial growth factor, a possible paracrine growth factor in human acute myeloid leukemia. *Blood* **89,** 1870–1875.

Fielder, W., Graeven, U., Ergun, S., Verago, S., Kilic, N., Stockschlader, M., and Hossfeld, D. K. (1997). Expression of FLT4 and its ligand VEGF-C in acute myeloid leukemia. *Leukemia* **11,** 1234–1237.

Foran, J., Paquette, R., Cooper, M. A., Jacobs, M., O'Farrell, A. M., Kim, H., Cherrington, J. M., and Scigalla, P. (2002). A phase I study of repeated oral dosing with SU11248 for the treatment of patients with acute myeloid leukemia who have failed, or are not eligible for, conventional chemotherapy. *Blood* **100,** 558a.

Frost, M. J., Ferrao, P. T., Hughes, T. P., and Ashman, L. K. (2002). Juxtamembrane mutant V560GKit is more sensitive to Imatinib (STI571) compared with wild-type c-kit whereas the kinase domain mutant D816VKit is resistant. *Mol. Cancer Ther.* **1,** 1115–1124.

Furitsu, T., Tsujimura, T., Tono, T., Ikeda, H., Kitayama, H., Koshimizu, U., Sugahara, H., Butterfield, J. H., Ashman, L. K., and Kanayama, Y. *et al.* (1993). Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. *J. Clin. Invest.* **92,** 1736–1744.

Gambacorti-Passerini, C., Barni, R., le Coutre, P., Zucchetti, M., Cabrita, G., Cleris, L., Rossi, F., Gianazza, E., Brueggen, J., Cozens, R., Pioltelli, P., Pogliani, E., Corneo, G., Formelli, F., and D'Incalci, M. (2000). Role of alpha1 acid glycoprotein in the *in vivo* resistance of human BCR-ABL(+) leukemic cells to the abl inhibitor STI571. *J. Natl. Cancer Inst.* **92,** 1641–1650.

Gazit, A., Yee, K., Uecker, A., Bohmer, F. D., Sjoblom, T., Ostman, A., Waltenberger, J., Golomb, G., Banai, S., Heinrich, M. C., and Levitzki, A. (2003). Tricyclic quinoxalines as potent kinase inhibitors of PDGFR kinase, Flt3 and Kit. *Bioorg. Med. Chem.* **11,** 2007–2018.

George, D. J., Dionne, C. A., Jani, J., Angeles, T., Murakata, C., Lamb, J., and Isaacs, J. T. (1999). Sustained *in vivo* regression of Dunning H rat prostate cancers treated with combinations of androgen ablation and Trk tyrosine kinase inhibitors, CEP-751 (KT-6587) or CEP-701 (KT-5555). *Cancer Res.* **59,** 2395–2401.

Gerber, H. P., Malik, A. K., Solar, G. P., Sherman, D., Liang, X. H., Meng, G., Hong, K., Marsters, J. C., and Ferrara, N. (2002). VEGF regulates haematopoietic stem cell survival by an internal autocrine loop mechanism. *Nature* **417,** 954–958.

Gilliland, D. G., and Griffin, J. D. (2002). The roles of FLT3 in hematopoiesis and leukemia. *Blood* **100,** 1532–1542.

Gleich, G. J., Leiferman, K. M., Pardanani, A., Tefferi, A., and Butterfield, J. H. (2002). Treatment of hypereosinophilic syndrome with imatinib mesilate. *Lancet* **359,** 1577–1578.

Golub, T. R., Barker, G. F., Lovett, M., and Gilliland, D. G. (1994). Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell* **77,** 307–316.

Gorre, M. E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P. N., and Sawyers, C. L. (2001). Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science* **293,** 876–880.

Gotze, K. S., Ramirez, M., Tabor, K., Small, D., Matthews, W., and Civin, C. I. (1998). Flt3high and Flt3low CD34+ progenitor cells isolated from human bone marrow are functionally distinct. *Blood* **91,** 1947–1958.

Graham, S. M., Jorgensen, H. G., Allan, E., Pearson, C., Alcorn, M. J., Richmond, L., and Holyoake, T. L. (2002). Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 *in vitro*. *Blood* **99,** 319–325.

Griffin, J. H., Leung, J., Bruner, R. J., Caligiuri, M. A., and Briesewitz, R. (2003). Discovery of a fusion kinase in EOL-1 cells and idiopathic hypereosinophilic syndrome. *Proc. Natl. Acad. Sci. USA* **100,** 7830–7835.

Grundler, R., Thiede, C., Miething, C., Steudel, C., Peschel, C., and Duyster, J. (2003). Sensitivity toward tyrosine kinase inhibitors varies between different activating mutations of the FLT3 receptor. *Blood* **102,** 646–651.

Gunby, R. H., Cazzaniga, G., Tassi, E., Le Coutre, P., Pogliani, E., Specchia, G., Biondi, A., and Gambacorti- Passerini, C. (2003). Sensitivity to imatinib but low frequency of the TEL/PDGFRb fusion protein in chronic myelomonocytic leukemia. *Haematologica* **88,** 408–415.

Hayakawa, F., Towatari, M., Kiyoi, H., Tanimoto, M., Kitamura, T., Saito, H., and Naoe, T. (2000). Tandem-duplicated Flt3 constitutively activates STAT5 and MAP kinase and introduces autonomous cell growth in IL-3-dependent cell lines. *Oncogene* **19,** 624–631.

Heinrich, M. C., Blanke, C. D., Druker, B. J., and Corless, C. L. (2002a). Inhibition of KIT tyrosine kinase activity: A novel molecular approach to the treatment of KIT-positive malignancies. *J. Clin. Oncol.* **20,** 1692–1703.

Heinrich, M. C., Druker, B., Curtin, P., Paquette, R., Sawyers, C., CeAngelo, D., Gilliland, D. G., Stone, R., Caligiuri, M., Byrd, J., Heaney, M., Nimer, S., Romanko, K., Lambing, J. L., Lokker, N. A., Giese, N. A., and Gretler, D. (2002b). A "first in man" study of the safety and PK/PD of an oral FLT3 inhibitor (MLN518) in patients with AML or high risk myelodysplasia. *Blood* **100,** 336a.

Hirayama, F., Lyman, S. D., Clark, S. C., and Ogawa, M. (1995). The flt3 ligand supports proliferation of lymphohematopoietic progenitors and early B-lymphoid progenitors. *Blood* **85,** 1762–1768.

Hirota, S., Isozaki, K., Moriyama, Y., Hashimoto, K., Nishida, T., Ishiguro, S., Kawano, K., Hanada, M., Kurata, A., Takeda, M., Muhammad Tunio, G., Matsuzawa, Y., Kanakura, Y., Shinomura, Y., and Kitamura, Y. (1998). Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* **279,** 577–580.

Hirota, S., Ohashi, A., Nishida, T., Isozaki, K., Kinoshita, K., Shinomura, Y., and Kitamura, Y. (2003). Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. *Gastroenterology* **125,** 660–667.

Hochhaus, A., Kreil, S., Corbin, A. S., La Rosee, P., Muller, M. C., Lahaye, T., Hanfstein, B., Schoch, C., Cross, N. C., Berger, U., Gschaidmeier, H., Druker, B. J., and Hehlmann, R. (2002). Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia* **16,** 2190–2196.

Hubbard, S. R. (2001). Theme and variations: Juxtamembrane regulation of receptor protein kinases. *Mol. Cell* **8,** 481–482.

Hubbard, S. R., Wei, L., Ellis, L., and Hendrickson, W. A. (1994). Crystal structure of the tyrosine kinase domain of the human insulin receptor. *Nature* **372,** 746–754.

Ikeda, H., Kanakura, Y., Tamaki, T., Kuriu, A., Kitayama, H., Ishikawa, J., Kanayama, Y., Yonezawa, T., Tarui, S., and Griffin, J. D. (1991). Expression and functional role of the proto-oncogene c-kit in acute myeloblastic leukemia cells. *Blood* **78,** 2962–2968.

Iwai, T., Yokota, S., Nakao, M., Okamoto, T., Taniwaki, M., Onodera, N., Watanabe, A., Kikuta, A., Tanaka, A., Asami, K., Sekine, I., Mugishima, H., Nishimura, Y., Koizumi, S., Horikoshi, Y., Mimaya, J., Ohta, S., Nishikawa, K., Iwai, A., Shimokawa, T., Nakayama, M., Kawakami, K.,

Gushiken, T., Hyakuna, N., and Fujimoto, T. *et al.* (1999). Internal tandem duplication of the FLT3 gene and clinical evaluation in childhood acute myeloid leukemia. The Children's Cancer and Leukemia Study Group, Japan. *Leukemia* **13,** 38–43.

Jeffers, M., Schmidt, L., Nakaigawa, N., Webb, C. P., Weirich, G., Kishida, T., Zbar, B., and Vande Woude, G. F. (1997). Activating mutations for the met tyrosine kinase receptor in human cancer. *Proc. Natl. Acad. Sci. USA* **94,** 11445–11450.

Johnson, J. R., Bross, P., Cohen, M., Rothmann, M., Chen, G., Zajicek, A., Gobburu, J., Rahman, A., Staten, A., and Pazdur, R. (2003). Approval Summary: Imatinib mesylate capsules for treatment of adult patients with newly diagnosed Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase. *Clin. Cancer Res.* **9,** 1972–1979.

Kantarjian, H., Sawyers, C., Hochhaus, A., Guilhot, F., Schiffer, C., Gambacorti-Passerini, C., Niederwieser, D., Resta, D., Capdeville, R., Zoellner, U., Talpaz, M., Druker, B., Goldman, J., O'Brien, S. G., Russell, N., Fischer, T., Ottmann, O., Cony-Makhoul, P., Facon, T., Stone, R., Miller, C., Tallman, M., Brown, R., Schuster, M., Loughran, T., Gratwohl, A., Mandelli, F., Saglio, G., Lazzarino, M., Russo, D., Baccarani, M., and Morra, E. (2002). Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N. Engl. J. Med.* **346,** 645–652.

Kelly, L. M., Yu, J. C., Boulton, C. L., Apatira, M., Li, J., Sullivan, C. M., Williams, I., Amaral, S. M., Curley, D. P., Duclos, N., Neuberg, D., Scarborough, R. M., Pandey, A., Hollenbach, S., Abe, K., Lokker, N. A., Gilliland, D. G., and Giese, N. A. (2002). CT53518, a novel selective FLT3 antagonist for the treatment of acute myelogenous leukemia (AML). *Cancer Cell* **1,** 421–432.

Kindler, T., Breitenbuecher, F., Marx, A., Hess, G., Gschaidmeier, H., Gamm, H., Kirkpatrick, C. J., Huber, C., and Fischer, T. (2003). Sustained complete hematologic remission after administration of the tyrosine kinase inhibitor imatinib mesylate in a patient with refractory, secondary AML. *Blood* **101,** 2960–2962.

Kiyoi, H., Naoe, T., Nakano, Y., Yokota, S., Minami, S., Miyawaki, S., Asou, N., Kuriyama, K., Jinnai, I., Shimazaki, C., Akiyama, H., Saito, K., Oh, H., Motoji, T., Omoto, E., Saito, H., Ohno, R., and Ueda, R. (1999). Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. *Blood* **93,** 3074–3080.

Kiyoi, H., Ohno, R., Ueda, R., Saito, H., and Naoe, T. (2002). Mechanism of constitutive activation of FLT3 with internal tandem duplication in the juxtamembrane domain. *Oncogene* **21,** 2555–2563.

Kiyoi, H., Towatari, M., Yokota, S., Hamaguchi, M., Ohno, R., Saito, H., and Naoe, T. (1998). Internal tandem duplication of the FLT3 gene is a novel modality of elongation mutation which causes constitutive activation of the product. *Leukemia* **12,** 1333–1337.

Kondo, M., Horibe, K., Takahashi, Y., Matsumoto, K., Fukuda, M., Inaba, J., Kato, K., Kojima, S., and Matsuyama, T. (1999). Prognostic value of internal tandem duplication of the FLT3 gene in childhood acute myelogenous leukemia. *Med. Pediatr. Oncol.* **33,** 525–529.

Kottaridis, P. D., Gale, R. E., Frew, M. E., Harrison, G., Langabeer, S. E., Belton, A. A., Walker, H., Wheatley, K., Bowen, D. T., Burnett, A. K., Goldstone, A. H., and Linch, D. C. (2001). The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: Analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood* **98,** 1752–1759.

La Rosee, P., Corbin, A. S., Stoffregen, E. P., Deininger, M. W., and Druker, B. J. (2002). Activity of the Bcr-Abl kinase inhibitor PD180970 against clinically relevant Bcr-Abl isoforms that cause resistance to matinib mesylate (Gleevec, STI571). *Cancer Res.* **62,** 7149–7153.

Lange, T., Gunther, C., Kohler, T., Krahl, R., Musiol, S., Leiblein, S., Al-Ali, H. K., van Hoomissen, I., Niederwieser, D., and Deininger, M. W. (2003). High levels of BAX, low levels of MRP-1, and high platelets are independent predictors of response to imatinib in myeloid blast crisis of CML. *Blood* **101,** 2152–2155.

Lavagna-Sevenier, C., Marchetto, S., Birnbaum, D., and Rosnet, O. (1998). FLT3 signaling in hematopoietic cells involves CBL, SHC and an unknown P115 as prominent tyrosine-phosphorylated substrates. *Leukemia* **12,** 301–310.

le Coutre, P., Mologni, L., Cleris, L., Marchesi, E., Buchdunger, E., Giardini, R., Formelli, F., and Gambacorti- Passerini, C. (1999). *In vivo* eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. *J. Natl. Cancer Inst.* **91,** 163–168.

le Coutre, P., Tassi, E., Varella-Garcia, M., Barni, R., Mologni, L., Cabrita, G., Marchesi, E., Supino, R., and Gambacorti-Passerini, C. (2000). Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. *Blood* **95,** 1758–1766.

Levis, M., Allebach, J., Tse, K. F., Zheng, R., Baldwin, B. R., Smith, B. D., Jones-Bolin, S., Ruggeri, B., Dionne, C., and Small, D. (2002). A FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukemia cells in vitro and in vivo. *Blood* **99,** 3885–3891.

Levis, M., and Small, D. (2003). FLT3: ITDoes matter in leukemia. *Leukemia* **17,** 1738–1752.

Levis, M., Tse, K. F., Smith, B. D., Garrett, E., and Small, D. (2001). A FLT3 tyrosine kinase inhibitor is selectively cytotoxic to acute myeloid leukemia blasts harboring FLT3 internal tandem duplication mutations. *Blood* **98,** 885–887.

Li, Z., Zhu, Y. X., Plowright, E. E., Bergsagel, P. L., Chesi, M., Patterson, B., Hawley, T. S., Hawley, R. G., and Stewart, A. K. (2001). The myeloma-associated oncogene fibroblast growth factor receptor 3 is transforming in hematopoietic cells. *Blood* **97,** 2413–2419.

Longley, B. J., Reguera, M. J., and Ma, Y. (2001). Classes of c-KIT activating mutations: Proposed mechanisms of action and implications for disease classification and therapy. *Leuk. Res.* **25,** 571–576.

Lugo, T. G., and Witte, O. N. (1989). The BCR-ABL oncogene transforms Rat-1 cells and cooperates with v-myc. *Mol. Cell. Biol.* **9,** 1263–1270.

Lux, M. L., Rubin, B. P., Biase, T. L., Chen, C. J., Maclure, T., Demetri, G., Xiao, S., Singer, S., Fletcher, C. D., and Fletcher, J. A. (2000). KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. *Am. J. Pathol.* **156,** 791–795.

Lyons, J. F., Wilhelm, S., Hibner, B., and Bollag, G. (2001). Discovery of a novel Raf kinase inhibitor. *Endocr. Relat. Cancer* **8,** 219–225.

Ma, Y., Longley, B. J., Wang, X., Blount, J. L., Langley, K., and Caughey, G. H. (1999). Clustering of activating mutations in c-KIT's juxtamembrane coding region in canine mast cell neoplasms. *J. Invest. Dermatol.* **112,** 165–170.

Mackarehtschian, K., Hardin, J. D., Moore, K. A., Boast, S., Goff, S. P., and Lemischka, I. R. (1995). Targeted disruption of the flk2/flt3 gene leads to deficiencies in primitive hematopoietic progenitors. *Immunity* **3,** 147–161.

Magnusson, M. K., Meade, K. E., Nakamura, R., Barrett, J., and Dunbar, C. E. (2002). Activity of STI571 in chronic myelomonocytic leukemia with a platelet-derived growth factor beta receptor fusion oncogene. *Blood* **100,** 1088–1091.

Mahon, F. X., Deininger, M. W., Schultheis, B., Chabrol, J., Reiffers, J., Goldman, J. M., and Melo, J. V. (2000). Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: Diverse mechanisms of resistance. *Blood* **96,** 1070–1079.

Mauro, M. M., Druker, B., Kuyl, G., Kurilik, R., and Maziarz, T. (2003). Increasing levels of detectable leukemia in imatinib treated CML patients with previously undetectable or very low levels of BCR-ABL. *American Society of Clinical Oncology, Meeting Proceedings* **22,** 569a.

McKenna, H. J., Stocking, K. L., Miller, R. E., Brasel, K., De Smedt, T., Maraskovsky, E., Maliszewski, C. R., Lynch, D. H., Smith, J., Pulendran, B., Roux, E. R., Teepe, M.,

Lyman, S. D., and Peschon, J. J. (2000). Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood* **95,** 3489–3497.

McLaughlin, J., Chianese, E., and Witte, O. N. (1987). *In vitro* transformation of immature hematopoietic cells by the P210 BCR/ABL oncogene product of the Philadelphia chromosome. *Proc. Natl. Acad. Sci. USA* **84,** 6558–6562.

McWhirter, J. R., Galasso, D. L., and Wang, J. Y. (1993). A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr-Abl oncoproteins. *Mol. Cell. Biol.* **13,** 7587–7595.

Meierhoff, G., Dehmel, U., Gruss, H. J., Rosnet, O., Birnbaum, D., Quentmeier, H., Dirks, W., and Drexler, H. G. (1995). Expression of FLT3 receptor and FLT3-ligand in human leukemia-lymphoma cell lines. *Leukemia* **9,** 1368–1372.

Mercer, K. E., and Pritchard, C. A. (2003). Raf proteins and cancer: B-Raf is identified as a mutational target. *Biochim. Biophys. Acta* **1653,** 25–40.

Meshinchi, S., Stirewalt, D. L., Alonzo, T. A., Zhang, Q., Sweetser, D. A., Woods, W. G., Bernstein, I. D., Arceci, R. J., and Radich, J. P. (2003). Activating mutations of RTK/*ras* signal transduction pathway in pediatric acute myeloid leukemia. *Blood* **102**(4), 1474–1479.

Meshinchi, S., Woods, W. G., Stirewalt, D. L., Sweetser, D. A., Buckley, J. D., Tjoa, T. K., Bernstein, I. D., and Radich, J. P. (2001). Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. *Blood* **97,** 89–94.

Mesters, R. M., Padro, T., Bieker, R., Steins, M., Kreuter, M., Goner, M., Kelsey, S., Scigalla, P., Fiedler, W., Buchner, T., and Berdel, W. E. (2001). Stable remission after administration of the receptor tyrosine kinase inhibitor SU5416 in a patient with refractory acute myeloid leukemia. *Blood* **98,** 241–243.

Mizuki, M., Fenski, R., Halfter, H., Matsumura, I., Schmidt, R., Muller, C., Gruning, W., Kratz-Albers, K., Serve, H., Steur, C., Buchner, T., Kienast, J., Kanakura, Y., Berdel, W. E., and Serve, H. (2000). Flt3 mutations from patients with acute myeloid leukemia induce transformation of 32D cells mediated by the Ras and STAT5 pathways. *Blood* **96,** 3907–3914.

Morris, S. W., Kirstein, M. N., Valentine, M. B., Dittmer, K. G., Shapiro, D. N., Saltman, D. L., and Look, A. T. (1994). Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science* **263,** 1281–1284.

Murata, K., Kumagai, H., Kawashima, T., Tamitsu, K., Irie, M., Nakajima, H., Suzu, S., Shibuya, M., Kamihira, S., Nosaka, T., Asano, S., and Kitamura, T. (2003). Selective cytotoxic mechanism of GTP-14564, a novel tyrosine kinase inhibitor in leukemia cells expressing a constitutively active Fms-like tyrosine kinase 3 (FLT3). *J. Biol. Chem.* **278,** 32892–32898.

Nagar, B., Bornmann, W. G., Pellicena, P., Schindler, T., Veach, D. R., Miller, W. T., Clarkson, B., and Kuriyan, J. (2002). Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res.* **62,** 4236–4243.

Nagata, H., Worobec, A. S., Oh, C. K., Chowdhury, B. A., Tannenbaum, S., Suzuki, Y., and Metcalfe, D. D. (1995). Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. *Proc. Natl. Acad. Sci. USA* **92,** 10560–10564.

Nakao, M., Yokota, S., Iwai, T., Kaneko, H., Horiike, S., Kashima, K., Sonoda, Y., Fujimoto, T., and Misawa, S. (1996). Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. *Leukemia* **10,** 1911–1918.

Nicholls, S. E., Winter, S., Mottram, R., Miyan, J. A., and Whetton, A. D. (1999). Flt3 ligand can promote survival and macrophage development without proliferation in myeloid progenitor cells. *Exp. Haematol.* **27,** 663–672.

Nowell, P., and Hungerford, D. (1960). Chromosome Studies on Normal and Leukemic Human Leukocytes. *J. Natl. Cancer Inst.* **25,** 85–109.

O'Brien, S. G., Guilhot, F., Larson, R. A., Gathmann, I., Baccarani, M., Cervantes, F., Cornelissen, J. J., Fischer, T., Hochhaus, A., Hughes, T., Lechner, K., Nielsen, J. L., Rousselot, P., Reiffers, J., Saglio, G., Shepherd, J., Simonsson, B., Gratwohl, A., Goldman, J. M., Kantarjian, H., Taylor, K., Verhoef, G., Bolton, A. E., Capdeville, R., and Druker, B. J. (2003). Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N. Engl. J. Med.* **348,** 994–1004.

O'Farrell, A. M., Abrams, T. J., Yuen, H. A., Ngai, T. J., Louie, S. G., Yee, K. W., Wong, L. M., Hong, W., Lee, L. B., Town, A., Smolich, B. D., Manning, W. C., Murray, L. J., Heinrich, M. C., and Cherrington, J. M. (2003). SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity *in vitro* and *in vivo*. *Blood* **101,** 3597–3605.

Okuda, K., Weisberg, E., Gilliland, D. G., and Griffin, J. D. (2001). ARG tyrosine kinase activity is inhibited by STI571. *Blood* **97,** 2440–2448.

Ottmann, O. G., Druker, B. J., Sawyers, C. L., Goldman, J. M., Reiffers, J., Silver, R. T., Tura, S., Fischer, T., Deininger, M. W., Schiffer, C. A., Baccarani, M., Gratwohl, A., Hochhaus, A., Hoelzer, D., Fernandes-Reese, S., Gathmann, I., Capdeville, R., and O'Brien, S. G. (2002). A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. *Blood* **100,** 1965–1971.

Padro, T., Bieker, R., Ruiz, S., Steins, M., Retzlaff, S., Burger, H., Buchner, T., Kessler, T., Herrera, F., Kienast, J., Muller-Tidow, C., Serve, H., Berdel, W. E., and Mesters, R. M. (2002). Overexpression of vascular endothelial growth factor (VEGF) and its cellular receptor KDR (VEGFR-2) in the bone marrow of patients with acute myeloid leukemia. *Leukemia* **16,** 1302–1310.

Pane, F., Frigeri, F., Sindona, M., Luciano, L., Ferrara, F., Cimino, R., Meloni, G., Saglio, G., Salvatore, F., and Rotoli, B. (1996). Neutrophilic-chronic myeloid leukemia: A distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). *Blood* **88,** 2410–2414.

Pardanani, A., Ketterling, R. P., Brockman, S. R., Flynn, H. C., Paternoster, S. F., Shearer, B. M., Reeder, T. L., Li, C. Y., Cross, N. C., Cools, J., Gilliland, D. G., Dewald, G. W., and Tefferi, A. (2003). CHIC2 deletion, a surrogate for FIP1L1-PDGFRA fusion, occurs in systemic mastocytosis associated with eosinophilia and predicts response to imatinib mesylate therapy. *Blood* **102,** 3093–3096.

Pitini, V., Arrigo, C., Teti, D., Barresi, G., Righi, M., and Alo, G. (2003). Response to STI571 in chronic myelomonocytic leukemia with platelet derived growth factor beta receptor involvement: a new case report. *Haematologica* **88,** ECR18.

Quackenbush, R. C., Reuther, G. W., Miller, J. P., Courtney, K. D., Pear, W. S., and Pendergast, A. M. (2000). Analysis of the biologic properties of p230 Bcr-Abl reveals unique and overlapping properties with the oncogenic p185 and p210 Bcr-Abl tyrosine kinases. *Blood* **95,** 2913–2921.

Ray, R. J., Paige, C. J., Furlonger, C., Lyman, S. D., and Rottapel, R. (1996). Flt3 ligand supports the differentiation of early B cell progenitors in the presence of interleukin-11 and interleukin-7. *Eur. J. Immunol.* **26,** 1504–1510.

Reuther, G. W., Lambert, Q. T., Caligiuri, M. A., and Der, C. J. (2000). Identification and characterization of an activating TrkA deletion mutation in acute myeloid leukemia. *Mol. Cell. Biol.* **20,** 8655–8666.

Reya, T., Morrison, S. J., Clarke, M. F., and Weissman, I. L. (2001). Stem cells, cancer, and cancer stem cells. *Nature* **414,** 105–111.

Roche-Lestienne, C., Soenen-Cornu, V., Grardel-Duflos, N., Lai, J. L., Philippe, N., Facon, T., Fenaux, P., and Preudhomme, C. (2002). Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood* **100,** 1014–1018.

Rombouts, W. J., Blokland, I., Lowenberg, B., and Ploemacher, R. E. (2000). Biological characteristics and prognosis of adult acute myeloid leukemia with internal tandem duplications in the Flt3 gene. *Leukemia* **14,** 675–683.

Rosnet, O., Buhring, H. J., deLapeyriere, O., Beslu, N., Lavagna, C., Marchetto, S., Rappold, I., Drexler, H. G., Birg, F., Rottapel, R., Hannum, C., Dubreuil, P., and Birnbaum, D. (1996a). Expression and signal transduction of the FLT3 tyrosine kinase receptor. *Acta Haematol.* **95,** 218–223.

Rosnet, O., Buhring, H. J., Marchetto, S., Rappold, I., Lavagna, C., Sainty, D., Arnoulet, C., Chabannon, C., Kanz, L., Hannum, C., and Birnbaum, D. (1996b). Human FLT3/FLK2 receptor tyrosine kinase is expressed at the surface of normal and malignant hematopoietic cells. *Leukemia* **10,** 238–248.

Rowley, J. D. (1973). Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* **243,** 290–293.

Saito, H., Bourinbaiar, A., Ginsburg, M., Minato, K., Ceresi, E., Yamada, K., Machover, D., Breard, J., and Mathe, G. (1985). Establishment and characterization of a new human eosinophilic leukemia cell line. *Blood* **66,** 1233–1240.

Santoro, M., Carlomagno, F., Romano, A., Bottaro, D. P., Dathan, N. A., Grieco, M., Fusco, A., Vecchio, G., Matoskova, B., and Kraus, M. H. *et al.* (1995). Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. *Science* **267,** 381–383.

Sausville, E. A. (2000). Dragons 'round the fleece again: STI571 versus alpha1 acid glycoprotein. *J. Natl. Cancer Inst.* **92,** 1626–1627.

Savage, D. G., and Goldman, J. M. (1997). Allografting for chronic myeloid leukemia. *Curr. Opin. Haematol.* **4,** 369–376.

Sawyers, C. L. (1999). Chronic myeloid leukemia. *N. Engl. J. Med.* **340,** 1330–1340.

Schaller, J. L., and Burkland, G. A. (2001). Case report: Rapid and complete control of idiopathic hypereosinophilia with imatinib mesylate. *Med. Gen. Med.* **3,** 9.

Schindler, T., Bornmann, W., Pellicena, P., Miller, W. T., Clarkson, B., and Kuriyan, J. (2000). Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* **289,** 1938–1942.

Schittenhelm, M., Aichele, O., Krober, S. M., Brummendorf, T., Kanz, L., and Denzlinger, C. (2003). Complete remission of third recurrence of acute myeloid leukemia after treatment with imatinib (STI-571). *Leuk. Lymphoma* **44,** 1251–1253.

Schnittger, S., Schoch, C., Dugas, M., Kern, W., Staib, P., Wuchter, C., Loffler, H., Sauerland, C. M., Serve, H., Buchner, T., Haferlach, T., and Hiddemann, W. (2002). Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: Correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood* **100,** 59–66.

Sefton, B. M., Hunter, T., Beemon, K., and Eckhart, W. (1980). Evidence that the phosphorylation of tyrosine is essential for cellular transformation by Rous sarcoma virus. *Cell* **20,** 807–816.

Shah, N. P., Nicoll, J. M., Nagar, B., Gorre, M. E., Paquette, R. L., Kuriyan, J., and Sawyers, C. L. (2002). Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* **2,** 117–125.

Silver, R. T., Woolf, S. H., Hehlmann, R., Appelbaum, F. R., Anderson, J., Bennett, C., Goldman, J. M., Guilhot, F., Kantarjian, H. M., Lichtin, A. E., Talpaz, M., and Tura, S. (1999). An evidence-based analysis of the effect of busulfan, hydroxyurea, interferon, and allogeneic bone marrow transplantation in treating the chronic phase of chronic myeloid leukemia: developed for the American Society of Hematology. *Blood* **94,** 1517–1536.

Sitnicka, E., Buza-Vidas, N., Larsson, S., Nygren, J. M., Liuba, K., and Jacobsen, S. E. (2003). Human CD34+ hematopoietic stem cells capable of multilineage engrafting NOD/SCID mice express flt3: Distinct flt3 and c-kit expression and response patterns on mouse and candidate human hematopoietic stem cells. *Blood* **102,** 881–886.

Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., and McGuire, W. L. (1987). Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* **235,** 177–182.

Small, D., Levenstein, M., Kim, E., Carow, C., Amin, S., Rockwell, P., Witte, L., Burrow, C., Ratajczak, M. Z., and Gewirtz, A. M. *et al.* (1994). STK-1, the human homolog of Flk-2/Flt-3, is selectively expressed in CD34+ human bone marrow cells and is involved in the proliferation of early progenitor/stem cells. *Proc. Natl. Acad. Sci. USA* **91,** 459–463.

Smith, B. D., Levis, M., Brown, P., Russsell, L., Hellreigel, E., Dauses, T., Allebach, J., and Small, D. (2002). Single agent CEP-701, a novel FLT-3 inhibitor, shows initial response in patients with refractory acute myeloid leukemia. *Blood* **100,** 85a.

Smith, M. L., Snaddon, J., Neat, M., Cambal-Parrales, M., Arch, R., Lister, T. A., and Fitzgibbon, J. (2003). Mutation of BRAF is uncommon in AML FAB type M1 and M2. *Leukemia* **17,** 274–275.

Spiekermann, K., Bagrintseva, K., Schoch, C., Haferlach, T., Hiddemann, W., and Schnittger, S. (2002). A new and recurrent activating length mutation in exon 20 of the FLT3 gene in acute myeloid leukemia. *Blood* **100,** 3423–3425.

Stirewalt, D. L., Kopecky, K. J., Meshinchi, S., Appelbaum, F. R., Slovak, M. L., Willman, C. L., and Radich, J. P. (2001). FLT3, RAS, and TP53 mutations in elderly patients with acute myeloid leukemia. *Blood* **97,** 3589–3595.

Stone, R., Klimeck, V., DeAngelo, D. J., Nimer, S., Estey, E., Galinsky, I., Neuberg, D., Yap, A., Fox, E., and Gilliland, D. G. (2002). PKC412, an oral FLT3 inhibitor, has activity in mutant FLT3 acute myeloid leukemia (AML): A phase II clinical trial. *Blood* **100,** 86a.

Teller, S., Kramer, D., Bohmer, S. A., Tse, K. F., Small, D., Mahboobi, S., Wallrapp, C., Beckers, T., Kratz-Albers, K., Schwable, J., Serve, H., and Bohmer, F. D. (2002). Bis(1H-2-indolyl)-1-methanones as inhibitors of the hematopoietic tyrosine kinase Flt3. *Leukemia* **16,** 1528–1534.

Thiede, C., Steudel, C., Mohr, B., Schaich, M., Schakel, U., Platzbecker, U., Wermke, M., Bornhauser, M., Ritter, M., Neubauer, A., Ehninger, G., and Illmer, T. (2002). Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: Association with FAB subtypes and identification of subgroups with poor prognosis. *Blood* **99,** 4326–4335.

Till, J. H., Ablooglu, A. J., Frankel, M., Bishop, S. M., Kohanski, R. A., and Hubbard, S. R. (2001). Crystallographic and solution studies of an activation loop mutant of the insulin receptor tyrosine kinase: Insights into kinase mechanism. *J. Biol. Chem.* **276,** 10049–10055.

Tomasson, M. H., Williams, I. R., Hasserjian, R., Udomsakdi, C., McGrath, S. M., Schwaller, J., Druker, B., and Gilliland, D. G. (1999). TEL/PDGFbetaR induces hematologic malignancies in mice that respond to a specific tyrosine kinase inhibitor. *Blood* **93,** 1707–1714.

Tse, K. F., Novelli, E., Civin, C. I., Bohmer, F. D., and Small, D. (2001). Inhibition of FLT3-mediated transformation by use of a tyrosine kinase inhibitor. *Leukemia* **15,** 1001–1010.

Tsujimura, T., Furitsu, T., Morimoto, M., Isozaki, K., Nomura, S., Matsuzawa, Y., Kitamura, Y., and Kanakura, Y. (1994). Ligand-independent activation of c- kit receptor tyrosine kinase in a murine mastocytoma cell line P- 815 generated by a point mutation. *Blood* **83,** 2619–2626.

Valent, P., Horny, H. P., Escribano, L., Longley, B. J., Li, C. Y., Schwartz, L. B., Marone, G., Nunez, R., Akin, C., Sotlar, K., Sperr, W. R., Wolff, K., Brunning, R. D., Parwaresch, R. M., Austen, K. F., Lennert, K., Metcalfe, D. D., Vardiman, J. W., and Bennett, J. M.

(2001). Diagnostic criteria and classification of mastocytosis: A consensus proposal. *Leuk. Res.* **25,** 603–625.

van der Geer, P., Hunter, T., and Lindberg, R. A. (1994). Receptor protein- tyrosine kinases and their signal transduction pathways. *Annu. Rev. Cell Biol.* **10,** 251–337.

Van Etten, R. A., Jackson, P., and Baltimore, D. (1989). The mouse type IV c-abl gene product is a nuclear protein, and activation of transforming ability is associated with cytoplasmic localization. *Cell* **58,** 669–678.

Veiby, O. P., Jacobsen, F. W., Cui, L., Lyman, S. D., and Jacobsen, S. E. (1996). The flt3 ligand promotes the survival of primitive hemopoietic progenitor cells with myeloid as well as B lymphoid potential. Suppression of apoptosis and counteraction by TNF-alpha and TGF-beta. *J. Immunol.* **157,** 2953–2960.

Verstovsek, S., Estey, E., Manshouri, T., Giles, F. J., Cortes, J., Beran, M., Rogers, A., Keating, M., Kantarjian, H., and Albitar, M. (2002). Clinical relevance of vascular endothelial growth factor receptors 1 and 2 in acute myeloid leukaemia and myelodysplastic syndrome. *Br. J. Haematol.* **118,** 151–156.

Vogelstein, B., and Kinzler, K. W. (1993). The multistep nature of cancer. *Trends Genet.* **9,** 138–141.

Von Bubnoff, N., Veach, D. R., Miller, W. T., Li, W., Sanger, J., Peschel, C., Bornmann, W. G., Clarkson, B., and Duyster, J. (2003). Inhibition of wild-type and mutant bcr-abl by pyrido-pyrimidine-type small molecule kinase inhibitors. *Cancer Res.* **63,** 6395–6404.

Wang, J. Y. (2000). Regulation of cell death by the Abl tyrosine kinase. *Oncogene* **19,** 5643–5650.

Weisberg, E., Boulton, C., Kelly, L. M., Manley, P., Fabbro, D., Meyer, T., Gilliland, D. G., and Griffin, J. D. (2002). Inhibition of mutant FLT3 receptors in leukemia cells by the small molecule tyrosine kinase inhibitor PKC412. *Cancer Cell* **1,** 433–443.

Weisberg, E., and Griffin, J. D. (2000). Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. *Blood* **95,** 3498–3505.

Westbrook, C. A., Hooberman, A. L., Spino, C., Dodge, R. K., Larson, R. A., Davey, F., Wurster-Hill, D. H., Sobol, R. E., Schiffer, C., and Bloomfield, C. D. (1992). Clinical significance of the BCR-ABL fusion gene in adult acute lymphoblastic leukemia: A cancer and leukemia group B study (8762). *Blood* **80,** 2983–2990.

Whitman, S. P., Archer, K. J., Feng, L., Baldus, C., Becknell, B., Carlson, B. D., Carroll, A. J., Mrozek, K., Vardiman, J. W., George, S. L., Kolitz, J. E., Larson, R. A., Bloomfield, C. D., and Caligiuri, M. A. (2001). Absence of the wild-type allele predicts poor prognosis in adult *de novo* acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: A cancer and leukemia group B study. *Cancer Res.* **61,** 7233–7239.

Wolff, N. C., and Ilaria, R. L., Jr. (2001). Establishment of a murine model for therapy-treated chronic myelogenous leukemia using the tyrosine kinase inhibitor STI571. *Blood* **98,** 2808–2816.

Wybenga-Groot, L. E., Baskin, B., Ong, S. H., Tong, J., Pawson, T., and Sicheri, F. (2001). Structural basis for autoinhibition of the Ephb2 receptor tyrosine kinase by the unphosphorylated juxtamembrane region. *Cell* **106,** 745–757.

Xu, F., Taki, T., Yang, H. W., Hanada, R., Hongo, T., Ohnishi, H., Kobayashi, M., Bessho, F., Yanagisawa, M., and Hayashi, Y. (1999). Tandem duplication of the FLT3 gene is found in acute lymphoblastic leukaemia as well as acute myeloid leukaemia but not in myelodysplastic syndrome or juvenile chronic myelogenous leukaemia in children. *Br. J. Haematol.* **105,** 155–162.

Yamamoto, Y., Kiyoi, H., Nakano, Y., Suzuki, R., Kodera, Y., Miyawaki, S., Asou, N., Kuriyama, K., Yagasaki, F., Shimazaki, C., Akiyama, H., Saito, K., Nishimura, M., Motoji, T., Shinagawa, K., Takeshita, A., Saito, H., Ueda, R., Ohno, R., and Naoe, T.

(2001). Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood* **97,** 2434–2439.

Yang, J. C., Haworth, L., Sherry, R. M., Hwu, P., Schwartzentruber, D. J., Topalian, S. L., Steinberg, S. M., Chen, H. X., and Rosenberg, S. A. (2003). A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N. Engl. J. Med.* **349,** 427–434.

Yarden, Y., Kuang, W. J., Yang-Feng, T., Coussens, L., Munemitsu, S., Dull, T. J., Chen, E., Schlessinger, J., Francke, U., and Ullrich, A. (1987). Human proto-oncogene c-kit: A new cell surface receptor tyrosine kinase for an unidentified ligand. *Embo. J.* **6,** 3341–3351.

Yee, K. W., O'Farrell, A. M., Smolich, B. D., Cherrington, J. M., McMahon, G., Wait, C. L., McGreevey, L. S., Griffith, D. J., and Heinrich, M. C. (2002). SU5416 and SU5614 inhibit kinase activity of wild-type and mutant FLT3 receptor tyrosine kinase. *Blood* **100,** 2941–2949.

Yeni, P. G., Hammer, S. M., Carpenter, C. C., Cooper, D. A., Fischl, M. A., Gatell, J. M., Gazzard, B. G., Hirsch, M. S., Jacobsen, D. M., Katzenstein, D. A., Montaner, J. S., Richman, D. D., Saag, M. S., Schechter, M., Schooley, R. T., Thompson, M. A., Vella, S., and Volberding, P. A. (2002). Antiretroviral treatment for adult HIV infection in 2002: Updated recommendations of the International AIDS Society-USA Panel. *Jama* **288,** 222–235.

Zermati, Y., De Sepulveda, P., Feger, F., Letard, S., Kersual, J., Casteran, N., Gorochov, G., Dy, M., Ribadeau Dumas, A., Dorgham, K., Parizot, C., Bieche, Y., Vidaud, M., Lortholary, O., Arock, M., Hermine, O., and Dubreuil, P. (2003). Effect of tyrosine kinase inhibitor STI571 on the kinase activity of wild-type and various mutated c-kit receptors found in mast cell neoplasms. *Oncogene* **22,** 660–664.

Zhang, S., Mantel, C., and Broxmeyer, H. E. (1999). Flt3 signaling involves tyrosyl-phosphorylation of SHP-2 and SHIP and their association with Grb2 and Shc in Baf3/Flt3 cells. *J. Leukoc. Biol.* **65,** 372–380.

Zheng, R., Levis, M., Piloto, O., Brown, P., Baldwin, B. R., Gorin, N. C., Beran, M., Zhu, Z., Ludwig, D., Hicklin, D., Witte, L., Li, Y., and Small, D. (2004). FLT3 ligand causes autocrine signaling in acute myeloid leukemia cells. *Blood* **103,** 267–274.

Steven Soignet* and Peter Maslak†

*The Arcus Group, LLC
New York, New York 10012
and
Department of Medicine
Memorial Sloan-Kettering Cancer Center
New York, New York 10021

†Hematology Laboratory Service
Memorial Sloan-Kettering Cancer Center
New York, New York 10021

Therapy of Acute Promyelocytic Leukemia

I. Chapter Overview

Acute promyelocytic leukemia (APL) is a form of myeloid leukemia for which the introduction of targeted therapies has radically altered the treatment paradigm. This chapter highlights the biology of this disease and reviews the therapeutic strategies currently employed to manage it.

II. Introduction

Although APL is a relatively rare disorder, it has proven invaluable as a model of targeted therapy in modern oncology. The initial experience in employing alternatives to the standard anthracycline–cytarabine-based regimens was tempered by empiricism borne out of a need to establish a therapeutic approach for a fulminant disorder in the face of rare resources.

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

Subsequently, the recognition that the clinical responses were intimately linked to the underlying biology of the disease led not only to the introduction of a new standard of care in treating leukemia but also to a further understanding of fundamental mechanisms of leukemogenesis.

III. Disease Background and Biology

The clinical syndrome of APL is often characterized by the patient presenting with the most common complications associated with acute leukemia: infection and hemorrhage. The incidence of hemorrhagic complications might be exceptionally severe and out of proportion to the thrombocytopenia, as patients often develop an additional coagulopathy (Drapkin *et al.*, 1978; Gralnick *et al.*, 1972). The initial diagnostic work-up includes examination of the peripheral blood and bone marrow. Peripheral white blood cell (WBC) counts are often below normal, but the bone marrow aspirate is generally replaced with abnormal promyelocytes with a characteristic hypergranular appearance. Some morphologic variants of the disorder exist, but the clinical course is determined primarily by the genotype (Golomb *et al.*, 1980; McKenna *et al.*, 1982; Sainty *et al.*, 2000).

More than 95% of APL cases are characterized by a balanced translocation between chromosomes 15 and 17 at the q22 and q21 loci on the respective chromosomes (Larson *et al.*, 1984; Rowley *et al.*, 1997). The ability to detect this translocation by conventional cytogenetics is diagnostic of APL and is often used in cases in which the morphology might be problematic. On a molecular level, the chromosomal translocation results in fusion of RARα gene on chromosome 17 to a portion of the PML gene on chromosome 15 (de Thé *et al.*, 1990; Kakizuka *et al.*, 1991). This chimeric fusion gene product has profound implications for the cell and has been established to be a key event in leukemogenesis.

A model for the ability of the PML/RARα to transform normal cells has been developed by correlating clinical data with experimental data generated by using cell lines and transgeneic mice (Guidez *et al.*, 1998; He *et al.*, 1998). Under normal physiologic conditions, RARα is thought to play an important role in myeloid differentiation because of its ability to bind nuclear corepressors, which in turn control the regulation of transcription by binding histone deacetylases. Nuclear corepressors and histone deacetylases regulate transcription through their effects on chromatin conformation. Binding of the corepressor complex results in a closed conformation of the chromatin, shutting down transcription of functionally active genes. Physiologic binding of retinoic acid causes a dissociation of the corepressor complex, allowing various transcriptional activators access to an open conformation of chromatin, facilitating gene transcription. The genes downstream from this regulatory switch are thought to be

important in the process of myeloid maturation. In the pathologic state, the PML/RARα gene product is substituted for RARα. This fusion protein has different properties, the most important being a greater affinity for the corepressor complex. Therefore, the stronger avidity of binding is not responsive to physiologic concentrations of retinoic acid. Instead, the supraphysiologic concentration achieved by the administration of all-*trans* retinoic acid (ATRA) is required to recapitulate the physiologic response and allow normal differentiation to proceed.

Although the vast majority of APL is defined by the t(15;17) resulting in the critical PML/RAR abnormality, variant translocations have been described (Arnould *et al*., 1999; Licht *et al*., 1995; Redner *et al*., 2000; Wells *et al*., 1997). These cytogenetic/molecular variants are clinically rare, but useful in further refining the model of transcriptional repression suggested by the experimental data. The most common variants involve translocations between chromosome 17 and either chromosome 11 or 5. Although the breakpoint in the RARα gene on chromosome 17 is invariable, the different "partner" chromosomes contain different "partner" genes. The molecular consequences of these variant translocations have distinct effects on the phenotype of the leukemia, particularly with regard to the response to ATRA. The (11;17) (q23q21) translocation is most notable in this regard, because resistance to the differentiating effects of ATRA is well described. In the new chimeric combination, PML is replaced by the leucine zinc finger PLZF as the molecular partner gene for RARα. PLZF interacts differently with the nuclear corepressor complex by binding in multiple sites, rendering the retinoic acid alone insufficient to reverse transcriptional inhibition. This translocation also renders the patient insensitive to standard chemotherapy, although the actual mechanism behind this resistance is not well understood.

The molecular genetics of APL has significance beyond mechanistic explanations of transformation in that the novel fusion gene can be used to clinically diagnose and manage patients with this disorder. In morphologic variants in which the characteristic microscopic features are not readily apparent, detecting either t(15;17) by conventional cytogenetics or PML/RARα through the use of reverse transcriptase polymerase chain reaction (RT-PCR) confirms the diagnosis of APL (LoCoco *et al*., 1992; Miller *et al*., 1992). The molecular technique has the advantage in this setting, because the turnaround time tends to be more rapid and the results are available in a timeframe that is meaningful for the clinician. The presence of PML/RARα in the setting of morphologic remission provides evidence of minimal residual disease. Patients in whom PML/RARα is able to be detected following completion of therapy have a poor prognosis and ultimately relapse with overt disease (Diverio *et al*., 1998; Jurcic *et al*., 2001). Other patients who become negative for PML/RARα by RT-PCR but later convert back to a positive result also ultimately relapse with clinically apparent disease.

Therefore, RT-PCR has become a standard tool for monitoring response, because this technique detects residual or recurrent disease before it can be detected by conventional microscopic means. The clinical advantage to this is that an improved outcome can be obtained if therapy is instituted at an earlier stage when the leukemia burden is at a minimum. This situation is in marked contrast to most other subtypes of acute myeloid leukemia (AML) for which no molecular markers exist or for which molecular monitoring of existing markers has not been validated by clinical data. In these forms of AML, the "gold standard" for response remains morphologic assessment of the bone marrow and peripheral blood. The ability to assess early relapse is, however, hampered by the inherent insensitivity of light microscopy, making the criteria for complete remission (CR) relatively imprecise with regard to the presence of minimal residual disease. The consequences of the inability to detect minimal disease can be seen in the high relapse rates and the inability to adapt the amount of therapy based on measurable criteria. For most patients with AML, "one size fits all" and patients receive the same amount of therapy whether they are cured of all traces of disease or have remnants of the original clone in the bone marrow. In APL, the ability to detect minimal residual disease through molecular techniques has allowed therapy to be adapted to the relative amount of disease present and has led to a new definition of response known as molecular remission. For cure to be accomplished in APL, molecular remission is required. In the modern era, the means toward this end is achieved through a combination of conventional chemotherapeutic agents, retinoids, and, in the relapsed setting, arsenicals.

IV. Treatment Approaches

APL is a subtype of AML, and prior to the late 1980s this disease was treated with the standard therapy for AML. Treatment for AML is generally divided into two phases of therapy: induction and postremission therapy. Induction therapy traditionally consists of intensive chemotherapy designed to produce aplasia, eradicate morphologically apparent disease, and result in CR. Postremission therapy can take a variety of forms, but the ultimate purpose of this continuing treatment past CR is to eradicate minimal residual disease and effect cure. A number of terms have been adopted to describe the various forms of postremission therapy (Bloomfield, 1985). Consolidation therapy describes chemotherapy that is similar to induction therapy but given in the immediate postremission setting. Intensification is a form of high-dose consolidation, and maintenance therapy is dose-attenuated treatment generally given over a prolonged period of time (typically more than 6 months).

In the modern era, standard induction therapy for AML consists of cytosine arabinoside (Ara-C) combined with an anthracycline or anthracenedione (Scheinberg *et al.*, 2001). Ara-C is generally administered as a continuous infusion over 7 days, whereas daunorubicin (or idarubicin or mitoxantrone) is given by intravenous bolus or push on the first 3 days of therapy. The daunorubicin/Ara-C regimen has become known as 7 + 3 or 3 + 7. Minor variations of this schedule exist, but the original daunorubicin/Ara-C is still widely employed.

Given the profound coagulopathy that accompanies the diagnosis of APL, the ability to distinguish APL from the other forms of AML at a relatively early time in the patient's presentation is key in the clinical management of the disorder (Drapkin *et al.*, 1978; Gralnick *et al.*, 1972). Historically, the CR rate in APL with standard chemotherapy might have been slightly lower than that achieved in other forms of AML because of the morbidity or mortality associated with the institution of therapy (Kantarjian *et al.*, 1986). However, as supportive care improved, many more patients could be sustained through the coagulopathy and CR rates increased to as high as 80%. Despite the relatively high CR rate, the long-term disease-free survival for patients with APL treated with standard therapy was 30–40% (Marty *et al.*, 1984). This outcome was superior to the survival data for AML as a whole, but it underscored the need to develop new treatment strategies to improve cure rate.

One such strategy was in a reevaluation of the standard chemotherapy regimen with modification of the doses used. Data from various clinical trials suggest that APL is particularly sensitive to the anthracycline component of induction therapy. This may be reflective of the underlying biology of the disease as demonstrated by the rare occurrence of drug-resistant phenotypes in *de novo* disease. A number of trials have reported CR rates comparable to standard induction regimens using monotherapy with either daunorubicin or idarubicin (Avvisati *et al.*, 1990; Petti *et al.*, 1987). Several other retrospective studies suggested a positive effect on survival when dose intensification of the anthracycline was employed. In the study from the Southwest Oncology Group (SWOG), this effect was seen in patients who received the higher cumulative anthracycline dose but not in those who received high-dose cytarabine (Head *et al.*, 1995). This finding is in contrast to the data in other forms of AML in which high-dose cytarabine has been emphasized in various treatment regimens. Another randomized study compared high-dose single-agent idarubicin with a standard idarubicin/cytarabine combination and showed no difference in CR rate. Therefore, these data suggest that anthracycline is of primary importance in this disease and a strategy of dose-intensive single-agent anthracycline as part of the modern treatment regimen for APL might be beneficial both in terms of toxicity and efficacy. The choice of the optimal anthracycline, however, remains controversial.

A. All-*trans* Retinoic Acid

The addition of ATRA into the treatment strategy of APL has greatly improved the outcome of this disease. ATRA was the first successful clinical application of differentiation therapy. The initial series of patients treated with ATRA was reported in 1987 (Huang *et al*., 1987), and by the mid 1990s, more than 3000 APL patients worldwide had been treated with this drug. Collectively, these studies with ATRA had a reported median complete remission rate in excess of 85% (Fenaux *et al*., 1993; Huang *et al*., 1987, 1988; Lo Coco *et al*., 1991; Warrell *et al*., 1991). These responses were obtained with doses of ATRA up to 100 mg/m^2/day. However, no particular dose–effect correlation was determined. Most clinical experience has been obtained with a dose of 45 mg/m^2/day administered as a single daily dose or in two equally divided doses given approximately 12 h apart. Although lower doses of ATRA given daily have less typical retinoid side effects (i.e., cheilitis and headaches) and are similar in terms of CR rates and pharmacokinetic parameters to ATRA at 45 mg/m^2/day, the incidence of life-threatening adverse events unique to APL (hyperleukocytosis and retinoic acid syndrome) was not reduced. In addition, the long-term therapeutic effects of a lower dose of ATRA have not been established, and therefore 45 mg/m^2/day is used as the standard dose for treatment of this disease.

Importantly, the clinical response to ATRA is correlated with the presence of the 15;17 chromosomal translocation assessed either by conventional cytogenetics or by RT-PCR (Diverio *et al*., 1998; Lo Coco *et al*., 1992; Miller *et al*., 1992). This translocation involves the molecular rearrangement of RARα, which seems to be the link to the responsiveness of APL to ATRA therapy. For example, patients with equivocal cellular morphology and normal karyotypes show typical rearrangements of RARα by molecular testing and are clinically responsive to ATRA (Lo Coco *et al*., 1992; Miller *et al*., 1993). Alternatively, patients who are negative by RT-PCR for PML/RARα and/or do not exhibit the karyotypic t(15;17) do not respond to ATRA (Lo Coco *et al*., 1992; Miller *et al*., 1992). Therefore, patients with acute leukemia with cytogenetic or molecular findings other than those distinctly found in APL should be treated with standard antileukemic therapy and not with ATRA.

Despite the high remission rate obtained with single-agent ATRA, initial studies revealed that remissions induced and maintained exclusively by ATRA are brief in duration (median duration <6 months) (Frankel *et al*., 1994; Warrell *et al*., 1994). In the series of studies in which ATRA was used alone, rarely did any patient maintain a remission of more than 1 year (Di Bona *et al*., 2000; Lo Coco *et al*., 1991). Subsequently, both randomized and nonrandomized studies have shown that by combining ATRA with standard induction therapy, followed by consolidation treatments using several

cycles of anthracycline-based regimens, remissions were not only durable but also superior to those achieved by chemotherapy alone (Avvisati *et al.*, 1998; Castaigne *et al.*, 1990; Warrell *et al.*, 1994).

In initial APL trials incorporating ATRA, the drug was given as a single agent until CR was achieved; then patients were consolidated with chemotherapy. However, the optimal schedule and duration of ATRA therapy needed to be determined to obtain the best clinical outcome. Subsequently, many large randomized studies have been conducted worldwide.

In the U.S. Intergroup study (Tallman *et al.*, 1997), 346 newly diagnosed APL patients were randomized to receive either ATRA or daunorubicin plus Ara-C for remission induction. Patients who obtained a CR received two cycles of consolidation therapy: the first cycle of treatment was identical to induction chemotherapy and the second cycle of consolidation consisted of high-dose Ara-C plus daunorubicin. Patients who remained in remission after completing consolidation therapy were randomized to either observation only or maintenance treatment with ATRA. Therefore, the majority of patients in this trial received ATRA, either as induction therapy or maintenance or both. Although there was no significant difference in the CR rate between the ATRA (72%) and chemotherapy (69%) induction groups, the 3-year disease-free survival (DFS) was statistically improved in ATRA-treated patients than in those treated with standard antileukemic therapy (72% vs. 32%, $p < 0.001$). The group of patients who received no ATRA as either induction or maintenance had a 3-year DFS of only 18%, which was consistent with the historical survival rate of APL patients. This trial clearly defined the benefit of ATRA in the management of APL, particularly when ATRA was incorporated as part of induction therapy.

A large-scale European study (Fenaux *et al.*, 1999) evaluated the clinical impact of the scheduling of ATRA/chemotherapy by prospectively randomizing 413 untreated APL patients between concurrent ATRA plus chemotherapy (daunorubicin and Ara-C) and sequential ATRA followed by the same chemotherapy. Induction therapy was stratified based on both age (>65 years or ≤ 65 years) and on presenting WBC count (>5000 or $\leq 5000/\mu$l). Patients who had WBC counts $>5000/\mu$l and were younger than 65 years (163 patients) were not randomized but were treated with concurrent ATRA and chemotherapy starting on Day 1. Patients who were older than 65 years (66 patients) were not randomized but were treated with ATRA followed sequentially by chemotherapy. Patients who achieved a CR irrespective of their induction regimen received one or two additional courses of consolidation chemotherapy (one course if older than 65) and then were randomized to receive either 2 years of maintenance chemotherapy consisting of ATRA alone, methotrexate plus 6-mercaptopurine (6-MP), ATRA plus methotrexate and 6-MP, or no maintenance chemotherapy (observation only). The proportion of patients achieving a CR in these two induction groups was not different, with an overall CR rate of 92%.

However, the event-free survival (EFS) at 2 years was estimated at 84% in the concurrent ATRA plus chemotherapy group vs. 77% in the ATRA followed by chemotherapy group ($p = 0.1$). This benefit appears to occur from a reduction in the risk of relapse, which at 2 years was 6% in the concurrent arm and 16% in the sequential arm ($p = 0.04$). An added possible advantage from the concurrent ATRA plus chemotherapy treatment approach appears to be a >50% reduction in the incidence of the potential fatal retinoic acid syndrome (RAS) (Tallman *et al.*, 2002).

The Italian Cooperative Group [Gruppo Italiano Malattie Ematologiche maligne dell' Adulto (GIMEMA)] eliminated Ara-C from induction therapy and used the combination of ATRA and idarubicin (referred to as the AIDA for all-*trans* retinoic acid plus idarubicin) for remission induction in newly diagnosed APL (Mandelli *et al.*, 1997). In the GIMEMA multicenter trial, 253 patients with cytogenetics positive for t(15;17) and/or molecularly positive by RT-PCR for the PML/RARα fusion gene were treated with ATRA until CR. Intravenous idarubicin (12 mg/m^2/day) was given concurrently with ATRA on Days 2, 4, 6, and 8. Patients who achieved a CR were consolidated with three cycles of combination cytotoxic chemotherapy. With this induction regimen, 229 of 240 (95%) evaluable patients achieved a CR. There were 11 deaths related to early complications. Molecular conversion rate from PCR positive to PCR negative for PML/RARα after induction therapy was 60.5%, which increased to 95% by the completion of the third cycle of consolidation therapy. The estimated actuarial EFS for all 253 patients was 83% and 79% at 1 and 2 years, respectively. These were unequivocally the best results reported for treating adults with acute leukemia.

B. Duration of ATRA

ATRA is recommended as part of the induction regimen and as maintenance therapy, which is initiated after the completion of consolidation chemotherapy. There is no gain from ongoing treatment with ATRA in induction once patients have achieved complete remission. Continuous ATRA therapy beyond complete remission can potentially have negative clinical implications by losing the benefits ATRA provides as maintenance. Generally, patients who relapse while taking ATRA or shortly after discontinuing ATRA fail to respond to further treatment with standard or high-dose ATRA therapy (Huang *et al.*, 1987; Warrell *et al.*, 1994). It would be exceptionally unusual for patients with a molecular diagnosis of APL who are retinoid naïve to be resistant to ATRA. Considering the limited duration of remission with ATRA as a single agent, acquired resistance to ATRA could theoretically result from genetic or epigenetic events (Warrell, 1993). Although acquired resistance in HL-60, a retinoid-sensitive leukemic cell line, has been associated with point mutations the in RARα fusion gene (Li

et al., 1994; Robertson *et al*., 1992), clinical specimens collected from patients prior to treatment with ATRA and at the time of relapse have not shown additional mutations in PML/RARα (Warrell, 1996). Therefore, it appears that multiple mechanisms are likely involved in the development of resistance.

Continuous daily treatment with ATRA is associated with a marked decrease in plasma drug concentrations occurring as early as 1–2 weeks of initiation of therapy (Muindi *et al*., 1992). The mechanisms by which this occurs include induction of cytochrome-P450 catabolic enzymes, increased expression of oxidative cofactors, and/or upregulation of cellular retinoic acid binding proteins (Muindi *et al*., 1992, 1994). These biologic mechanisms function cooperatively to modulate intracellular retinoid concentrations. As a result, it is plausible that prolonged administration of ATRA could result in the development of clinical resistance because of an inability to sustain effective concentrations *in vivo* that would be required to achieve cytodifferentiation. Also, upregulation of the respective metabolic enzymes reverses after a defined period from discontinuation of therapy (Adamson *et al*., 1995; Delva *et al*., 1993). When reinstituting therapy with standard doses of ATRA, the therapeutic levels needed clinically to induce myeloid differentiation are once again obtained. Therefore, ATRA therapy should be discontinued once a complete remission has been achieved and not used again until maintenance therapy is initiated.

C. Postremission Consolidation Therapy

As discussed previously, treatment of APL with ATRA alone or in combination with chemotherapy yields a complete clinical remission rate as high as 85–95%. However, minimal residual disease (MRD) detected by a positive RT-PCR for the PML/RARα transcript is present in 80–90% of patients following induction therapy with ATRA alone and in approximately 50% of patients when ATRA is given with chemotherapy (Jurcic *et al*., 2001; Sanz *et al*., 1999). Consistently, patients with positive cytogenetics for t(15:17) or MRD determined by RT-PCR clinically relapse (Fenaux *et al*., 1994; Grimwade *et al*., 1996; Lo Coco *et al*., 1992; Miller *et al*., 1992). The proportion of patients with MRD is considerably reduced by the administration of postremission chemotherapy. Jurcic *et al*. (2001) did serial PCR analysis on the bone marrow of 47 patients with untreated APL who received ATRA induction followed by consolidation treatment with chemotherapy and biologic agents. In this study, 40 of 47 (85%) patients had MRD detectable by RT-PCR after ATRA induction and only 4 (10%) patients after completing three cycles of consolidation therapy. This benefit likely results in a higher percentage of patients with APL cured (Grimwade *et al*., 1996; Lo Coco *et al*., 1992; Sanz *et al*., 1999).

Although several groups have incorporated high-dose Ara-C as part of the consolidation strategy (Burnett *et al*., 1999; Lengfelder *et al*., 2000; Tallman *et al*., 1997), there have been no substantial data to support the benefit of this approach in either induction or consolidation. The exact role of cytarabine in the treatment of APL is questionable. In a recent nonrandomized prospective study conducted by the Spanish PETHEMA group, 123 newly diagnosed molecularly confirmed APL patients were treated with a modified AIDA regimen for induction and consolidation (Ara-C and etoposide were eliminated from consolidation) (Sanz *et al*., 1999). Complete hematologic remission was obtained in 89% (109) of the patients. In addition, 51% of patients after induction therapy and 93% after the completion of consolidation treatment achieved a molecular remission. The 2-year DFS and overall survival (OS) estimates were 92% and 82%, respectively. These results are comparable to those obtained (CR 92%, DFS 96%, OS 88%) in the German AML Cooperative Group study (Lengfelder *et al*., 2000), in which intensified double-induction therapy with high-dose Ara-C with ATRA was administered. Similar results were observed in other trials that employed dose-intense Ara-C.

Currently, for newly diagnosed APL untreated patients, most clinicians use a combination of ATRA and chemotherapy (anthracycline based) for remission induction. Once remission is achieved, ATRA is discontinued and an anthracycline-based regimen is administered as consolidation therapy, with the goal of eliminating MRD. As previously discussed, results of this approach have been extremely good, with 80–85% of patients achieving long-term DFS and in all likelihood cured (Fenaux *et al*., 1994; Warrell *et al*., 1994). However, patients in remission should be monitored by RT-PCR for evidence of MRD. The experience to date shows that patients who remain positive during clinical remission or those who convert from negative to positive will ultimately relapse clinically (Jurcic *et al*., 2001; Lo Coco *et al*., 1992; Miller *et al*., 1992). Therefore, it is important that sequential PCR analysis for MRD be part of the routine monitoring of patients with APL. The results of such analyses drive important clinical decisions, such as which patients could potentially benefit from additional therapy, and, equally important, which patients can be spared the toxicity and risks of further treatment, such as in allogeneic bone marrow or stem cell transplantation.

D. Maintenance Therapy

Conventionally, maintenance therapy has not been a critical component of the management of AML. However in APL, recent studies have suggested a possible benefit of maintenance therapy (De Botton *et al*., 1998; Kantarjian *et al*., 1987). In a study reported by Kantarjian *et al*. (1987), 39 of 70 patients with APL who received 6-MP and methotrexate as maintenance had an approximate twofold (56% vs. 30%, $p < 0.01$) higher

sustained 3-year remission rate than patients who did not receive maintenance. Subsequently, results of a single nonrandomized (Sanz *et al.*, 1999) and two randomized trials (Fenaux *et al.*, 1999; Tallman *et al.*, 1997) have shown a reduction in the risk of relapse in patients, treated with ATRA or chemotherapy, or both, as maintenance. In the largest of these three studies, the European APL 93 trial (Fenaux *et al.*, 1999), 289 patients, after completing their consolidation therapy, were randomized to observation only or to maintenance therapy with either ATRA (45 mg/m^2/day for 15 days every 3 months) or continuous low-dose chemotherapy with 6-MP (90 mg/m^2/day) plus methotrexate (50 mg/m^2 given weekly), or both. The 2-year relapse incidence was 13% compared with 25% ($p = 0.2$) and 11% compared with 27% ($p = 0.0003$) in patients who received vs. those who did not receive ATRA and in patients who received vs. those who did not receive chemotherapy as maintenance, respectively. Notably, the relapse rate (6 of 74 patients, 8%) was lowest in patients who were randomized to both ATRA and low-dose chemotherapy. In addition, there was an improvement in overall survival ($p = 0.01$) in patients who received chemotherapy maintenance and a similar trend in patients who received ATRA maintenance ($p = 0.22$). Of interest, high-risk patients (presenting WBC counts >5000, and >60 years old) who received both ATRA and low-dose chemotherapy as maintenance seemed to benefit the most. At present, there are two large ongoing randomized trials further evaluating the dose, schedule, and role of ATRA and chemotherapy maintenance in APL. The GIMEMA Cooperative Group is randomizing newly diagnosed APL patients to either no maintenance or maintenance therapy using the same dose and schedules used in the APL 93 study, and the North American Intergroup is evaluating ATRA given alone every other week vs. ATRA every other week with continuous low-dose 6-MP plus methotrexate every week.

E. Adverse Effects of ATRA

In the initial studies using ATRA in the treatment of APL, two unique adverse events emerged: hyperleukocytosis and the RAS, which often in the initial experience with ATRA proved fatal. Subsequently, a number of successful treatment strategies have been developed that have reduced the associated morbidity mortality of these adverse events.

In general, the toxicity profile of ATRA is comparable to that of other retinoids. As mentioned previously, APL patients are uniquely prone to the development of a hyperleukocytosis and the RAS, with a reported incidence as high at 50% (Frankel *et al.*, 1992). RAS is characterized by fever, respiratory distress, radiographic pulmonary infiltrates, pleural or pericardial effusions, weight gain due to fluid overload, episodic hypotension, and acute renal failure. Clinical diagnosis can be difficult, because this patient population is at risk to develop pneumonia, sepsis, and congestive heart failure as a

result of the disease or because of complications of cytotoxic chemotherapy. The first signs and or symptoms of RAS can occur any time during therapy. Most often, they occurs within the first few days to weeks of initiating ATRA therapy (Warrell, 1993), but have also occurred toward the end of induction therapy in patients maintained on ATRA and whose marrow was recovering from postmyelosuppressive doses of chemotherapy (De Botton *et al*., 1998). Although hyperleukocytosis is frequently observed preceding RAS, up to one-third of patients who have RAS have a normal leukocyte count (Frankel *et al*., 1992, 1994; Vahdat *et al*., 1994).

The basis of RAS is unknown; however, clinically it resembles a capillary leak syndrome. Postmortem examinations revealed extensive infiltration of maturing myeloid cells into lung, skin, kidney, liver, and lymph nodes of patients who died with progressive hypoxemia and multiorgan failure (Frankel *et al*., 1992). Several potential contributing mechanisms have been proposed, including release of vasoactive cytokines, increased expression of adhesion molecules on myeloid cell surfaces, and attainment of migratory capabilities by malignant promyelocytes as they undergo differentiation (Frankel *et al*., 1992; Vahdat *et al*., 1994). ATRA has been shown to increase the expression of the surface integrin ICAM-1 in certain cell lines. Of clinical importance is that this effect can be blocked by treatment with dexamethasone (Dedhar *et al*., 1991; Zhang *et al*., 1993). Development of RAS is also correlated with expression of CD13 (aminopeptidase N; Vahdat *et al*., 1994), which is has been associated with a poor outcome in patients with AML. These observations suggest a association with the clinical experience of extravascular adhesion and migration of differentiating cells in this reaction.

Progression of RAS can be abated by early intervention with a brief course of high-dose dexamethasone (10 mg twice a day for 3 days), and, as a result, the morbidity and mortality from this adverse event has decreased significantly (Tallman *et al*., 2002). Most important is early recognition accompanied by prompt intervention. Development of any unexplained signs or symptoms, particularly fluid retention, hectic fevers, and pulmonary infiltrates, should prompt immediate dexamethasone treatment. Once RAS is fully established, it is especially difficult to manage and often results in significant morbidity and/or death. Therefore, the benefits of empiric steroid therapy far outweigh the risk of complications associated with its use in leukemic patients. Some groups have advocated prophylactic use of corticosteroids, based on a nonrandomized prospective study reported by the Australian Study Group in which patients were treated with prophylaxis corticosteroids (prednisone 75 mg/day) and reported a lower incidence of pulmonary toxicity and the RAS (Wiley and Firkin, 1995). However, most groups have not adopted this as a standard approach, given the risks and benefits of corticosteroids, but instead administer dexamethasone at the earliest appearance of any of the signs or symptoms suggestive of RAS.

APL is generally associated with leukopenia at the time of presentation; however, leukocytosis ($\geq$10,000 cells/mm^3) frequently occurs in these patients when treated with ATRA alone. Although leukocytosis in itself might not result in any immediate consequences such as leukostasis, it has been suggested that development of leukocytosis and the RAS is associated with a higher risk of extramedullary relapse (Ko *et al*., 1999). The prevention and management of leukocytosis involves using full-dose chemotherapy along with ATRA. The concurrent administration of these agents not only eliminates the occurrence of leukocytosis but also appears to result in a lower incidence of RAS. Whereas with ATRA alone the incidence of RAS is approximately 25%, when given concurrently with chemotherapy (the GIMEMA trial, the Japanese Adult Leukemia Study Group, and the European APL study) the reported incidence ranged between 6 and 15% (Fenaux *et al*., 1999; Sanz *et al*., 1999; Tallman *et al*., 1997).

F. Management of Relapsed APL

Although there has been significant improvement in the cure rate of patients with APL since the addition of ATRA therapy, still 15–20% of the patients relapse (Fenaux *et al*., 1993, 1994; Warrell *et al*., 1991). Some patients, particularly those who have relapsed >6 months after completing their last ATRA therapy, can achieve a second CR when retreated with ATRA (Fenaux *et al*., 2001). However, patients who achieve a second CR, irrespective of agent(s) used, need additional curative therapy. Salvage therapy often involves high doses of cytotoxic chemotherapy followed by allogeneic bone marrow or stem cell transplantation, which is contingent on finding an HLA-matched donor. However, such an approach carries a risk of significant morbidity and mortality and might not be appropriate for all patients, particularly the very young or old.

There are evolving clinical data assessing the role of dose intense chemotherapy followed by transplantation in patients who have relapsed from prior ATRA-based treatment. In the European APL 91 trial (Fenaux *et al*., 1999), 4 of 5 patients who underwent allografts in second CR obtained a prolonged CR. Similarly, 6 of 15 patients in the Italian GIMEMA study (Mandelli *et al*., 1997) who underwent such therapy in second CR benefited. More recently, Thomas *et al*. (2000) treated 50 APL patients in the first relapse with single-agent ATRA and induced a second CR in 45 (90%) patients. With an intent to transplant, those patients in CR received sequential dose-intense chemotherapy (etoposide 200 mg/m^2/day for 3 days, mitoxantrone 12 mg/m^{2}day for 3 days, and Ara-C 500 mg/m^2/day for two sequences of 3 days). Subsequently, 11 patients underwent HLA-identical allogeneic transplant and had a median DFS of 8.2 months and 22 patients underwent autologous transplants and had a 3-year DFS rate of 77%. An important consideration for patients undergoing an autologous

transplantation is the RT-PCR status for PML/RARα. Patients who have evidence for molecular disease are less likely to benefit from an autologous transplantation. Meloni *et al.* (1997) evaluated the outcomes of 15 relapsed APL patients who underwent autologous transplantation in second CR. All patients whose stem cells were positive for PML/RARα relapsed in less than 9 months posttransplant. In comparison, only 1 of 8 patients whose cells were RT-PCR negative for PML/RARα relapsed.

1. Arsenic Trioxide

Based on initial reports from China (Shen *et al.*, 1997; Sun *et al.*, 1992; Zhang *et al.*, 1996), a pilot trial conducted at Sloan-Kettering, using arsenic trioxide (ATO) in patients with relapsed APL, resulted in a complete remission rate of 92% (Soignet *et al.*, 1998). This was followed by a U.S. multicenter study (Soignet *et al.*, 2001) that was designed to evaluate the efficacy of ATO for remission induction and consolidation in patients with APL who had relapsed from prior retinoid- and anthracycline-based therapy. In this study, 34 (85%) of 40 patients achieved a hematological CR. The median time to bone marrow remission was 35 days (range 20/85 days), and the median time to clinical CR was 59 days (range 28/85 days). In addition to the high CR rate, 78% of the patients who achieved a CR also converted from positive to negative for the PML-RARα transcript by RT-PCR by the completion of their consolidation therapy. Of the 34 patients who achieved a CR, 18 patients received additional ATO as maintenance on a different protocol and 12 patients underwent allogeneic ($N = 9$) or autologous ($N = 3$) transplant post ATO treatment while in CR. When the data from the 12 patients treated in the original pilot study of ATO are combined with results from these 40 patients, the Kaplan–Meier 18-month estimate of overall and relapse-free survival was 66% and 50%, respectively (Soignet *et al.*, 2001). More than half of these patients were alive at the 18-month follow-up, irrespective of age or number of prior relapses.

Based on the results of the U.S. pilot and multicenter trials, ATO (Trisenox®) received approval in the U.S. and in Europe for patients with relapse APL. The best treatment strategy, however, for patients who achieve a CR with ATO remains to be determined. Although 10 of 21 patients treated on the U.S. multicenter study who received ATO only were without evidence of either clinical or molecular recurrence at a median of the 18-month follow-up, there is little data on the long-term outcome. Therefore, patients who relapse and are candidates for either allogeneic or autologous transplants should be managed accordingly. Subsequently, many groups have reported their successful experience with ATO as a single agent or in combination with ATRA for remission induction (Raffoux *et al.*, 2003) in patients with relapsed APL. In addition, the potential role of ATO in newly diagnosed APL patients is being explored.

In the initial pilot APL study of ATO conducted by Soignet *et al.* (1998a) a flat dose of 10 mg/day for up to 60 doses was employed until bone marrow remission was achieved. Subsequently, 0.15 mg/kg/day was established as the standard daily dose for the treatment of APL. Considering the extensive use of ATO in the treatment of relapsed APL, as well as the broader application of this agent, a limited amount of pharmacokinetic data is available for ATO. The metabolism of arsenic in humans involves the cycling of arsenic between trivalent (As^{+3}) and pentavalent (As^{+5}) states and conversion to mono-, di-, and trimethylated metabolites. Previous studies have shown that when As^{+3} is ingested, a small amount is excreted in the urine as As^{+3} and a larger proportion is methylated and excreted as dimethylarsonic and methylarsonic acids (Crecelius, 1977; Mann *et al.*, 1996). Urinary excretion of As^{+3} occurred rapidly (within 5 h of ingestion) and was usually undetectable 60 h after ingestion. The urinary excretion of methylated arsenic species was also detectable within the first 6 h and declined at 85 h, with an apparent half-life of 30 h (Crecelius, 1977).

In preclinical models, a wide range of arsenic concentrations have been reported to have antiproliferative effects. ATO has demonstrated *in vitro* activity against bladder, ovary, breast, kidney, cervix, and stomach cancer cell lines at concentrations from 0.34 to 2.84 μM (Crecelius, 1977). NB4 cells, an APL cell line, are sensitive to the effects of ATO at concentrations from 0.5 to 3 μM (Chen *et al.*, 1996). Shen *et al.* (1997) reported the pharmacokinetics of ATO on blood samples taken from eight patients with APL who received daily doses of 10 mg. In their analysis, they deserved peak levels of 5.54 μM/l to 7.30 μM/l followed by a rapid decline in plasma arsenic concentrations. Coinciding urine samples from these patients showed rising daily urinary excretion levels of arsenic ranging from 1 to 8% of the total daily dose of ATO administered. Also, continuous administration of ATO did not appear to alter its pharmacokinetic behavior. In a study conducted by the Sloan-Kettering group, using a daily administration schedule, concentrations of total arsenic from 0.14 to 3.0 μM ranging over a 5-day period were reported (Soignet *et al.*, 1998b, 2000). After a single intravenous dose of ATO, blood samples for pharmacokinetic analyses were collected at time points out to 168 h. Soignet and coworkers reported peak plasma arsenic concentrations occurring near the end of infusion and declined with at $t_{1/2}$ of ~92 h and volume of distribution >400 l. $AUC_{0-24\ h}$ data suggested a dose–concentration relationship (Soignet, 1998b). These early pharmacokinetic studies show that *in vivo* concentrations of ATO can be achieved to match the concentrations that have shown *in vitro* activity; however, the clinical relevance of these findings in diseases other than APL is unknown.

The most common adverse events observed with ATO in the U.S. pilot and multicenter studies included leukocytosis (>10,000 WBC/mm^3) during induction therapy, mild hyperglycemia, nausea, and fatigue (Soignet *et al.*,

2001). Also, 30% of patients developed one or more signs or symptoms, or both, suggestive of the RAS and were effectively treated with dexamethasone (Camacho *et al*., 2000; Soignet, 2001). One of the most concerning and frequent side effects of ATO therapy observed is QT prolongation on the electrocardiogram. This adverse event was observed to some degree in more than 60% of the patients treated with ATO in either Phase I or II clinical studies, which included the APL trials (Barbey *et al*., 2003). This effect does not appear to be dose related and occurs more frequently in female patients. Although all the patients were asymptomatic, including one patient who developed a brief episode of *torsades de pointe*, more recently there have been reports of sudden cardiac death (Unnikrishnan *et al*., 2001; Westervelt *et al*., 2001) associated with ATO therapy. Therefore, close monitoring, including aggressive management of electrolytes, particularly potassium and magnesium, is recommended in conjunction with ATO therapy (Trisenox package insert, Cell Therapeutics, Inc; Rust and Soignet, 2001). Both intravenous and oral supplements are liberally provided to maintain the serum potassium >4 mEq/l and the magnesium level >1.8 mg/dl (Rust and Soignet, 2001). In addition, the concomitant use of other agents known to prolong QT intervals or induce ventricular arrhythmias should be avoided (see www.arizonacert.org for listings).

2. *Other Agents for Relapse APL*

Gemtuzumab ozogamicin (Mylotarg®) is an anti-CD33 antibody conjugated with calicheamicin, a potent antitumor antibiotic. This agent binds to the CD33 antigen, which is highly expressed on the surface of the majority of APL cells universally. The group at the M. D. Anderson Cancer Center reported a clinical CR rate of 84% in newly diagnosed APL patients when combining gemtuzumab ozogamicin with ATRA (Estey *et al*., 2002). In addition, it observed a 100% molecular remission rate in 12 evaluable patients. Although this clinical response rate is similar to what would be expected with ATRA alone in treating naïve APL patients, the molecular remission rate is several-fold higher than what is traditionally expected from single-agent ATRA. Also, others have reported induction of a clinical and molecular remission in patients with multiple-relapsed APL (Petti *et al*., 2001). However, definitive studies are needed to establish the efficacy of gemtuzumab alone or in combination in patients with relapsed APL.

Another potential agent that warrants further consideration for relapsed APL is liposomal ATRA. Douer *et al*. (2001) reported intravenously administering liposomal ATRA in 69 patients with newly diagnosed (32 patients) or relapsed (35 patients) APL and achieving a CR in 62% and 70%, respectively, of patients with the first relapse who were ATRA-naïve or off oral ATRA for more than 1 year. Of the patients who were in the first relapse and off oral ATRA for less than 1 year or in their second or higher relapse,

20% achieved a CR. This agent clearly has a role in a patient population in which oral ATRA is not ideal, such as patients who are not able to tolerate or absorb the oral formulation. However, the exact benefit in relapsed APL needs to be defined.

3. *Extramedullary Relapse*

Until recently, the incidence of extramedullary disease (EMD) in patients with APL was considered rare (Liso *et al.*, 1998; Marra *et al.*, 1989). However, the reported incidence has increased since the early 1990s, with numerous cases being diagnosed at the time of clinical relapse (Ko *et al.*, 1999; Liso *et al.*, 1998; Menendez *et al.*, 2000; Weiss and Warrell, 1994). In the Italian GIMEMA study and the European APL 93 trial, EMD was detected in 13 of 97 patients and 3 of 75 cases, respectively. Central nervous system (CNS) involvement appears to be more common than either skin or organ infiltration. The relationship of the incorporation of ATRA in the treatment of APL and the subsequent rising incidence of extramedullary relapse is unclear. In an evaluation of the incidence of EMD involvement in a series of APL patients treated with either chemotherapy alone or with ATRA and idarubicin (AIDA regimen) and subsequently relapsed (Specchia *et al.*, 2001), there were no conclusive data to support a higher incidence of EMD in patients treated with ATRA. However, the proportion of patients with EMD that had CNS involvement was significantly higher in the group that received ATRA as part of its induction therapy compared with the group that received chemotherapy only. One explanation for this observation is that ATRA therapy induces the expression of adhesion molecules such as CD11c, CD13, and CD56 in the malignant promyelocytes and thereby might facilitate CNS infiltration (Di Noto *et al.*, 1996; Evans *et al.*, 1999; Ferrara *et al.*, 2000). There was no comparative difference in the incidence of the other site of EMD.

Of clinical importance is that 14 of the 16 patients on the AIDA trial with EMD found at relapse also had hematologic or molecular evidence of disease. Treatment for CNS relapse requires systemic reinduction along with four to six cycles of intrathecal methotrexate or Ara-C. Some patients might benefit from additional cranial–spinal radiation. To date, there is no specific risk factor identified to predict CNS relapse. In addition, CNS prophylaxis with either intrathecal chemotherapy alone or combined with cranial irradiation does not improve DFS in adults with AML. This is likely because the majority of the patients have systemic relapse in addition to CNS or EMD.

G. Summary

The advances in the treatment of APL exemplify the recent progress in medical science and serve as a model for modern medicine. APL is unique in that its etiology evolves from a specific cytogenetic alteration, t(15;17). With

current technologies, we are able to use this genotype information to diagnose, monitor minimal residual disease, and detect early relapse. It has also resulted in a greater understanding of the fundamental biology of APL, which serves as a template for the development of targeted therapies. For example, transgenic mice models that stably carry the t(15;17) genotype and express a malignant phenotype have been developed and are currently used to test potential novel agents and identify new targets for future clinical development (Kogan *et al.*, 2000). Also, APL is the first successful clinical application of differentiation therapy, and, as a result, the conventional approach for the treatment of leukemia has been irrevocably altered. As medical science evolves through the era of genomics, proteomics, and rationale drug design, the models of pathogenesis based on interactions between the PML/RARα fusion products, corepressor-binding proteins, and histone deacetylase will no doubt further our understanding and treatment of other forms of cancer.

References

Adamson, P. C., Bailey, J., Pluda, J., Poplack, D. G., Bauza, S., Murphy, R. F., Yarchoan, R., and Balis, F. M. (1995). Pharmacokinetics of all-*trans* retinoic acid administered on an intermittent schedule. *J. Clin. Oncol.* **13,** 1238–1241.

Arnould, C., Phillippe, C., Bourdon, V., Grgoire, M. J., Berger, R., and Jonveaux, P. (1999). The signal transducer and activator of transcription STAT5b gene is a new partner of retinoic acid receptor alpha in acute promyelocytic-like leukaemia. *Hum. Mol. Genet.* **8,** 1741–1749.

Avvisati, G. (1998). AIDA protocol: The Italian way of treating APL [abstract]. *Br. J. Hematol.* **102,** 593a.

Avvisati, G., Mandelli, F., Petti, M. C., Vegna, M. L., Spadea, A., Liso, V., Specchia, G., Bernasconi, C., Alessandrino, E. P., and Piatti, C. (1990). Idarubicin (4-demethoxydaunorubicin) as single agent for remission induction of previously untreated acute promyelocytic leukemia: A pilot study of the Italian cooperative group GIMEMA. *Eur. J. Haematol.* **44,** 257–260.

Barbey, J. T., Pezzullo, J. C., and Soignet, S. L. (2003). Effects of arsenic trioxide on QT interval in patients with advanced malignancies. *J. Clin. Oncol.* **21,** 19.

Bloomfield, C. D. (1985). Post-remission therapy in acute myeloid leukemia. *J. Clin. Oncol.* **3,** 1570–1572.

Burnett, A. K., Grimwade, D., Solomon, E., Wheatley, K., and Goldstone, A. H. (1999). Presenting white blood cell count and kinetics of molecular remission predicts prognosis in acute promyelocytic leukemia treated with all-*trans* retinoic acid: Results of the randomized MRC trial. *Blood* **93,** 4131–4143.

Camacho, I. H., Soignet, S. L., Chanel, S., Ho, R., and Warrell, R. P., Jr. (2000). Leukocytosis and the retinoic acid syndrome in patients with acute promyelocytic leukemia treated with arsenic trioxide. *J. Clin. Oncol.* **18,** 2620–2625.

Castaigne, S., Chomienne, C., Daniel, M. T., Ballerini, P., Berger, R., Fenaux, P., and Degos, L. (1990). All-*trans* retinoic acid as a differentiating therapy for acute promyelocytic leukemias. I. Clinical results. *Blood* **76,** 1704–1713.

Chen, G.-Q., Zhu, J., Shi, X.-G., Ni, J.-H., Zhong, H.-J., Si, G.-Y., Jin, X.-L., Tang, W., Li, X.-S., Xong, S.-M., Shen, Z.-X., Sun, G.-L., Ma, J., Zhang, P., Zhang, T.-D., Gazin, C., Naoe, T.,

Chen, S.-J., Wang, Z.-Y., and Chen, Z. (1996). *In vitro* studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB_4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RARα/PML proteins. *Blood* **88,** 1052–1061.

Crecelius, F. A. (1977). Changes in the chemical speciation of arsenic following ingestion by man. *Environ. Health Perspect.* **19,** 147–150.

De Botton, S., Dombret, H., Sanz, M., Miguel, J. S., Caillot, D., Zittoun, R., Gardembas, M., Stamatoulas, A., Conde, E., Guerci, A., Gardin, C., Geiser, K., Makhoul, D. C., Reman, O., de la Serna, J., Lefrere, F., Chomienne, C., Chastang, C., Degos, L., and Fenaux, P. (1998). Incidence, clinical features, and outcome of all-*trans*-retinoic acid syndrome in 413 cases of newly diagnosed acute promyelocytic leukemia. *Blood* **92,** 2712–2718.

Dedhar, S., Robertson, K., and Gray, V. (1991). Induction of expression of the $\alpha_{v\beta}$ and $\alpha_{v\beta}$ integrin heterodimers during retinoic acid-induced neuronal differentiation of murine embryonal carcinoma cells. *J. Biol. Chem.* **266,** 21846–21852.

Delva, L., Cornic, M., Balitrand, N., Guidez, F., Miclea, J. M., Delmer, A., Teillet, F., Fenaux, P., Caistaigne, S., and Degos, L. (1993). Resistance to all-*trans* retinoic acid (ATRA) therapy in relapsing acute promyelocytic leukemia: Study of *in vitro* ATRA sensitivity and cellular retinoic acid binding protein levels in leukemic cells. *Blood* **82,** 2175–2181.

de Thé, H., Chomienne, C., Lanotte, M., Degos, L., and Dejean, A. (1990). The t(15;17) translocation of acute promyelocytic leukemia fuses the retinoic acid receptor alpha gene to a novel transcribed locus. *Nature* **347,** 558–561.

Di Bona, E., Avvisati, G., Castaman, G., Luce, V., Vegna, M., De Sanctis, V., Rodeghiero, F., and Mandelli, F. (2000). Early haemorrhagic morbidity and mortality during remission induction with or without all-*trans* retinoic acid in acute promyelocytic leukemia. *Br. J. Haematol.* **108,** 689–695.

Di Noto, R., Lo Pardo, C., Schiavone, F. M., Ferrara, F., Manzo, C., Vacca, C., and Del Vecchio, L. (1996). All-*trans* retinoic acid (ATRA) and the regulation of adhesion molecules in acute myeloid leukemia. *Leuk. Lymphoma* **2,** 201–209.

Diverio, D., Rossi, V., Avvisati, G., DeSantis, S., Pistilli, A., Pane, F., Saglio, G., Martinelli, G., Petti, M. C., Santoro, A., Pelicei, P. G., Mandelli, F., Biondi, A., and Lo Coco, F. (1998). Early detection of relapse by prospective reverse transcriptase-polymerase chain reaction analysis of the PML/RARα fusion gene in patients with acute promyelocytic leukemia enrolled in the GIMEMA-AIEOP multicenter "AIDA" trial *Blood* **92,** 784–789.

Douer, D., Estey, E., Santillana, S., Bennett, J. M., Lopez-Bernstein, G., Boehm, K. N., and Williams, T. (2001). Treatment of newly diagnosed and relapsed acute promyelocytic leukemia with intravenous liposomal all-*trans* retinoic acid. *Blood* **97**(1), 73–80.

Drapkin, R. I., Gee, T. S., Dowling, M. D., Arlin, Z., McKenzie, S., Kempin, S., and Clarkson, B. (1978). Prophylactic heparin therapy in acute promyelocytic leukemia. *Cancer* **41,** 2484–2490.

Estey, E. H., Giles, F. J., Beran, M., O'Brien, S., Pierce, S. A., Faderl, S. H., Cortes, J. E., and Kantarjian, H. M. (2002). Experience with gemtuzumab ozogamycin ("myelotarg") and all-*trans* retinoic acid in untreated acute promyelocytic leukemia. *Blood* **99**(11), 4222–4224.

Evans, G. D., and Grimwade, D. J. (1999). Extramedullary disease in acute promyelocytic leukemia. *Leuk. Lymphoma* **33**(3/4), 219–229.

Fenaux, P., Chastang, C., Chevret, S., Sanz, M., Dombert, H., Archimbaud, E., Fey, M., Rayon, C., Huguet, F., Sotto, J. J., Gardin, C., Makhoul, P. C., Travade, P., Solary, E., Fegueux, N., Bordessoule, D., Miguel, J. S., Link, H., Desablens, B., Stamatoullas, A., Deconinck, E., Maloisel, F., Castaigne, S., Preudhomme, C., and Degos, L. (1999). A randomized comparison of all *trans*-retinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly

diagnosed acute promyelocytic leukemia. The European APL Group. *Blood* **94**(4), 1192–1200.

Fenaux, P., Chastang, C., Chomienne, C., and Degos, L. (1994). Tretinoin with chemotherapy in newly diagnosed acute promyelocytic leukemia: Results. *Lancet* **343,** 1033.

Fenaux, P., Chomienne, C., and Degos, L. (2001). All-*trans* retinoic acid and chemotherapy in the treatment of acute promyelocytic leukemia. *Semin. Hematol.* **38**(1), 13–25.

Fenaux, P., Le Dely, M. C., Castaigne, S., Archimbaud, E., Chomienne, C., Link, H., Guerci, A., Duarte, M., Daniel, M. T., and Bowe, D. (1993). Effect of all-*trans* retinoic acid in newly diagnosed acute promyelocytic leukemia: Results of a multicenter randomized trial. *Blood* **82,** 3241–3249.

Ferrara, F., Morabito, F., Martino, B., Specchia, G., Liso, V., Nobile, F., Boccuni, P., DiNoto, R., Pane, F., Annunziata, M., Schiavone, E. M., De Simone, M., Guglielmi, C., Del Vecchio, L., and Lo Coco, F. (2000). CD56 expression is an indicator of poor clinical outcome in patients with acute promyelocytic leukaemia treated with simultaneous ATRA and chemotherapy. *J. Clin. Oncol.* **18,** 1295–1300.

Frankel, S. R., Eardley, A., Heller, G., Berman, E., Miller, W. H., Jr., Dmitrovsky, E., and Warrell, R. P., Jr. (1994). All-*trans* retinoic acid for acute promyelocytic leukemia: Results of the New York study. *Ann. Intern. Med.* **120,** 278–286.

Frankel, S. R., Eardley, A., Lauwers, G., Weiss, M., and Warrell, R. P., Jr. (1992). The "retinoic acid syndrome" in acute promyelocytic leukemia. *Ann. Intern. Med.* **117,** 292–296.

Golomb, H. M., Rowley, J. D., Vardiman, J. W., Testa, J. R., and Butler, A. (1980). Microgranular acute promyelocytic leukaemia: A distinct clinical, ultrastructural, and cytogenetic entity. *Blood* **55,** 253–259.

Gralnick, H. R., Bagley, J., and Abrell, E. (1972). Heparin treatment for the hemorrhagic diathesis of acute promyelocytic leukemia. *Am. J. Med.* **52,** 167–174.

Grimwade, D., Howe, K., Langabeer, S., Davies, L., Oliver, F., Walker, H., Swirsky, D., Wheatley, K., Goldstone, A., Burnett, A., and Solomon, E. (1996). Minimal residual disease detection in acute promyelocytic leukemia by reverse-transcriptase PCR: Evaluation of PML-RARα and RARα-PML assessment in patients who ultimately relapse. *Leukemia* **10,** 61–66.

Guidez, F., Ivins, S., Zhu, J., Soderstrom, M., Waxman, S., and Zelent, A. (1998). Reduced retinoic acid-sensitivities of nuclear receptor corepressor binding to PML- and PLZF-RARalpha underlie molecular pathogenesis and treatment of acute promyelocytic leukemia. *Blood* **91,** 2634–2642.

He, I. Z., Guidez, F., Tribioli, C., Peruzzi, D., Ruthardt, M., Zelent, A., and Pandolfi, P. P. (1998). Distinct interactions of PML-RARalpha and PLZF-RARalpha with co-repressors determine differential responses to RA in APL. *Nat. Genet.* **18,** 126–135.

Head, D., Kopecky, K. J., Weick, J., Files, J. C., Ryan, D., Foucar, K., Montiel, M., Bickers, J., Fishleder, A., and Miller, M. (1995). Effect of aggressive daunorubicin therapy on survival in acute promyelocytic leukemia. *Blood* **86,** 1717–1728.

Huang, M. E., Ye, Y. C., Chen, S. R., Chai, J. R., Lu, J. X., Zhoa, L., Gu, L. J., and Wang, Z. Y. (1988). Use of all-*trans* retinoic acid in treatment of acute promyelocytic leukemia. *Blood* **72,** 567–572.

Huang, M. E., Ye, Y. C., Chen, S. R., Zhoa, J. C., Gu, L. J., Cai, J. R., Zhao, L., Xie, J. X., Shen, Z. X., and Wang, Z. Y. (1987). All-*trans* retinoic acid with or without low dose cytosine arabinoside in acute promyelocytic leukemia—report of 6 cases. *Chin. Med. J.* **100,** 949–953.

Jurcic, J. G., Nimer, S. D., Scheinberg, D., DeBlasio, T., Warrell, R. P., Jr., and Miller, W. H., Jr. (2001). Prognostic significance of minimal residual disease detection and PML/RAR-isoform type: Long-term follow-up in acute promyelocytic leukemia. *Blood* **98,** 2651–2656.

Kakizuka, A., Miller, W. H., Umesono, K., Warrell, R. P., Jr., Frankel, S. R., Murty, V. V., Dmitrovsky, E., and Evans, R. M. (1991). Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RARα with a novel putative transcription factor, PLL. *Cell* **66,** 663–674.

Kantarjian, H., Keating, M. J., Walters, R. S., Estey, E., McCredie, K. B., Smith, T. L., Dalton, W. T., Jr., Cork, A., Trujillo, J. M., and Freireich, E. J. (1986). Acute promyelocytic leukemia: MD Anderson Hospital experience. *Am. J. Med.* **80,** 789–797.

Kantarjian, H. M., Keating, M. J., Walters, R. S., Smith, T. L., McCredie, K. B., and Freireich, E. J. (1987). Role of maintenance chemotherapy in acute promyelocytic leukemia. *Cancer* **59**(7), 1258–1263.

Ko, B.-S., Tang, G.-L., Chen, Y.-C., Yao, M., Wang, G. H., Shen, M. C., and Tien, H. F. (1999). Extramedullary relapse after all-*trans* retinoic treatment in acute promyelocytic leukemia: The occurrence of retinoic acid syndrome is a risk factor. *Leukemia* **13,** 1406–1408.

Kogan, S. C. (2000). Acute promyelocytic leukemia: A view from a mouse. *Blood Cells Mol. Dis.* **26,** 620–625.

Larson, R. A., Kondo, K., Vardiman, J. W., Butler, A. E., Golomb, H. M., and Rowley, J. D. (1984). Evidence for a 15;17 translocation in every patient with acute promyelocytic leukemia. *Am. J. Med.* **76,** 827–841.

Lengfelder, E., Reichert, A., Schoch, C., Haase, D., Haferlach, T., Loffler, H., Staib, P., Heyll, A., Seifarth, W., Saussele, S., Fonatsch, C., Gassmann, W., Ludwig, W. D., Hochhaus, A., Beelen, D., Aul, C., Sauerland, M. C., Heinecke, A., Hehlmann, R., Wormann, B., Hiddemann, W., and Buchner, T. (2000). Double induction strategy including high-dose cytarabine in combination with all-*trans* retinoic acid: Effects in patients with newly diagnosed acute promyelocytic leukemia. *Leukemia* **14,** 1362–1370.

Li, Y.-P., Said, F., and Gallagher, R. E. (1994). Retinoic acid-resistant HL-60 cells exclusively contain mutant retinoic acid receptor. *Blood* **11,** 3298–3302.

Licht, J. D., Chomienne, C., Goy, A., Chen, A., Scott, A. A., Head, D. R., Michaux, J. L., Wu, Y., DeBlasio, A., and Miller, W. H., Jr. (1995). Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukemia associated with *trans*-location (11;17). *Blood* **85,** 1083–1094.

Liso, V., Specchia, G., Pogliani, E. M., Palumbo, G., Mininni, D., Rossi, V., Teruzzi, E., Mestice, A., Coppi, M. R., and Biondi, A. (1998). Extramedullary involvement in patients with acute promyelocytic leukemia: A report of seven cases. *Cancer* **83,** 1522–1528.

Lo Coco, F., Avvisati, G., Diverio, D., Petti, M. C., Alcalay, M., Pandolfi, P. P., Zangrilli, D., Biondi, A., Rambaldi, A., and Moleti, M. (1991). Molecular evaluation of response to all-*trans* retinoic acid therapy in patients with acute promyelocytic leukemia. *Blood* **77,** 1657–1661.

Lo Coco, F., Diverio, D., Pandolfi, P. P., Biondi, A., Rossi, V., Avvisati, G., Rambaldi, A., Arcese, W., Petti, M. C., and Melani, G. (1992). Molecular evaluation of residual disease as a predictor of relapse in acute promyelocytic leukemia. *Lancet* **340,** 1437–1438.

Mandelli, F., Diverio, D., Avvisati, G., Luciano, A., Barbui, T., Bernasconi, C., Broccia, G., Falda, M., Fioritoni, G., Leoni, F., Liso, V., Petti, M. C., Rodeghiero, F., Saglio, G., Vegna, M. L., Visani, G., Jehn, U., Willemze, R., Muus, P., Pelicci, P. G., Biondi, A., and Lo Coco, F. (1997). Molecular remission in PML/RAR alpha-positive acute promyelocytic leukemia by combined all-*trans* retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. *Blood* **90**(3), 1014–1021.

Mann, S., Droz, P. O., and Vahter, M. (1996). A physiologically based pharmacokinetic model for arsenic exposure. II. Validation and application in humans. *Toxicol. Appl. Pharmacol.* **140,** 471–486.

Marra, R., Storti, S., Pagano, L., Fioritoni, G., Rabitti, C., Sica, S., Leone, G., Torlontano, G., and Bizzi, B. (1989). Central nervous system acute promyelocytic leukemia: A report of three cases. *Haematologica* **22,** 195–199.

Marty, M., Ganem, G., Fischer, J., Flandrin, G., Berger, R., Schaison, G., Degos, L., and Boiron, M. (1984). Acute promyelocytic leukemia: Retrospective study of 119 patients treated with daunorubicin. *Nouv. Rev. Fr. Hematol.* **26,** 371–387.

McKenna, R. W., Parkin, J., Bloomfield, C. D., Sundberg, R. D., and Brunning, R. D. (1982). Acute promyelocytic leukaemia: A study of 39 cases with identification of a hyperbasophilic microgranular variant. *Br. J. Haematol.* **50,** 201–214.

Meloni, G., Diverio, D., Vignetti, M., Avvisati, G., Capria, S., Petti, M. C., Mandelli, F., and Lo Coco, F. (1997). Autologous bone marrow transplantation for acute promyelocytic leukemia in second remission: Prognostic relevance of pretransplant minimal residual disease assessment by reverse-transcription polymerase chain reaction of the PML/RAR alpha fusion gene. *Blood* **90,** 1321–1325.

Menendez, A., Gonzales, A., Cabrera, H., Mesa, J., Martinez, G., Espinoza, E., and Hernandez, P. (2000). Clinical spectrum of extramedullary acute promyelocytic leukemia. *Eur. J. Haematol.* **64,** 201–203.

Miller, W. H., Jr., Kakizuka, A., Frankel, S. R., Warrell, R. P., Jr., DeBlasio, A., Levine, K., Evans, R. M., and Dmitrovsky, E. (1992). Reverse transcription polymerase chain reaction for the re-arranged retinoic acid receptor clarifies diagnosis and detects minimal residual disease in acute promyelocytic leukemia. *Proc. Natl. Acad. Sci. USA* **89,** 2694–2698.

Miller, W. H., Jr., Levine, K., De Blasio, A., Frankel, S. R., Dmitrovsky, E., and Warrell, R. P., Jr. (1993). Detection of minimal residual disease in acute promyelocytic leukemia by a reverse transcription polymerase chain reaction. *Blood* **82,** 1689–1694.

Muindi, J., Frankel, S. R., Miller, W. H., Jr., Jakubowski, A., Scheinberg, D. A., Young, C. W., Dmitrovsky, E., and Warrell, R. P., Jr. (1992). Continuous treatment with all-*trans* retinoic acid results in a progressive decrease in plasma concentrations: Implications for relapse and "resistance" in acute promyelocytic leukemia. *Blood* **79,** 299–303.

Muindi, J. F., Scher, H. I., Rigas, J. R., Warrell, R. P., Jr., and Young, C. W. (1994). Elevated plasma lipid peroxide content correlates with rapid plasma clearance of all-*trans* retinoic acid in patients with advanced cancer. *Cancer Res.* **54,** 2125–2158.

Petti, M. C., Avvisati, G., Amadori, S., Baccarani, M., Guarini, A. R., Papa, G., Rosti, G. A., Tura, S., and Mandelli, F. (1987). Acute promyelocytic leukemia: Clinical aspects and results of treatment in 62 patients. *Haematologica* **72,** 151–155.

Petti, M. C., Pinazzi, M. B., Diverio, D., Romano, A., Petrucci, M. T., De Santis, S., Meloni, G., Tafuri, A., Mandelli, F., and Lo Coco, F. (2001). Prolonged molecular remission in advanced acute promyelocytic leukaemia after treatment with gemtuzumab ozogamicin (Mylotarg CMA-676). *Br. J. Haematol.* **115**(1), 63–65.

Raffoux, E., Rousselot, P., Poupon, J., Daniel, M. T., Cassinat, B., Delarue, R., Taksin, A. L., Rea, D., Buzyn, A., Tibi, A., Lebbe, G., Cimerman, P., Chomienne, C., Fermand, J. P., de The, H., Degos, L., Hermine, O., and Dombret, H. (2003). Combined treatment with arsenic trioxide and all-*trans*-retinoic acid in patients with relapsed acute promyelocytic leukemia. *J. Clin. Oncol.* **21**(12), 2326–2334.

Redner, R. L., Chen, J. D., Rush, E. A., Li, H., and Pollock, S. L. (2000). The t(5;17) acute promyelocytic leukemia fusion protein NPM-RAR interacts with co-repressor and co-activator proteins and exhibits both positive and negative transcriptional properties. *Blood* **95,** 2683–2690.

Robertson, K. A., Emami, B., and Collins, S. J. (1992). Retinoic acid-resistant HL-60R cells harbor a point mutation in the retinoic acid receptor ligand-binding domain that confers dominant negative activity. *Blood* **80,** 1885–1889.

Rowley, J., Golomb, H. M., and Dougherty, C. (1997). 15/17 *trans*-location, a consistent chromosomal change in acute promyelocytic leukemia. *Lancet* **1,** 549–550.

Rust, D. M., and Soignet, S. L. (2001). Risk/benefit profile of arsenic trioxide. *The Oncologist* **6**(Suppl. 2), 29–32.

Sainty, D., Liso, V., Cantu-Rajnoldi, A., Head, D., Mozziconacci, M. J., Arnoulet, C., Benattas, L., Fenu, S., Mancini, M., Duchayne, E., Mahon, E. X., Gutierrez, N., Birg, E., Biondi, A., Grimwade, D., Lafage-Pochitaloff, M., and Hagemijer, F. G. (2000). A new morphological classification system for acute promyelocytic distinguishes cases with underlying PLZF-RAR_rearrangements. *Blood* **96,** 1287–1296.

Sanz, M. A., Martin, G., Rayon, C., Esteve, J., Gonzalez, M., Diaz-Mediavilla, J., Bolufer, P., Barragan, E., Terol, M. J., Gonzalez, J. D., Colomer, D., Chillon, C., Rivas, C., Gomez, T., Ribera, J. M., Bornstein, R., Roman, J., Calasanz, M. J., Arias, J., Alvarez, Ramos, F., and Deben, G. (1999). A modified AIDA protocol with anthracycline-based consolidation results in high antileukemic efficacy and reduced toxicity in newly diagnosed PML/RAR-alpha-positive acute promyelocytic leukemia. *Blood* **94,** 3015–3021.

Scheinberg, D. A. S., Maslak, P., and Weiss, M. A. (2001). Acute leukemias. *In* "Cancer: Principles and Practice of Oncology" (V. De Vita, S. Hellman, and S. A. Rosenberg, Eds.), 6th ed. Lippincott Williams & Wilkins, Philadelphia, PA.

Shen, Z.-X., Chen, G.-O., Ni, J.-H., Li, X. S., Xiong, S. M., Qiu, O. Y., Zhu, J., Tang, W., Sun, G. L., Yang, K. Q., Chen, Y., Zhou, L., Fang, Z. W., Wang, Y. J., Zhang, P., Zhang, T. D., Chen, S. J., Chen, Z., and Wang, Z. Y. (1997). Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. *Blood* **89,** 3354–3360.

Soignet, S. L. (2001). Clinical experience of arsenic trioxide in relapsed acute promyelocytic leukemia. *The Oncologist* **6**(Suppl. 2), 11–16.

Soignet, S. L., Frankel, S. R., Douer, D., Tallman, M. S., Kantarjian, H., Calleja, E., Stone, R. M., Kalaycio, M., Scheinberg, D. A., Steinherz, P., Sievers, E. L., Coutre, S., Dahlberg, S., Ellison, R., and Warrell, R. P., Jr. (2001). United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. *J. Clin. Oncol.* **19,** 3852–3860.

Soignet, S. L., Maslak, P., Wang, Z.-G., Jhanwar, S., Calleja, E., Dandasht, L. J., Corso, D., De Blasio, A., Gabrilove, J., Scheinberg, D. A., Pandolfi, P. P., and Warrell, R. P., Jr. (1998a). Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. *N. Engl. J. Med.* **339,** 1341–1348.

Soignet, S., Novick, S., Bienvenu, B., Chanel, S., Ho, R., Spriggs, D., Ellison, R., and Warrell, R. P., Jr. (2000). Arsenic trioxide (As_2O_3): A dose-ranging study and clinical pharmacologic study in patients with advanced hematologic cancers. *Proc. Am. Assoc. Cancer. Res.*, 3462 (abs).

Soignet, S., Wang, Z.-G., Nagy, J., Maslak, P., Chanel, S., Ho, R., Spriggs, D., Ellison, R., Pandolfi, P. P., and Warrell, R. P., Jr. (1998b). Dose-ranging study of arsenic trioxide in advanced hematologic cancers: Clinical pharmacokinetics and biological effects. *Blood* **92**(Suppl. 1), 2466 (abs).

Specchia, G., Lo Coco, E., Vignetti, M., Avyisati, G., Fazi, P., Albano, F., Di Raimondo, F., Martino, B., Ferrara, F., Selleri, C., Liso, V., and Mandelli, F. (2001). Extramedullary involvement at relapse in acute promyelocytic leukemia patients treated or not treated with all-*trans* retinoic acid: A report by the Gruppo Italiano Malattie Ematologiche dell'Adulto. *J. Clin. Oncol.* **19**(20), 4023–4028.

Sun, H. D., Ma, L., Hu, X. C., and Zhang, T. D. (1992). Ai-Lin 1 treated 32 cases of acute promyelocytic leukemia. *Chin. J. Integrat. Chin. West. Med.* **12,** 170–171.

Tallman, M. S., Andersen, J. W., Schiffer, C. A., Appelbaum, F. R., Feusner, J. H., Ogden, A., Shepard, L., Willman, C., Bloomfield, C. D., Rowe, J. M., and Wiernik, P. H. (1997). All-*trans* retinoic acid in acute promyelocytic leukemia. *N. Eng. J. Med.* **337**(15), 1021–1028.

Tallman, M. S., Nabhan, C., Feusner, J. H., and Rowe, J. (2002). Acute promyelocytic leukemia: evolving therapeutic strategies. *Blood* **99**(3), 759–767.

Thomas, X., Dombret, H., Cordonnier, C., Pigneux, A., Gardin, C., Guerci, A., Vekhoff, A., Sadoun, A., Stamatoullas, A., Fegueux, N., Maloisel, E., Cahn, J. T., Reman, O., Gratecos, N., Berthou, C., Huguet, F., Kotoucek, P., Trauade, P., Buzyn, A., de Rowel, T., Vilque, J. P., Naccache, P., Chomienne, C., Degos, L., and Fenaux, P. (2000). Treatment of relapsing acute promyelocytic leukemia by all-*trans* retinoic acid therapy followed by timed sequential chemotherapy and stem cell transplantation. *Leukemia* **12,** 1006–1013.

Unnikrishnan, D., Dutcher, J. P., Varshneya, N., Lucariello, R., Api, M., Garl, S., Wiernik, P. H., and Chiaramida, S. (2001). Torsades de pointes in 3 patients with leukemia treated with arsenic trioxide. *Blood* **97,** 1514–1516.

Vahdat, L., Maslak, P., Miller, W. H., Jr., Eardley, A., Heller, G., Scheinberg, D. A., and Warrell, R. P., Jr. (1994). Early mortality and the retinoic acid syndrome in acute promyelocytic leukemia: Impact of leukocytosis, low-dose chemotherapy, PMN/RAR-alpha isoform, and CD13 expression in patients treated with all-*trans* retinoic acid. *Blood* **84,** 3843–3849.

Warrell, R. P., Jr. (1996). Pathogenesis and management of acute promyelocytic leukemia. *Annu. Rev. Med.* **47,** 555–565.

Warrell, R. P., Jr. (1993). Retinoid resistance in acute promyelocytic leukemia: New mechanisms, strategies, and implications [editorial]. *Blood* **82,** 1949–1953.

Warrell, R. P., Jr., Frankel, S. R., Miller, W. H., Jr., Scheinberg, D. A., Itri, L., Hittelman, W. N., Vyas, R., Andreeff, M., Tafuri, A., Jakubowski, A., Gabrilove, J., Gordon, M., and Dmitrovsky, E. (1991). Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-*trans*-retinoid acid). *N. Eng. J. Med.* **324,** 1385–1393.

Warrell, R. P., Jr., Maslak, P., Eardley, A., Heller, G., Miller, W. H., Jr., and Frankel, S. R. (1994). Treatment of acute promyelocytic leukemia with all-*trans* retinoic acid: An update of the New York experience. *Leukemia* **8,** 929–933.

Wells, R. A., Catzavelos, C., and Kamel-Reid, S. (1997). Fusion of retinoic acid receptorα to NUMA, the nuclear mitotic apparatus protein, by a variant translocation in acute promyelocytic leukaemia. *Nat Genet* **17,** 109–113.

Weiss, M. A., and Warrell, R. P., Jr. (1994). Two cases of extramedullary acute promyelocytic leukemia. *Cancer* **74,** 1882–1886.

Westervelt, P., Brown, R. A., Adkins, D. R., Khury, H., Curtin, P., Hurd, D., Luger, S. M., Ma, M. K., Ley, T. J., and DiPersio, J. F. (2001). Sudden cardiac death among patients with acute promyelocytic leukemia treated with arsenic trioxide. *Blood* **98,** 266–271.

Wiley, J. S., and Firkin, F. C. (1995). Reduction of pulmonary toxicity by prednisone prophylaxis during all-*trans* retinoic acid treatment of acute promyelocytic leukemia. *Leukemia* **9,** 774–778.

Zhang, Z., Tarone, G., and Turner, D. C. (1993). Expression of integrin $\alpha 1\beta 1$ is regulated by nerve growth factor and dexamethasone in PC12 cells: Critical consequences for adhesion and neurite outgrowth. *J. Biol. Chem.* **268,** 5557–5565.

Zhang, P., Wang, S. Y., and Hu, L. H. (1996). Arsenic trioxide treated 72 cases of acute promyelocytic leukemia. *Chin. J. Hematol.* **2,** 58–62.

Farhad Ravandi and Jorge Cortes

Department of Leukemia
The University of Texas M. D. Anderson Cancer Center
Houston, Texas 77030

Investigational Agents in Myeloid Disorders

I. Chapter Overview

Advances in molecular diagnostics and understanding of the biology of neoplastic transformation have begun to provide clinicians treating myeloid disorders with new therapeutic tools. The progress in treating myeloid disorders over the past several decades can be largely attributed to improved supportive care. New cytotoxic agents with minor differences in their structure and mechanism of action have yet to translate to meaningful improvements in survival. With the realization of the activity of all-*trans* retinoic acid (ATRA) and later the description of its mechanism of action in acute promyelocytic leukemia, the benefits of a truly targeted agent have become clear. Furthermore, scientific progress in understanding mechanisms of drug resistance and means of overcoming them has led to the modification of some of the existing cytotoxic agents. This, together with the discovery of new targets and new agents aimed at these targets, has led to the current availability of a large

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

number of potentially useful agents. Learning from the lessons of the past, our challenge would be to design and conduct rational clinical trials, which would ensure that the full potential of these new agents is recognized. More importantly, the intelligent development of these agents could result in a significant improvement in the prognosis of patients with myeloid malignancies.

II. Introduction

Myeloid hematological disorders, including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myeloproliferative disorders (MPDs), have traditionally been classified based on morphologic features in systems such as the French–American–British (FAB) classification. With increased understanding of the biology of these disorders, it is becoming clear that these classifications require reevaluation. New molecular techniques have led to the discovery of specific molecular abnormalities that might define specific disorders (e.g., acute promyelocytic leukemia (APL) and core-binding factor leukemias) within the previous broad categories. Thus, the heterogeneity of AML and MDS is increasingly apparent. Therefore, myeloid neoplasms can be considered a spectrum of diseases ranging from the more indolent to the highly proliferative and aggressive entities, in which various molecular events probably contribute at different steps of their pathogenesis.

The increased awareness of molecular abnormalities occurring in myeloid disorders has led to the identification of multiple potential agents for therapeutic intervention in these diseases. Traditional therapy for these disorders (i.e., cytotoxic chemotherapeutic agents) has been for the most part unsatisfactory, with the majority of patients eventually succumbing to their disease. Thus, the need for new therapeutic strategies in myeloid disorders is unquestionable.

In this chapter, some of the new promising agents undergoing evaluation in treating myeloid malignancies are discussed (Table I). Some of these drugs are addressed in more detail in other chapters. These agents are divided arbitrarily into those with the more traditional cytotoxic activity, agents with a putative biological mode of action, and agents that target cellular signaling pathways. There is significant overlap among these groups, and this division by no means assumes the exact mechanism of action, which for most agents described is largely unknown.

III. Nucleoside Analogs

Nucleoside analogs (NAs) in general and cytosine arabinoside (Ara-C) in particular have been essential components of therapy of myeloid malignancies. These compounds mimic physiologic nucleosides in terms of uptake by transporters and metabolism by intracellular enzymes and are incorporated

TABLE I Examples of New Agents in Myeloid Malignancies

New nucleoside analogs	Hypomethylating agents
	Decitabine
	5AZA
	Structural modifications
	Gemcitabine
	Clofarabine
	Troxacitabine
Reversal of multidrug resistance	Cyclosporine
	PSC-833
	Others
Biological agents	Monoclonal antibodies
	Myelotarg
	HuM195
	Immunoconjugates
	Radioimmunoisotopes
	Angiogenesis inhibitors
	Anti Bcl-2 antisense
	Histone deacetylase inhibitors
Signaling pathway/cell cycle modulators	Kinase inhibitors
	Farnesyltransferase inhibitors
	Proteasome inhibitors
	Others

into newly synthesized DNA, resulting in chain termination and inhibition of DNA synthesis. The cytotoxic activity of Ara-C, as well as that of other NAs, is dependent on conversion to its active phosphorylated metabolites by intracellular enzymes such as deoxycytidine kinase (dCK) (Galmarini *et al.*, 2001). Deamination by enzymes such as deoxycytidine deaminase (dCD) to nontoxic metabolites is one mechanism of inactivation. Also, cytoplasmic 5′-nucleotidase activity opposes that of dCK by dephosphorylating 5′-monophosphate derivatives, thereby preventing the synthesis of active forms (Galmarini *et al.*, 2001). Over the past 2 decades, there has been significant increase in the understanding of mechanisms of action and resistance to NAs (Galmarini *et al.*, 2001). To overcome resistance, modifications of their structure have been attempted. Several new agents have been developed and investigated in preclinical models and clinical trials. Some of these agents have already demonstrated significant activity in myeloid (as well as some lymphoid) malignancies.

A. Troxacitabine

The naturally occurring nucleosides, as well as the majority of NAs used in cancer therapy, are D-enantiomers. Until recently, L-enantiomers were thought to be inactive, as they would not be recognized by the activating

enzymes (Giles *et al*., 2001a). Troxacitabine is a NA developed by the exchange of the sulfur endocyclic atom with an oxygen in the structure of the antiviral drug lamivudine (Mansour *et al*., 1995). It is the first nucleoside L-enantiomer with substantial cytotoxic activity (Grove and Cheng, 1996; Grove *et al*., 1995) and has been shown to have significant activity in myeloid disorders (Giles *et al*., 2001a, 2002, 2003a). As with ara-C, dCK catalyzes the phosphorylation of troxacitabine to its active metabolite, whereas dCD is unable to inactivate it by deamination because of its chiral specificity (Grove and Cheng, 1996; Grove *et al*., 1995). In an initial Phase I study, escalating doses of troxacitabine were administered to 42 patients with refractory or advanced hematological malignancies, including 31 patients with AML and 6 patients with MDS. Stomatitis and hand–foot syndrome were dose limiting, with a maximum tolerated dose of 8 mg/m^2/day × 5. Three complete remissions (CRs) and one partial remission (PR) were observed in 30 assessable patients with AML (Giles *et al*., 2001a). These results led to a Phase II study of 8 mg/m^2/day troxacitabine administered as an intravenous infusion to 42 patients with refractory or relapsed hematological malignancies. Among 16 evaluable patients with AML, 2 achieved CR and 1 PR (Giles *et al*., 2002). Six (37%) of 16 assessable patients with blast phase-chronic myeloid leukemia (CML-BP) returned to chronic phase. Combinations of troxacitabine with other cytotoxic agents have been evaluated in patients with refractory or relapsed AML, MDS, or CML-BP (Giles *et al*., 2003a). Giles *et al*. (2003a) reported their experience combining troxacitabine with Ara-C (TA), idarubicin (TI), or topotecan (TT). Among 74 evaluable patients with AML or MDS, 10 (13%) achieved CR (Giles *et al*., 2003a). These included 7 (11%) of 66 evaluable patients with AML: 4 patients received TA, 2 received TI, and 1 received TT. Four additional patients with AML (all receiving TA) showed hematological improvement (Giles *et al*., 2003a). In addition, 3 patients with advanced MDS obtained CR: 2 received TA and 1 TI. Based on the evidence of clinical activity and acceptable toxicity profile in this population of heavily pretreated patients, a study in patients with previously untreated AML or MDS was initiated. In this adaptively randomized prospective study, standard chemotherapy (IA) was compared with TA and TI in older patients (≥50 years) with unfavorable karyotype AML (Of the patients, 53% had monosomies of chromosome 5 or 7, or both, or deletions of the long arms of these chromosomes) (Giles *et al*., 2003b). The CR rates were 10/18 for IA, 5/11 for TA, and 1/5 for TI. In this study, the patients randomized to receive IA had an overall better profile with respect to cytogenetics and presence of antecedent hematological disorder, but poorer performance status and an age distribution similar to that of the TA and TI groups. However, because of the small number of patients randomized, no definite conclusions regarding the possibility of imbalances in the distribution of important prognostic covariates could be made. Overall, troxacitabine-based regimens were

deemed equivalent to standard chemotherapy (Giles *et al.*, 2003b). A larger randomized trial comparing troxacitabine-based regimens vs. standard chemotherapy is ongoing to better define the role of troxacitabine in AML.

B. Gemcitabine

Gemcitabine has been shown to have significant activity, alone or in combination with other agents, in a number of solid tumors and lymphoid malignancies (Perez-Manga *et al.*, 2000; Santoro *et al.*, 2000; Zinzani *et al.*, 2000). Recent studies have reported clinical activity in myeloid malignancies also. Gandhi *et al.* (2002) administered gemcitabine at a fixed dose of 10 mg/m^2/min to patients with relapsed or refractory AML and investigated dose escalation by prolonging the duration of administration in a Phase I setting. They proposed that a continuous infusion of gemcitabine at the given schedule would allow plasma gemcitabine concentrations sufficient to maximize the rate of gemcitabine triphosphate accumulation, which would then optimize any antileukemia effect. The dose escalation in this study was done by increasing the duration of infusion while keeping the dose and rate constant. With this approach, gemcitabine could be administered for longer than 12 h without significant toxicity. However, with more prolonged infusions, grade 3 mucositis and rash were observed. The authors concluded that although it was difficult to ascertain the exact maximum tolerated duration (MTD), it appeared that the MTD of infusion at the fixed rate of 10 mg/m^2/min is between 12 and 15.4 h. Among 19 patients treated, 1 had a PR and 2 hematological improvement (Gandhi *et al.*, 2002). Combinations of gemcitabine with other cytotoxic agents have been investigated in patients with refractory acute leukemia. Rizzieri *et al.* (2002) used a combination of gemcitabine at a fixed rate of 10 mg/m^2/min, for an escalating duration of infusion (based on the study by Gandhi and coworkers), with 12 mg/m^2/day of mitoxantrone for 3 days. They reported a maximum recommended duration of gemcitabine of 12 h (total dose of gemcitabine 7200 mg/m^2) with an overall response rate of 42% among 26 patients with relapsed or refractory leukemia, including a CR rate of 25% in the 25 patients treated at the recommended Phase II dose (Rizzieri *et al.*, 2002). The most common dose-limiting toxic effects of the regimen were severe myelosuppression and mucositis. A second study with a similar regimen reported a CR rate of 11% among 18 patients with advanced leukemia (Apostolidou *et al.*, 2003b). Combinations of prolonged-infusion gemcitabine with irinotecan and prolonged-infusion gemcitabine with fludarabine have also been investigated, with reported response rates of 18% and 28%, respectively (Bass *et al.*, 2002; Rizzieri *et al.*, 2003). These studies suggest that gemcitabine might have more clinical activity in AML and MDS than previously recognized, and further studies are warranted.

C. Clofarabine

Clofarabine (2-chloro-2′-fluoro-deoxy-9-β-D-arabinofuranosyladenine) has been developed to incorporate some of the favorable antitumor properties of fludarabine and cladribine (Kantarjian *et al*., 2003b). A chloride chain in the adenosine ring makes clofarabine, like cladribine, resistant to deamination by adenosine deaminase. A fluoride in the 2′ position of the arabinofuranosyl ring makes it resistant to phosphorolysis by purine nucleoside phosphorylase (PNP). As with other NAs, clofarabine is phosphorylated to its active metabolite by dCK. Clofarabine triphosphate inhibits DNA polymerases and ribonucleotide reductase, leading to cell death (Kantarjian *et al*., 2003b; Parker *et al*., 1991). In a Phase I study of this drug, reversible dose-limiting hepatotoxicity was noted at a dose of 55 mg/m^2 and the recommended dose for acute leukemia studies in adults was 40 mg/m^2 daily for 5 days (Kantarjian *et al*., 2003b). Among 32 patients with acute leukemia, 2 achieved a CR and 3 had a marrow remission without a platelet recovery, for an overall response rate of 16% (Kantarjian *et al*., 2003b). A single-institution Phase II study using clofarabine 40 mg/m^2 daily for 5 days every 3–4 weeks included 62 patients with relapsed or refractory acute leukemia, high-risk MDS, or CML-BP (Cortes *et al*., 2002d; Kantarjian *et al*., 2003a). Among 31 evaluable patients with AML, 17 (55%) patients with AML achieved CR. Similarly, 4 of 8 (50%) patients with MDS and 7 of 11 (64%) patients with CML-BP achieved CR. A further 9 patients (15%) achieved CR without full platelet recovery. The regimen was well tolerated with few greater than or equal to grade 3 toxicities, including skin rash (10%), transient elevation of transaminases (24%), palmoplantar erythrodysesthesia (11%), and nausea and vomiting (3%) (Cortes *et al*., 2002d; Kantarjian *et al*., 2003a). In addition to its significant antileukemia activity, clofarabine has significant synergy with Ara-C through increased Ara-C triphosphate formation by dCK. Thus, combination studies of clofarabine and ara-C have been initiated. This combination is currently being evaluated in frontline therapy of AML and high-risk MDS.

D. Hypomethylating Agents (Decitabine and 5-Azacytidine)

Epigenetic events, such as aberrant DNA methylation, can be important in the progression of a number of human neoplasms (Jones and Laird, 1999; Santini *et al*., 2001; Singal and Ginder, 1999). Hypermethylation of promoter-associated CpG-rich regions (CpG islands) can result in silencing of genes such as tumor suppressor genes (as an alternative mechanism to deletions and mutations), leading to their inactivation (Baylin *et al*., 1998; Toyota *et al*., 2001). Increased methylation of promoters of genes such as p15 have been associated with disease progression in myeloid malignancies

and correlate with an inferior outcome (Au *et al*., 2003; Teofili *et al*., 2003). Decitabine [5-aza-2′-deoxycitidine (DAC)] is a pyrimidine analog with significant antileukemic activity (Pinto and Zagonel, 1993). Although it is a, NA, its mechanism of action is believed to be related to the inhibition of the enzyme cytosine methyltransferase, which results in the reduction of methylation of CpG dinucleotide islands in the DNA and activation of silenced genes (Issa *et al*., 1997; Momparler *et al*., 1984; Santi *et al*., 1983). This leads to the terminal differentiation of leukemic cells (Issa *et al*., 1997; Momparler *et al*., 1984; Santi *et al*., 1983). Decitabine is an S-phase specific agent, which is activated by dCK and then incorporated into DNA strands by a DNA polymerase.

Decitabine has demonstrated clinical efficacy in achieving responses in patients with relapsed or refractory leukemia (Momparler *et al*., 1985; Richel *et al*., 1991; Rivard *et al*., 1981). Initial dose-finding studies demonstrated minimal extramedullary toxicity, with the dose-limiting toxicity being prolonged myelosuppression (De Lima *et al*., 2003; Kantarjian *et al*., 1997a; Petti *et al*., 1993; Pinto and Zagonel, 1993; Ravandi *et al*., 2001; Willemze *et al*., 1993; Zagonel *et al*., 1993). Kantarjian *et al*. (1997b) administered decitabine at a dose of 100 mg/m^2 every 12 h for a total of 10 doses (total dose 1000 mg/m^2) to 37 patients with accelerated ($n = 17$) or blast phase ($n = 20$) CML and observed an overall response rate of 25% in blast phase and 53% in accelerated phase. The median time to recovery of more than 500 μl granulocytes was 48 days (Kantarjian *et al*., 1997b). Other investigators have combined decitabine with other antileukemia agents (amsacrine, idarubicin, and daunorubicin) and have reported clinical activity in AML (Schwartsmann *et al*., 1997; Willemze *et al*., 1997). These studies have generally used the MTD defined in the Phase I setting. However, an optimal biologic (hypomethylating) effect can be achieved *in vitro* with low concentrations of decitabine. Increasing concentrations do not result in more hypomethylation, but rather result in a direct cytotoxic effect. Thus, Issa *et al*. (2001) conducted a Phase I study to determine the optimal biologic dose in patients with relapsed or refractory myeloid malignancies. The starting dose was 5 mg/m^2 daily for 10 days, with escalation until maximal hypomethylation was achieved. A total of 39 patients were treated and responses were seen at all dose levels for an overall response rate of 39% (95% CI 28–61%). A dose of 15 mg/m^2 for 10 days gave the best response rate and was associated with minimal toxicity. This total dose was only 15% of that used in previous studies, with higher doses associated with increased toxicity and lower response rates (Issa *et al*., 2001).

Decitabine and the related drug 5-azacytidine (AZA) have also demonstrated significant activity in patients with MDS. Silverman *et al*. (2002) recently reported the results of a study by the Cancer and Leukemia Group B (CALGB) in which 191 patients with MDS were randomized to receive AZA (75 mg/m^2/day subcutaneously for 7 days every 28 days) vs. supportive

care with a crossover design. Responses occurred in 60% of the patients treated with AZA (7% CR, 16% PR, 37% hematological improvement). This resulted in a significant improvement in time to transformation to AML or death for patients treated with AZA ($p = 0.007$) (Silverman *et al.*, 2002). Landmark analysis after 6 months (to adjust for the confounding effect of early crossover to AZA) showed a significant improvement in median survival for patients randomized to receive the drug (18 months vs. 11 months, $p = 0.03$) (Silverman *et al.*, 2002). Furthermore, there was a significant difference in favor of the AZA-treated group with regard to a number of quality-of-life measures, such as fatigue and psychologic state (Kornblith *et al.*, 2002).

Decitabine has also been investigated for the therapy of elderly patients with MDS (Wijermans *et al.*, 1997, 2000). Decitabine was administered to 66 patients with MDS at a dose of 45 mg/m^2/day for 3 days every 6 weeks for a maximum of 6 cycles (Wijermans *et al.*, 2000). The overall response rate was 49%, with similar response rates across international prognostic scoring system (IPSS) risk groups. The median response duration was 31 weeks and median overall survival 15 months (Wijermans *et al.*, 2000). Responses were associated with the disappearance of chromosomal abnormalities in patients with abnormal cytogenetics before start of therapy (Lubbert *et al.*, 2001). In addition, reversal of hypermethylation was observed in responding patients (Daskalakis *et al.*, 2002; Sigalotti *et al.*, 2003). Treatment was well tolerated, with an induction mortality of 7% in a population with a median age of 68 years. On the basis of these encouraging results, a randomized multicenter study comparing decitabine to supportive care in patients with MDS is currently ongoing. Other studies are investigating alternative schedules, decitabine-based combinations, and the role of decitabine in AML and CML after failure to respond to imatinib mesylate.

IV. Reversal of Multidrug Resistance

Anthracyclines and epipodophyllotoxins have significant activity in myeloid malignancies and are usually used in combination with cytarabine as induction therapy for patients with AML and high-risk MDS. Resistance to these chemotherapeutic agents has been correlated with expression of P-glycoprotein (Pgp), as well as the multidrug resistance protein (MRP) and the major vault protein (LRP) in myeloid disorders and other malignancies (Sonneveld, 2000). Pgp is a highly conserved 170-kDa plasma membrane protein that functions as an ATP-dependent multidrug exporter with broad specificity for natural-product-derived agents (Ueda *et al.*, 1987). Overexpression of Pgp is associated with a relative *in vitro* resistance to anthracyclines as a result of decreased intracellular accumulation of the agents.

Agents such as cyclosporine A, verapamil, and PSC-833 that inhibit Pgp can overcome this resistance *in vitro* (Nooter *et al.*, 1990). Expression of the multidrug resistance (MDR1) gene coding for Pgp is high in elderly patients with AML and is associated with a significantly poorer CR rate (Leith *et al.*, 1997).

A. Cyclosporine A

List *et al.* (2001) investigated the benefit of adding cyclosporine A to therapy with cytarabine and daunorubicin in patients with poor-risk AML. A total of 226 patients were randomly assigned to sequential therapy with cytarabine and infusional daunorubicin with or without intravenous cyclosporine A. Patients randomized to receive cyclosporine A had a slightly higher CR rate (40% vs. 33%, $p = 0.14$) but a significantly better relapse-free (34% vs. 9% at 2 years, $p = 0.031$) and overall survival (22% vs. 12%, $p = 0.046$) (List *et al.*, 2001). The benefit of cyclosporine was seen in both MDR1-positive and MDR1-negative patients, but was more significant in the former (median survival 12 months vs. 4 months in the latter). Steady-state daunorubicin serum concentrations were higher for cyclosporine-treated patients, possibly because of inhibition of the hepatic metabolism of daunorubicin (List *et al.*, 2001).

B. PSC-833

PSC-833 is a nonimmunosuppressive cyclosporine analog that is 20 times more potent than cyclosporine in increasing daunorubicin retention in MDR cells (Boesch *et al.*, 1991). Moreover, PSC-833 lacks renal toxicity, and concentrations sufficient to block Pgp function *in vitro* are easily achieved in patients with doses that are relatively free of side effects (Tidefelt *et al.*, 2000). As PSC-833 can retard hepatic clearance of cytotoxic drugs, their dose often needs to be reduced when coadministered with PSC-833. Addition of PSC-833 to mitoxantrone, etoposide, and Ara-C (PSC–MEC) was found to be safe and induced a CR rate of 29% in patients with relapsed or refractory AML (Advani *et al.*, 1999). However, in a randomized trial by the Eastern Cooperative Oncology Group (ECOG) in 113 patients with relapsed or refractory AML, no advantage was reported for PSC–MEC over MEC alone (Greenberg *et al.*, 1999). Also Baer *et al.* (2002a) randomized 120 patients with AML who were older than 60 years between a combination of cytarabine, daunorubicin, and etoposide with (ADEP) or without (ADE) PSC-833. Excessive early mortality in the ADEP arm (despite dose adjustments for daunorubicin and etoposide) led to the closure of this arm. The outcomes of induction therapy were different in the two groups, with rates of CR and induction death of 46% and 20%, respectively, for ADE vs. 39% and 44%, respectively, for ADEP ($p = 0.008$)

(Baer *et al.*, 2002a). This group is currently undertaking a similar study in patients younger than 60 years with a reduction in the dose of etoposide (40 mg/m^2/day for 3 days) in order to avoid the early induction toxicity. More selective and specific MDR modulators such as pipecolinate derivatives VX-710 and VX-853 that target Pgp, as well as the multidrug resistance protein (MRP-1) and the breast cancer resistance protein (BCRP), are in development (Baer *et al.*, 2002b). A drawback of this approach is that it addresses only one of the many known mechanisms of drug resistance and does not address resistance to some of the most active agents in myeloid malignancies, such as cytarabine.

V. Biological Agents

A. Monoclonal Antibodies

A number of monoclonal antibodies against antigens expressed by tumor cells have been developed that are currently in clinical use (Caron and Scheinberg, 1997; Winter and Milstein, 1991). The prototypes were generally murine monoclonal antibodies raised against human antigens and were associated with the problem of immunogenicity. This obstacle has been overcome through genetic engineering and humanization of the parent mouse antibody, resulting in human–mouse chimeric antibodies with the human constant region and mouse hypervariable region (Caron *et al.*, 1992). Such a chimera has the advantage of eliciting very little human antimouse antibody (HAMA) neutralizing response. Avoidance of HAMA allows for repeated treatments without loss of effectiveness and a longer circulation time.

CD33 provides a useful target antigen for AML therapy because it is expressed on the leukemia cell surface in more than 90% of patients with AML, with an average antigen density of 10,000 sites/cell (Scheinberg *et al.*, 1989; Tanimoto *et al.*, 1989). It is also expressed on normal granulocyte/monocyte colony forming units (CFU-GM) and some primitive erythroid progenitors. However, it is not prominently expressed on tissues other than the hematopoietic system and normal pluripotent hematopoietic stem cells (Scheinberg *et al.*, 1989; Tanimoto *et al.*, 1989).

A naked antibody to CD33, HuM195, was developed at the Memorial Sloan-Kettering Cancer Center. Reports from early studies demonstrated minimal toxicity confined to infusion reactions associated with these antibodies (Caron *et al.*, 1994a,b). *In vitro* and *in vivo* studies have shown that HuM195 is capable of rapidly internalizing into target cells on binding to the antigen (Caron *et al.*, 1994b). Jurcic *et al.* (2000) have used HuM195 in treating patients with APL and reported a higher rate of PCR negativity (as compared with ATRA alone) when the antibody was given to PCR-positive

patients in hematologic CR after an induction course of ATRA with or without chemotherapy (Jurcic *et al.*, 2000). Feldman *et al.* (2003) have reported on their experience with HuM195 in 50 patients with relapsed or refractory AML. Two patients achieved CR, and one patient had a PR by using the antibody alone. Minimal toxicity related to infusion of first dose of the antibody was reported (Feldman *et al.*, 2003). More recently, addition of HuM195 to chemotherapy [mitoxantrone, etoposide, and cytarabine (MEC)] was compared with MEC alone in 191 adults with primary refractory or first relapse of AML (Feldman *et al.*, 2002). An overall response rate of 43% [27 CR and 13 CR with incomplete platelet recovery (CRp)] was reported in 94 patients receiving the combination vs. 26% (20 CR and 5 CRp) in 97 patients given chemotherapy alone ($p = 0.015$). Addition of HuM195 did not result in any increase in chemotherapy-related toxicity (Feldman *et al.*, 2002). Thus, this antibody has promising activity in myeloid malignancies, particularly when combined with chemotherapy.

Another approach has been to conjugate these monoclonal antibodies with toxins or radioisotopes (Sievers *et al.*, 1999). Such immunoconjugates would theoretically deliver the intended toxin or radiation selectively to the tumor and its vicinity and would be associated with improved efficacy and reduced toxicity.

Gemtuzumab ozogamicin (GO) is a conjugate of an antibody to CD33 and the antitumor drug calicheamicin (Sievers *et al.*, 2001). Calicheamicin is a potent toxin that is highly lethal in preclinical models. Its conjugation with the specific anti-CD33 antibody through a stable linker allows its delivery into a selected cell population. In the pivotal multicenter trial, 142 patients with AML in first relapse with no history of an antecedent hematologic disorder and a median age of 61 years were treated with GO given at a dose of 9 mg/m^2 at 2-week intervals for two doses. Of the patients, 30% obtained remission, as characterized by 5% or less blasts in the marrow, recovery of neutrophils to at least 1.5×10^9/l, and RBC and platelet transfusion independence. Approximately half these patients did not recover their platelet count to 100×10^9/l and were categorized as having a CRp. This newly defined category (CRp) translates into a survival advantage for patients compared to those with no response, although it is inferior to a CR as conventionally described. Of the patients, 23% (33 of 142) had grade 3 or 4 usually transient hyperbilirubinemia and 17% (24 of 142) grade 3 or 4 increases in AST or ALT levels (Sievers *et al.*, 2001).

There has been some concern that treatment with GO may induce veno-occlusive disease (VOD). Giles *et al.* (2001b) reported the occurrence of this phenomenon in 14 of 119 (12%) patients treated with GO alone or in combination with other chemotherapeutic agents. These included five patients who had received no prior antileukemic cytotoxic therapy. The diagnosis of VOD was made clinically in most patients. Later, Rajvanshi *et al.* (2002) reported liver toxicity in 11 of 23 patients who received GO for AML that

had relapsed after stem cell transplant. Histological examination of the liver demonstrated sinusoidal injury with extensive fibrosis, centrilobular congestion, and hepatocyte necrosis (Rajvanshi *et al*., 2002). Thus, it appears that VOD of the liver might occur in a small percentage of patients treated with GO. More recently, in a study of 62 patients with AML and MDS who underwent allogeneic stem cell transplantation, 13 (21%) developed VOD, including 9 of 14 (64%) with prior GO exposure and 4 of 48 (8%) without prior GO exposure ($p < 0.0001$) (Wadleigh *et al*., 2003). Therefore, the principal risk factor for VOD appears to be its administration before or after a stem cell transplant (Wadleigh *et al*., 2003). However, administration of GO with other chemotherapeutic agents (such as 6-thioguanine) has also been reported to increase the risk of VOD (Kell *et al*., 2002).

A study by Leopold and coworkers provides useful insights on the possible role of the agent in patients with relapsed AML (Estey, 2002). They compared GO to high-dose Ara-C-containing regimens in patients in first relapse after an initial CR duration of >3 months. GO was more likely than Ara-C to produce a second CR if the duration of CR1 was less than 6 months, whereas Ara-C-containing regimens were more likely to achieve a second CR if the duration of CR1 was >1 year (Estey, 2002). There was no statistically significant difference in the probability of achieving a second CR with either therapy for patients whose CR1 was 6–13 months (Estey, 2002). These data supported the use of GO in older patients in first relapse with a short duration of CR1. However, patients with long first CR and younger patients should probably still be treated with a high-dose Ara-C-based regimen instead (Estey, 2002).

Despite the results seen in the salvage setting, the use of this agent as first-line therapy for older patients with AML has been unsatisfactory. At the M. D. Anderson Cancer Center, 51 newly diagnosed patients with AML who were older than 64 years were treated with two doses of GO and the responses were compared with historical controls. CR rates of 45% in 20 patients with a normal karyotype and 6% of 31 patients with an unfavorable karyotype fared unfavorably as compared with historical controls treated with a combination of Ara-C and idarubicin in which the corresponding CR rates were 54% and 44%, respectively (Estey *et al*., 2002). This probably reflects the selection bias of the original studies in which only patients who had achieved a CR and relapsed after a minimum of 3 months (6 months for the younger) were included.

There has been considerable interest in exploring the use of GO in combination with other agents. De Angelo *et al*. (2002) have reported preliminary results of a Phase II study combining cytarabine and daunorubicin with GO in patients below 60 years with untreated AML. The maximum tolerated dose arrived at the Phase I portion of the study: 100 mg/m^2/day Ara-C on Days 1–7, 45 mg/m^2/day daunorubicin on Days 1–3, and 6 mg/m^2 GO on Day 4. At the time of reporting, 42 patients were enrolled and 15

of 18 (83%) evaluable patients achieved a CR. The regimen was well tolerated and no cases of VOD were reported (De Angelo *et al*., 2002). In another study, GO 3 mg/m^2 was administered on Day 1 of induction chemotherapy with one of three regimens: DAT, DA, or FALG-IDA (Kell *et al*., 2002). Of 55 patients treated, 41 (85%) entered CR with Course 1. Nonhematopoietic toxicity was confined to the liver, with the regimen containing both thioguanine and GO associated with a higher risk of liver toxicity. Administration of GO with consolidation courses was reported to be well tolerated, with VOD-like syndromes noted in 2 of 15 patients receiving GO with Courses 1 and 2 and none of the 17 patients receiving GO with Course 3 (Kell *et al*., 2002). Several other GO-based combinations have been reported (Alvarado *et al*., 2003; Apostolidou *et al*., 2003a; Cortes *et al*., 2002a; Tsimberidou *et al*., 2003a,b). The questions that need to be resolved include the ideal combination, the proper scheduling and the most appropriate dose of GO, and whether using GO in combination with other agents might increase the risk of toxicity, particularly VOD.

Monoclonal antibody/radioisotope combinations are also under investigation. Monoclonal antibodies conjugated to α-emitting radioisotopes such as ^{213}(Bi) and ^{211}(At) have been constructed and have shown preclinical activity (McDevitt *et al*., 2001). The HuM195-^{213}(Bi) conjugate was used in a Phase I study of 18 patients with relapsed for refractory AML or chronic myelomonocytic leukemia (CMML) (Jurcic *et al*., 2002). No significant extramedullary toxicity was observed and all the 17 evaluable patients became myelosuppressed. Reductions of circulating and bone marrow blasts were reported in 14 of 15 (93%) and 14 of 18 (78%) evaluable patients, respectively (Jurcic *et al*., 2002). β-emitting radioconjugates using ^{131}I and ^{90}Y have also been constructed and produce profound myelosuppression with elimination of large leukemic burdens, making them attractive for stem cell transplantation (Schwartz *et al*., 1993). Similarly, radiolabeled antibodies against CD45 have been studied in combination with high-dose cyclophosphamide and total body radiation as a preparative regimen for allogeneic transplantation (Matthews *et al*., 1999).

By using α-emitting constructs, the ratio of the amount of radiation delivered to the bone marrow, liver, and spleen relative to that delivered to the rest of the body is found to be 1000-fold greater than that achieved with β-emitting constructs. This reflects the shorter pathway length of α-emitters and suggests that α-emitting radioconjugates are likely to have lower toxicity and to be preferable at least in the nontransplant setting.

DT388–GMCSF is a fusion toxin consisting of the catalytic and translocation subunits of diphtheria toxin (DT388) linked to the human granulocyte-macrophage colony-stimulating factor (GMCSF) and directed against the GMCSF receptor that is strongly expressed by most human leukemic blasts (Hall *et al*., 1999). In preclinical studies, leukemic progenitors isolated from patients with AML were sensitive to DT388–GMCSF irrespective of

clinical responsiveness of the patients to standard chemotherapeutic agents (Hogge *et al*., 1998). In a Phase I clinical trial in 31 patients with AML who were resistant to chemotherapy, 1 had a CR and 2 had PR; all 3 responders were treated at or above the maximal tolerated dose and all had baseline marrow blast percentages of <30% (Frankel *et al*., 2002). Other fusion toxins such as DT(388)IL-3, composed of DT388 and human interleukin 3 (IL-3), have also been examined in preclinical models of leukemia (Black *et al*., 2003; Frankel *et al*., 2000). These agents are currently under evaluation in larger multicenter trials.

B. Angiogenesis

Although angiogenesis was first thought to have an important role in the pathogenesis and progression of solid tumors, it has become evident that angiogenesis and angiogenic cytokines play a major role in hematological malignancies (Aguayo *et al*., 2000; Giles, 2002; Hussong *et al*., 2000). Increased levels of angiogenic factors, as well as increased microvessel density, are identified in patients with AML, MDS, and CML (Aguayo *et al*., 2000). The vascular endothelial growth factor (VEGF) can stimulate the proliferation of leukemic progenitors, and this can be blocked by specific anti-VEGF antibodies in preclinical models (Ratajczak *et al*., 1998). Also, VEGF can inhibit the development and activation of dendritic cells, and antibodies to VEGF can enhance antitumor response by improving endogenous dendritic cell function (Gabrilovich *et al*., 1999). In addition, cellular receptors for VEGF are members of receptor tyrosine kinases. There has been growing interest in developing antiangiogenic strategies through one of two mechanisms: first is the inhibition of circulating angiogenic factors such as VEGF by using agents such as thalidomide or monoclonal antibodies, and the second is through direct inhibition of the VEGF receptor family. Thalidomide alone (Steins *et al*., 2002) or in combination with standard induction chemotherapy (Cortes *et al*., 2003d) has little if any activity in AML and MDS. Bevacizumab, an anti-VEGF monoclonal antibody, has undergone Phase I testing in solid tumors and is currently under evaluation in AML, MDS, and CML (Giles, 2002). Karp *et al*. (2002) reported their Phase II experience with bevacizumab administered in a time-sequential combination with ara-C and mitoxantrone. Thirty-two patients with high-risk refractory or relapsed AML were treated. Fourteen of 32 (44%) evaluable patients achieved CR (Karp *et al*., 2002). Several receptor tyrosine kinase inhibitors directed at the VEGF signaling pathway (see later), including SU5416, SU6668, ZD6474, ZK1222584, and CGP41251, are in development (Laird *et al*., 2000, Thavasu *et al*., 1999). SU5416 was associated with a stable PR in a patient with refractory AML together with a decrease in bone marrow microvessel density, a marker of angiogenesis (Mesters *et al*., 2001). More recently, Giles *et al*. (2003c) investigated this agent in 55 patients, including

33 with AML and 22 with MDS. Grade 3 or 4 toxicities included headache, infusion-related reactions, dyspnea, fatigue, thrombosis, and gastrointestinal disturbances. Three patients achieved PRs with two hematological improvements (Giles *et al.*, 2003c). These responses demonstrate the biological activity of these agents. However, they are mostly cytostatic in preclinical models. Thus, it is possible that either combinations with chemotherapy or the setting of minimal residual disease (i.e., maintenance therapy in CR) would be more appropriate.

C. Anti Bcl-2 Antisense

The process of leukemic transformation is dependent not only on increased proliferation but also, at least to some extent, on suppression of programmed cell death (apoptosis) (Reed, 1997, 1999). Dysregulation of the processes governing apoptosis can prolong the life span of the cell, resulting in leukemic cell expansion independently of cell division. Moreover, such defects might contribute to leukemogenesis by creating a permissive environment for genetic instability and accumulation of gene mutations. Furthermore, defects of apoptotic machinery promote resistance to immune-based destruction, facilitate growth factor-independent cell survival, and confer resistance to cytotoxic drugs (Reed, 1999). Thus, treatment strategies that target the apoptotic machinery are of great interest in myeloid malignancies.

Among the first candidates for therapy directed at apoptosis-regulating pathways is Bcl-2, which is frequently overexpressed in myeloid neoplasms. In addition to its antiapoptotic function, overexpression of Bcl-2 has been shown to be associated with resistance to chemotherapy (Reed, 1997, 1999). Therefore, it is conceivable that downregulation of Bcl-2 can reduce the threshold for chemotherapy resistance and restore chemosensitivity to leukemia cells (Andreeff *et al.*, 2002; Cotter, 1999; Gewirtz, 1998). Genasense (G3139) is an 18-mer phosphorothioate oligodeoxynucleotide (ODN) antisense designed to bind the first six codons of human Bcl-2 mRNA. Based on the potential increase of chemosensitivity, Marcucci *et al.* (2003) designed a study in which G3139 was given in combination with fludarabine and cytarabine and G-CSF (FLAG) to 20 patients with refractory AML or acute lymphoblastic leukemia (ALL) (Marcucci *et al.*, 2003) They demonstrated that G3139 could be administered in this setting with minimal increase in toxicity. Bcl-2 mRNA levels were downregulated in 75% (9 of 12) of the patients evaluated, proving the biological effect of G3139. Significant responses, including a CR rate of 35%, were encouraging in this refractory population (Marcucci *et al.*, 2003). However, it is unclear how much of the biological or clinical effects could be expected from the chemotherapy alone. Further studies examining the utility of this agent in leukemia and other hematological malignancies are therefore needed.

D. Histone Deacetylase Inhibitors

Hypomethylating agents, including 5-azacytidine and decitabine, have been discussed earlier. Their mechanism of action when administered at low doses is related to their ability to inhibit DNA methyltransferases and hence influence chromatin remodeling (Issa *et al*., 1997; Toyota *et al*., 2001; Villar-Garea and Esteller, 2003). DNA silencing of tumor suppressor genes by methylation of their promoter regions is one of the events in chromatin remodeling that might lead to carcinogenesis (Santini *et al*., 2001). In addition, abnormal recruitment of histone deacetylases (HDACs) to promoters of regulatory and other genes results in transcriptional repression (Amann *et al*., 2001; Faretta *et al*., 2001; Ferrara *et al*., 2001; Hiebert *et al*., 2001). Removal of acetyl groups by HDAC allows tighter binding of histones to DNA, hence preventing gene transcription. It has been suggested that this might work in a two-step process with DNA methylation toward gene silencing. Several HDAC inhibitors, such as phenylbutyrate, trichostatin A, SAHA, valproic acid, and depsipeptide, have been investigated *in vitro* for their ability to reverse this process (He *et al*., 2001; Klisovic *et al*., 2003; Kramer *et al*., 2003; Warrell *et al*., 1998). In a recent Phase I clinical study, depsipeptide, 3 mg/m^2 was infused on Days 1, 8, and 15 while repeating the cycle every 28 days (Marcucci *et al*., 2002). Nine patients, including six with relapsed AML, one with primary refractory disease, and two previously untreated patients older than 60 years, were treated with this regimen. None of the patients achieved a response, but some experienced transient declines in their blast count and one patient developed tumor lysis syndrome. The drug induced its classical biologic effect. The median increase in histone acetylation was 40% (range 10–160%) and 100% (range 0–240%) for H3 and H4 histone acetylation, respectively, at 4 h following depsipeptide infusion in 6 patients analyzed. Further dose escalation is underway to achieve the minimum effective pharmacologic dose. However, as the weekly schedule was associated with a cumulative increase in gastrointestinal and constitutional side effects, an alternative dosing schedule is also being explored (Marcucci *et al*., 2002). Studies to examine how these agents can be combined with chemotherapy and hypomethylating agents are currently in progress.

VI. Signaling Modulators

Molecules such as cytokines, as well as hormones and intercellular interactions mediated by neighboring cell surface antigens, influence cellular processes such as proliferation, differentiation, and cell death. Ultimately, these interactions result in gene transcription either directly, or indirectly by activating intracellular signaling pathways, which in turn activate appropriate

cellular machinery (Alberts *et al.*, 1994). Conversion of such external stimuli into intracellular signals by surface receptors is of pivotal importance in this process. These receptors can be activated by proteins with inherent enzymatic function or through mediators linked with other catalytic enzymes. Indeed, most growth factors and cytokines bind the cell surface receptors and exert their function through their activation, commonly by phosphorylation (Alberts *et al.*, 1994). These events usually trigger intracellular pathways that lead to proliferation, increased cell survival, and malignant transformation.

The normal functioning of the hematopoietic system depends on a number of intricately regulated signaling cascades that are mediated by cytokines and their receptors. As a result of orderly function of these pathways, an appropriate constellation of hematopoietic cells is produced; similarly, their abnormal activation results in neoplastic transformation.

Three major classes of proteins are involved in cellular signaling: kinases, adaptor or docking proteins, and transcription factors (Fig. 1). Inappropriate function of members of each group has been linked to a number of hematological malignancies (Ravandi *et al.*, 2003). A number of kinase cascades have been described. In general, these kinases mediate their action through phosphorylation of tyrosine or serine/threonine residues of their substrates. Also, disorderly function of a number of adaptor proteins and transcription factors (such as Ras and Myc) has been intimately linked with neoplastic transformation.

The description of a number of oncogenes with constitutive kinase activity has generated considerable interest as therapeutic targets. These

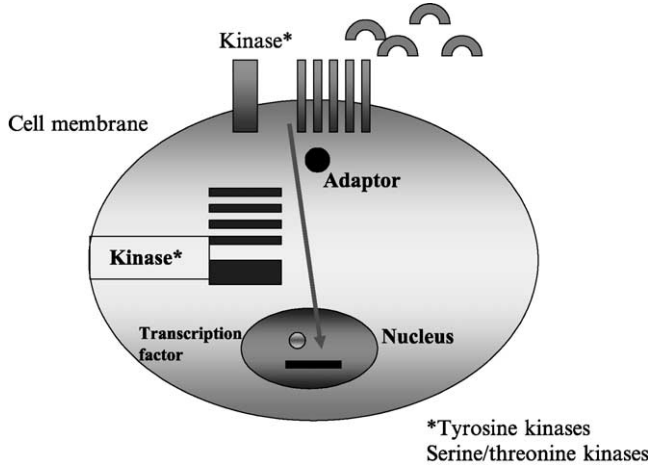

FIGURE 1 Signal transduction cascades.

oncogenes are derived from genes including c-ABL, c-FMS, FLT3, c-kit, PDGFRα and PDGFRβ, which are normally involved in the regulation of hematopoiesis (Scheijen and Griffin, 2002). Mutations that remove inhibitory domains of the molecule or induce the kinase domain to adopt an activated configuration lead to the constitutive activation of the protein product (Scheijen and Griffin, 2002). A number of downstream signaling cascades such as the Jak–Stat pathway, the Ras/Raf/MAPK pathway, and the PI3K pathway are then switched on and lead to inappropriate proliferation and cell survival.

Signaling pathways can be particularly attractive targets in treating myeloid malignancies because they are frequently inappropriately activated in leukemic cells. Therefore, their partial inhibition might be sufficient to interfere with malignant cell growth without causing significant toxicity (Frank, 1999). As a result, despite the pivotal role of these pathways in normal cellular function, their inhibition might not be associated with significant clinical toxicity.

A. Imatinib Mesylate

A clear example in which these pathways are activated and targeted therapeutic intervention has resulted in clinical benefit is the constitutive activation of the platelet-derived growth factor, PDGFRβ, in patients with CMML who have the translocation t(5;12)(q33;p13) generating the fusion protein TEL-PDGFRβ (Golub *et al*., 1994). This fusion leads to ligand-independent dimerization and autophosphorylation of the PDGF β-subunit and its constitutive activation. PDGF is able to stimulate the growth of primitive hematopoietic, erythroid, and megakaryocytic precursors, and TEL-PDGFRβ can confer cytokine-independent growth to Ba/F3 cells (Magnusson *et al*., 2001). Imatinib mesylate inhibits the kinase PDGFRβ and has been shown to be highly effective in patients with this translocation and other similar fusion genes (Apperley *et al*., 2002; Kulkarni *et al*., 2000; Magnusson *et al*., 2002; Ross *et al*., 1998). Similarly, a number of patients with the idiopathic hypereosino-philic syndrome (HES) have been shown to harbor the fusion gene FIP1-L1-PDGFRα in their neoplastic clone, leading to the constitutive activation of PDGFRα kinase. A fraction of patients with HES, including all those with this novel fusion gene, respond to therapy with imatinib mesylate (Cools *et al*., 2003; Cortes *et al*., 2003b). Patients with this clonal abnormality should indeed be now reclassified as chronic eosinophilic leukemia. Imatinib has also been used in AML because of its inhibition of the kinase activity of c-kit, which is expressed in more than 90% of patients with AML. Anecdotal responses have been reported (Kindler *et al*., 2003), but larger series demonstrated minimal or no activity (Cortes *et al*., 2003c).

B. FLT3 Inhibitors and Other Receptor Tyrosine Kinase Inhibitors

Receptor tyrosine kinases are membrane-bound enzymes with an extracellular ligand-binding domain, a transmembrane domain, and a highly conserved intracellular domain that mediates the activation, through tyrosine phosphorylation, of a number of downstream signaling proteins (Blume-Jensen and Hunter, 2001; Drexler, 1996; Porter and Vaillancourt, 1998). Ligand binding, as well as cell–cell interactions via cell adhesion molecules, can activate these enzymes (Weiss *et al*., 1997), and phosphorylated tyrosine residues serve as high-affinity docking sites for SH2-containing adaptor and effector proteins (Weiss and Schlessinger, 1998). Receptor tyrosine kinases include diverse molecules, which are considered as members of four distinct classes. Of particular interest in myeloid malignancies are the members of class III receptor tyrosine kinases, such as platelet-derived growth factor receptor (PDGFR), macrophage colony-stimulating factor (FMS-R or CSF-1R), stem cell factor receptor (kit), and FMS-like tyrosine kinase 3 receptor (FLT3R) (Drexler, 1996; Matthews *et al*., 1991; Sherr, 1990). Constitutive activation of FLT3R resulting in stimulation of multiple signaling pathways and leading to malignant transformation has been demonstrated in up to 30% of patients with AML (Gilliland and Griffin, 2002b; Tse *et al*., 2000). This figure might be an overrepresentation of the actual incidence of this phenomenon, and recent studies including all patients with newly diagnosed AML report a 15–20% incidence. This activation results from two well-described molecular events. One is internal tandem duplication of the FLT3R gene occurring at exons 11 and 12 of the gene that codes for the juxtamembrane domain of the receptor (Gilliland and Griffin, 2002a; Kelly *et al*., 2002; Kiyoi *et al*., 2002; Thiede *et al*., 2002). The second mechanism involves point mutations of codon 835 of the FLT3R receptor gene, located in the activation loop of its tyrosine kinase domain, and has been reported in up to 7% of patients with AML (Thiede *et al*., 2002; Yamamoto *et al*., 2001). Patients with these activating mutations of FLT3R have a significantly worse outcome, particularly when there is a homozygous inactivation (Abu-Duhier *et al*., 2000; Kottaridis *et al*., 2001; Whitman *et al*., 2001). Inhibitors of such aberrant activation are undergoing clinical evaluation (Levis *et al*., 2001, 2002). The preliminary results of these trials are summarized in Table II. Although these agents are considerably different in their chemical structure and selectivity for FLT3R, and despite the different designs, these trials have clearly demonstrated significant activity of FLT3 inhibitors, particularly in patients with FLT3R mutations. The *in vitro* and modest clinical activity in patients with wild-type FLT3R is intriguing and suggests a role in all patients with AML, although most likely in combination with other agents. Ongoing studies are further exploring the role of FLT3 inhibitors in leukemia, in particular in combination with chemotherapy and other targeted therapies.

TABLE II Recently Reported Trials of FLT3R Inhibitors

Reference	*Agent*	*FLT3R wild-type eligibility*	*Study phase*	*Dose schedule*	*Patients (n)*	*Response*	*Toxicity reported*
Stone *et al.*, 2002	PKC412	Yes	II	75 mg po tid	8	6 Pts with decreased PB blasts	
Foran *et al.*, 2002b	SU11248	Yes	I	25/50/75/100 mg po qd 2 weeks on/2 weeks off	32	13/16 Evaluable with >50% decreased PB blasts	One G3 fatigue each at 50 and 75 mg dose levels
Foran *et al.*, 2002a	SU11248	Yes	I	Single-dose po 50 to 350 mg	29	>50% Inhibition of FLT3R in all pts receiving ≥200 mg	
Smith *et al.*, 2002	CEP701	No	II	40/60/80 mg po bid	5	1 CRp at 60 mg	
Heinrich, *et al.*, 2002	MLN518	Yes	I	50 mg po q12h	6	2/3 Evaluable with >50% decreased PB blasts	

Note: Pts: patients; G3: grade 3; PB: peripheral blood; CRp: complete remission without platelet recovery to $>100 \times 10^9$/l.

C. Farnesyltransferase Inhibitors

Ras proteins play a significant role in human carcinogenesis, and inhibition of Ras signal transduction has been considered a target for antineoplastic therapy. Ras-mediated signaling can be inhibited by preventing its localization to cell membrane, by inhibition of Ras protein expression using antisense nucleotides, or by inhibition of its downstream targets (Adjei, 2001; Lee and McCubrey, 2002). Farnesyltransferase (Ftase) is an enzyme responsible for the C-terminal prenylation of Ras that is required for its association with the cell membrane, a prerequisite for its transforming activity. Several pharmacological inhibitors of Ftase have been developed, and clinical trials of their efficacy in leukemia are underway (Table III; Beaupre and Kurzrock, 1999; Cortes *et al*., 2003a; Gibbs, 1991; James *et al*., 1993; Karp *et al*., 2001; Kohl *et al*., 1993). Four such farnesyltransferase inhibitors are currently at various stages of clinical testing: R115777, SCH66336, L778123, and BMS214662 (Adjei *et al*., 2000; Cortes *et al*., 2003a; Karp, 2001; Lee and McCubrey, 2002). Karp *et al*. (2001) conducted a Phase I study using R115777 (Zarnestra®) administered orally for 21 days every 28 days for up to four cycles in patients with poor-risk acute leukemias. Reliable inhibition of Ftase activity occurred at or above the 300-mg bid dosing level, and dose-limiting toxicity, manifested as a reversible neurotoxicity, was observed at the 1200-mg bid dosing level. Clinical responses were observed in 10 of 34 (30%) patients, including two with CR. Interestingly, responses occurred across the entire range of dosing levels (100–900 mg bid) and independently of Ras mutational status (Karp *et al*., 2001). Lancet *et al*. (2002) have reported a Phase II study of Zarnestra 600 mg twice daily for 21 days in 41 patients with untreated poor-risk AML (defined as any of age $\geq$65 years, age $\geq$18 years with adverse cytogenetics, secondary AML) and MDS with IPSS score $\geq$1.5. Complete and partial hematological responses were observed in 10 of 30 (33%) evaluable patients, including 8 CRs and 2 PRs, 9 of which occurred after one cycle of therapy. An additional 12 patients had stable disease after one cycle of therapy (Lancet *et al*., 2002).

Kurzrock *et al*. (2001) have reported results of their Phase I and Phase II clinical trials using Zarnestra in MDS. In the Phase I study, using a 21-day cycle every 28 days, the dose-limiting toxicity was fatigue, occurring at 900 mg bid, although mostly among older patients. A response rate of 33% was reported. In the Phase II trial in patients with relapsed or poor-risk MDS, a more prolonged schedule was used (28 days every 42 days). This resulted in higher toxicity and a lower response rate, suggesting a dependence on the schedule. Similar responses have been obtained with other members of this family of drugs.

Although the intended target of these drugs is Ras, it is now clear that these agents might have Ras-independent effects on other cellular signaling

TABLE III Recently Reported Studies of Ftase Inhibitors

Reference	*Agent*	*Disease*	*Phase*	*Schedule*	*Patients (n)*	*Ras mutations present*	*Response*	*Toxicity*
Cortes *et al.*, 2002b	SCH66336	CML	Pilot	200 mg po bid	12	?	2	Grade 3 GI in 33%
Ravoet *et al.*, 2002	SCH66336	MDS, sAML	I/II	200 mg po bid for 4 weeks	16	5/11	PR in 2/12 evaluable	Diarrhea, rash, fatigue
Gotlib *et al.*, 2002	R115777	CML, Ph− CML, CMML	I/II	300 mg po bid for 3 weeks (400 mg in 7 patients)	13	0/9	8 Biological 4 Clinical	Grade 3 decreased platelets, increased SGOT, increased creatinine, tremor 6/36
Lancet *et al.*, 2002	R115777	AML, MDS	II	600 mg po bid for 3 weeks	41	2/13	10/30, 8CR, 2PR	Grade 4 NF

Note: CML: chronic myelogenous leukemia; MDS: myelodysplastic syndrome; sAML: secondary acute myeloid leukemia; Ph−: Philadelphia chromosome negative; CMML: chronic myelomonocytic leukemia; CR: complete remission; PR: partial remission; GI: gastrointestinal; NF: neutropenic fever.

components and that these effects might contribute to their antineoplastic action (Kurzrock *et al*., 2001). Some of the proposed targets that may be responsible for their clinical activity include RhoB and the mitotic kinasins, CENP-E and -F (Ashar *et al*., 2000). Ftase inhibitors are currently being further investigated in combination with chemotherapy and other targeted therapies and in the setting of minimal residual disease.

D. Proteasome Inhibitors

The ubiquitin–proteasome pathway is the central pathway for degradation of intracellular proteins (Almond and Cohen, 2002; Ciechanover, 1994). Targeting of proteins to proteasome is through covalent attachment of a polyubiquitin chain. The proteasome system can degrade decaying proteins (i.e., housekeeping function) as well as important proteins involved in the regulation of the cell cycle and other functions, thus regulating cell proliferation and maturation (Almond and Cohen, 2002). Important substrates for proteasome degradation include cyclins and CDKIs; transcription factors such as p53, NFκB, c-Myc, c-fos, and c-Jun; a number of apoptosis families of proteins; inhibitor of apoptosis proteins (IAPs); and some caspases (Almond and Cohen, 2002; Breitschopf *et al*., 2000; Chang *et al*., 1998; Karin and Ben-Neriah, 2000; King *et al*., 1996; Li and Dou, 2000; Marshansky *et al*., 2001). Different classes of proteasome inhibitors can therefore be used to differentially affect cellular levels of oncogenic proteins (Almond and Cohen, 2002). PS-341 (bortezomib) is a specific and potent inhibitor of proteasome (Gardner *et al*., 2000). It induces apoptosis and overcomes resistance in a number of cell lines, as well as primary cells from patients with CLL (Adams *et al*., 1999; Almond *et al*., 2001; Chandra *et al*., 1998; Masdehors *et al*., 1999, 2000). Its *in vitro* activity in myeloma has led to its ongoing examination in other hematological malignancies and solid tumors (Hideshima *et al*., 2001, 2002; Mitsiades *et al*., 2002). A Phase I clinical trial in 15 patients with acute leukemias and MDS showed significant proteasome inhibition but no significant clinical activity (Cortes *et al*., 2002c). However, there was significant apoptosis of leukemic cells in some patients. This and the reported synergy with agents such as topoisomerase I inhibitors have led to currently ongoing studies of bortezomib in combination with chemotherapy.

E. Other Potential Targets

Other steps in the serine/threonine cascades, from the cell surface receptor to gene transcription, are also being examined as possible targets for therapy (Lee and McCubrey, 2002) (Fig. 1). Compounds such as geldanamycin and derivatives of radicicol are known to destabilize Raf protein and interfere with Raf signaling (Blagosklonny *et al*., 2001; Shiotsu *et al*., 2000;

Soga *et al*., 1999). Several staurosporine derivatives, including UCN-O1, CGP41251, and PKC412, are able to inhibit PKC signaling and have been examined in cell lines and clinical studies (Propper *et al*., 2001; Seynaeve *et al*., 1994; Thavasu *et al*., 1999). Aberrant MEK and ERK activity has been demonstrated in AML and CML (Kang *et al*., 2000; Kim *et al*., 1999; Morgan *et al*., 2001; Okuda *et al*., 1994), and MEK inhibitors such as PD098059, PD184352, and UO126 are able to modulate cellular proliferation, differentiation, and apoptosis (Alessi *et al*., 1995; Dudley *et al*., 1995; Favata *et al*., 1998). PD184352 has been examined in Phase I trials in patients (Sebolt-Leopold, 2000). Pharmacological inhibitors of PI3K, wortmannin and LY294002, have shown significant potency in preclinical studies (Powis *et al*., 1994; Vlahos *et al*., 1994).

VII. Conclusions

Recent advances in the investigation of the biology of leukemias have led the way in the discovery of multiple potential targets for therapy. Many of these agents are currently under investigation, and some have already shown significant clinical activity. Many important questions have arisen and will need to be answered. Many of these agents can clearly produce the intended biological response (i.e., hit the target), but clinical responses might be modest at best. The questions then arise of whether a drug is worth developing further and how to best define responses to it. Furthermore, many of these agents affect only one of many steps in a specific pathway, whereas biology of cells, in particular malignant cells, is a complex and multistep process with multiple targets. Therefore, a multistep approach is likely needed. As we discover the ever-growing heterogeneity of myeloid malignancies, it is likely that treatments will have to be designed for specific subgroups of patients rather than a general approach encompassing all patients as previously used with combination chemotherapy (e.g., Ara-C and anthracyclines for all patients with AML). This brings another challenge—how to more effectively combine and sequence different biological agents. Although, these challenges are significant, continued translation of laboratory findings into the clinic and clinical findings into the laboratory will eventually lead to the promised cure of leukemias.

References

Abu-Duhier, F. M., Goodeve, A. C., Wilson, G. A., Gari, M. A., Peake, I. R., Rees, D. C., Vandenberghe, E. A., Winship, P. R., and Reilly, J. T. (2000). FLT3 internal tandem duplication mutations in adult acute myeloid leukaemia define a high-risk group. *Br. J. Haematol.* **111**, 190–195.

Adams, J., Palombella, V. J., Sausville, E. A., Johnson, J., Destree, A., Lazarus, D. D., Maas, J., Pien, C. S., Prakash, S., and Elliott, P. J. (1999). Proteasome inhibitors: A novel class of potent and effective antitumor agents. *Cancer Res.* **59,** 2615–2622.

Adjei, A. A. (2001). Blocking oncogenic Ras signaling for cancer therapy. *J. Natl. Cancer. Inst.* **93,** 1062–1074.

Adjei, A. A., Erlichman, C., Davis, J. N., Cutler, D. L., Sloan, J. A., Marks, R. S., Hanson, L. J., Svingen, P. A., Atherton, P., Bishop, W. R., Kirschmeier, P., and Kaufmann, S. H. (2000). A Phase I trial of the farnesyl transferase inhibitor SCH66336: Evidence for biological and clinical activity. *Cancer Res.* **60,** 1871–1877.

Advani, R., Visani, G., Milligan, D., Saba, H., Tallman, M., Rowe, J. M., Wiernik, P. H., Ramek, J., Dugan, K., Lum, B., Villena, J., Davis, E., Paietta, E., Litchman, M., Covelli, A., Sikic, B., and Greenberg, P. (1999). Treatment of poor prognosis AML patients using PSC833 (valspodar) plus mitoxantrone, etoposide, and cytarabine (PSC-MEC). *Adv. Exp. Med. Biol.* **457,** 47–56.

Aguayo, A., Kantarjian, H., Manshouri, T., Gidel, C., Estey, E., Thomas, D., Koller, C., Estrov, Z., O'Brien, S., Keating, M., Freireich, E., and Albitar, M. (2000). Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. *Blood* **96,** 2240–2245.

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. D. (1994). "Molecular Biology of the Cell." Garland Publishing, New York.

Alessi, D. R., Cuenda, A., Cohen, P., Dudley, D. T., and Saltiel, A. R. (1995). PD 098059 is a specific inhibitor of the activation of mitogen-activated protein kinase *in vitro* and *in vivo*. *J. Biol. Chem.* **270,** 27489–27494.

Almond, J. B., and Cohen, G. M. (2002). The proteasome: A novel target for cancer chemotherapy. *Leukemia* **16,** 433–443.

Almond, J. B., Snowden, R. T., Hunter, A., Dinsdale, D., Cain, K., and Cohen, G. M. (2001). Proteasome inhibitor-induced apoptosis of B-chronic lymphocytic leukaemia cells involves cytochrome c release and caspase activation, accompanied by formation of an approximately 700 kDa Apaf-1 containing apoptosome complex. *Leukemia* **15,** 1388–1397.

Alvarado, Y., Tsimberidou, A., Kantarjian, H., Cortes, J., Garcia-Manero, G., Faderl, S., Thomas, D., Estey, E., and Giles, F. J. (2003). Pilot study of Mylotarg, idarubicin and cytarabine combination regimen in patients with primary resistant or relapsed acute myeloid leukemia. *Cancer Chemother. Pharmacol.* **51,** 87–90.

Amann, J. M., Nip, J., Strom, D. K., Lutterbach, B., Harada, H., Lenny, N., Downing, J. R., Meyers, S., and Hiebert, S. W. (2001). ETO, a target of t(8;21) in acute leukemia, makes distinct contacts with multiple histone deacetylases and binds mSin3A through its oligomerization domain. *Mol. Cell. Biol.* **21,** 6470–6483.

Andreeff, M., Milella, M., and Konopleva, M. (2002). Induction of apoptosis in AML by HA14-1, a small molecule Bcl-2 antagonist is independent of caspase-8 and -9. *Blood* **100,** 543a.

Apostolidou, E., Cortes, J., Tsimberidou, A., Estey, E., Kantarjian, H., and Giles, F. J. (2003a). Pilot study of gemtuzumab ozogamicin, liposomal daunorubicin, cytarabine and cyclosporine regimen in patients with refractory acute myelogenous leukemia. *Leuk. Res.* **27,** 887–891.

Apostolidou, E., Estey, E., Cortes, J., Garcia-Manero, G., Faderl, S., Thomas, D., Tsimberidou, A., Kantarjian, H., and Giles, F. J. (2003b). Mitoxantrone and prolonged infusion gemcitabine as salvage therapy in patients with acute myelogenous leukemia. *Leuk. Res.* **27,** 301–304.

Apperley, J. F., Gardembas, M., Melo, J. V., Russell-Jones, R., Bain, B. J., Baxter, E. J., Chase, A., Chessells, J. M., Colombat, M., Dearden, C. E., Dimitrijevic, S., Mahon, F. X., Marin, D., Nikolova, Z., Olavarria, E., Silberman, S., Schultheis, B., Cross, N. C., and Goldman, J. M. (2002). Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. *N. Engl. J. Med.* **347,** 481–487.

Ashar, H. R., James, L., Gray, K., Carr, D, Black, S., Armstrong, L., Bishop, W. R., and Kirschmeier, P. (2000). Farnesyltransferase inhibitors block the farnesylation of CNEP_E and CNEP_F and alter the association of CNEP_E with the microtubules. *J. Biol. Chem.* **275**(39), 30451–30457.

Au, W. Y., Fung, A., Man, C., Ma, S. K., Wan, T. S., Liang, R., and Kwong, Y. L. (2003). Aberrant p15 gene promoter methylation in therapy-related myelodysplastic syndrome and acute myeloid leukaemia clinicopathological and karyotypic associations. *Br. J. Haematol.* **120,** 1062–1065.

Baer, M. R., George, S. L., Dodge, R. K., O'Loughlin, K. L., Minderman, H., Caligiuri, M. A., Anastasi, J., Powell, B. L., Kolitz, J. E., Schiffer, C. A., Bloomfield, C. D., and Larson, R. A. (2002a). Phase 3 study of the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age and older with acute myeloid leukemia. Cancer and Leukemia Group B Study 9720. *Blood* **100,** 1224–1232.

Baer, M. R., Suvannasankha, A., O'Loughlin, K. L., Greco, W. R., and Minderman, H. (2002b). The pipecolinate derivatives VX-710 (biricodar; Incel) and VX-853 are effective modulators of drug efflux mediated by the multidrug resistance proteins P-glycoprotein, multidrug resistance protein and breast cancer resistance protein in acute myeloid leukemia. *Blood* **100,** 67a.

Bass, A. J., Gockerman, J. P., Hammett, E., DeCastro, C. M., Adams, D. J., Rosner, G. L., Payne, N., Davis, P., Foster, T., Moore, J. O., and Rizzieri, D. A. (2002). Phase I evaluation of prolonged-infusion gemcitabine with irinotecan for relapsed or refractory leukemia or lymphoma. *J. Clin. Oncol.* **20,** 2995–3000.

Baylin, S. B., Herman, J. G., Graff, J. R., Vertino, P. M., and Issa, J. P. (1998). Alterations in DNA methylation: A fundamental aspect of neoplasia. *Adv. Cancer Res.* **72,** 141–196.

Beaupre, D. M., and Kurzrock, R. (1999). RAS and leukemia: From basic mechanisms to gene-directed therapy. *J. Clin. Oncol.* **17,** 1071–1079.

Black, J. H., McCubrey, J. A., Willingham, M. C., Ramage, J., Hogge, D. E., and Frankel, A. E. (2003). Diphtheria toxin-interleukin-3 fusion protein (DT(388)IL3) prolongs disease-free survival of leukemic immunocompromised mice. *Leukemia* **17,** 155–159.

Blagosklonny, M. V., Fojo, T., Bhalla, K. N., Kim, J. S., Trepel, J. B., Figg, W. D., Rivera, Y., and Neckers, L. M. (2001). The Hsp90 inhibitor geldanamycin selectively sensitizes Bcr-Abl-expressing leukemia cells to cytotoxic chemotherapy. *Leukemia* **15,** 1537–1543.

Blume-Jensen, P., and Hunter, T. (2001). Oncogenic kinase signalling. *Nature* **411,** 355–365.

Boesch, D., Gaveriaux, C., Jachez, B., Pourtier-Manzanedo, A., Bollinger, P., and Loor, F. (1991). *In vivo* circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. *Cancer Res.* **51,** 4226–4233.

Breitschopf, K., Zeiher, A. M., and Dimmeler, S. (2000). Ubiquitin-mediated degradation of the proapoptotic active form of bid. A functional consequence on apoptosis induction. *J. Biol. Chem.* **275,** 21648–21652.

Caron, P. C., and Scheinberg, D. A. (1997). The biological therapy of acute and chronic leukemia. *Cancer Invest.* **15,** 342–352.

Caron, P. C., Co, M. S., Bull, M. K., Avdalovic, N. M., Queen, C., and Scheinberg, D. A. (1992). Biological and immunological features of humanized M195 (anti-CD33) monoclonal antibodies. *Cancer Res.* **52,** 6761–6767.

Caron, P. C., Jurcic, J. G., Scott, A. M., Finn, R. D., Divgi, C. R., Graham, M. C., Jureidini, I. M., Sgouros, G., Tyson, D., Old, L. J. *et al.* (1994a). A phase 1B trial of humanized monoclonal antibody M195 (anti-CD33) in myeloid leukemia: Specific targeting without immunogenicity. *Blood* **83,** 1760–1768.

Caron, P. C., Schwartz, M. A., Co, M. S., Queen, C., Finn, R. D., Graham, M. C., Divgi, C. R., Larson, S. M., and Scheinberg, D. A. (1994b). Murine and humanized constructs of monoclonal antibody M195 (anti-CD33) for the therapy of acute myelogenous leukemia. *Cancer* **73,** 1049–1056.

Chandra, J., Niemer, I., Gilbreath, J., Kliche, K. O., Andreeff, M., Freireich, E. J., Keating, M., and McConkey, D. J. (1998). Proteasome inhibitors induce apoptosis in glucocorticoid-resistant chronic lymphocytic leukemic lymphocytes. *Blood* **92,** 4220–4229.

Chang, Y. C., Lee, Y. S., Tejima, T., Tanaka, K., Omura, S., Heintz, N. H., Mitsui, Y., and Magae, J. (1998). mdm2 and bax, downstream mediators of the p53 response, are degraded by the ubiquitin-proteasome pathway. *Cell Growth Differ.* **9,** 79–84.

Ciechanover, A. (1994). The ubiquitin-proteasome proteolytic pathway. *Cell* **79,** 13–21.

Cools, J., DeAngelo, D. J., Gotlib, J., Stover, E. H., Legare, R. D., Cortes, J., Kutok, J., Clark, J., Galinsky, I., Griffin, J. D., Cross, N. C., Tefferi, A., Malone, J., Alam, R., Schrier, S. L., Schmid, J., Rose, M., Vandenberghe, P., Verhoef, G., Boogaerts, M., Wlodarska, I., Kantarjian, H., Marynen, P., Coutre, S. E., Stone, R., and Gilliland, D. G. (2003). A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N. Engl. J. Med.* **348,** 1201–1214.

Cortes, J., Albitar, M., Thomas, D., Giles, F., Kurzrock, R., Thibault, A., Rackoff, W., Koller, C., O'Brien, S., Garcia-Manero, G., Talpaz, M., and Kantarjian, H. (2003a). Efficacy of the farnesyl transferase inhibitor R115777 in chronic myeloid leukemia and other hematologic malignancies. *Blood* **101,** 1692–1697.

Cortes, J., Ault, P., Koller, C., Thomas, D., Ferraioli, A., Wierda, W., Rios, M. B., Letvak, L., Kaled, E. S., and Kantarjian, H. (2003b). Efficacy of imatinib mesylate in the treatment of idiopathic hypereosinophilic syndrome. *Blood* **101,** 4714–4716.

Cortes, J., Giles, F., O'Brien, S., Thomas, D., Albitar, M., Rios, M. B., Talpaz, M., Garcia-Manero, G., Faderl, S., Letvak, L., Salvado, A., and Kantarjian, H. (2003c). Results of imatinib mesylate therapy in patients with refractory or recurrent acute myeloid leukemia, high-risk myelodysplastic syndrome, and myeloproliferative disorders. *Cancer* **97,** 2760–2766.

Cortes, J., Kantarjian, H., Albitar, M., Thomas, D., Faderl, S., Koller, C., Garcia-Manero, G., Giles, F., Andreeff, M., O'Brien, S., Keating, M., and Estey, E. (2003d). A randomized trial of liposomal daunorubicin and cytarabine versus liposomal daunorubicin and topotecan with or without thalidomide as initial therapy for patients with poor prognosis acute myelogenous leukemia or myelodysplastic syndrome. *Cancer* **97,** 1234–1241.

Cortes, J., Tsimberidou, A. M., Alvarez, R., Thomas, D., Beran, M., Kantarjian, H., Estey, E., and Giles, F. J. (2002a). Mylotarg combined with topotecan and cytarabine in patients with refractry acute myelogenous leukemia. *Cancer Chemother. Pharmacol.* **50,** 497–500.

Cortes, J. E., Daley, G., Talpaz, M., O'Brien, S., Garcia-Manero, G., Giles, F., Faderl, S., Pate, O., Zaknoen, S., and Kantarjian, H. (2002b). Pilot study of SCH66336 (Lonafarnib), a farnesyl transferase inhibitor (FTI), in patients with chronic myeloid leukemia (CML) in chronic or accelerated phase resistant or refractory to imatinib. *Blood* **100,** 164a.

Cortes, J. E., Estey, E., Giles, F. J., O'Brien, S., Keating, M., McConkey, D., Wright, J., Schenkein, D., and Kantarjian, H. (2002c). Phase I study of Bortezomib (PS-341, VELCADE), a proteasome inhibitor, in patients with refractory or relapsed acute leukemias and myelodysplastic syndromes. *Blood* **100,** 560a.

Cortes, J. E., Gandhi, V., Plunkett, W., Keating, M., Faderl, S., O'Brien, S., Giles, F., Garcia-Manero, G., Thomas, D., Du, M., Davis, J., Craig, A., Estey, E., and Kantarjian, H. (2002d). Clofarabine (2-clloro-9-(2-deoxy-2-fluoro-*b*-D-arabinofuranosyl adenine) is active for patients with refractory or relapsed acute leukemias, myelodysplastic syndrome and chronic myeloid leukemia in blast phase. *Blood* **100,** 197a.

Cotter, F. E. (1999). Antisense therapy of hematologic malignancies. *Semin. Hematol.* **36,** 9–14.

Daskalakis, M., Nguyen, T. T., Nguyen, C., Guldberg, P., Kohler, G., Wijermans, P., Jones, P. A., and Lubbert, M. (2002). Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2′-deoxycytidine (decitabine) treatment. *Blood* **100,** 2957–2964.

De Angelo, D. J., Schiffer, C., Stone, R., Amrein, P., Fernandez, H., Bradstock, K., Tallman, M., Foran, J., Juliusson, G., Liu, D., Paul, C., Russo, D., Stenke, L., Leopold, L., Stevenson, D., Richie, M., and Berger, M. (2002). Interim analysis of a phase II study of the safety and efficacy of gemtuzumab ozogamicin (Mylotarg) given in combination with cytarabine and daunorubicin in patients less than 60 years old with untreated acute myeloid leukemia. *Blood* **100,** 198a.

De Lima, M., Ravandi, F., Shahjahan, M., Andersson, B., Couriel, D., Donato, M., Khouri, I., Gajewski, J., Van Besien, K., Champlin, R., Giralt, S., and Kantarjian, H. (2003). Long-term follow-up of a phase I study of high-dose decitabine, busulfan, and cyclophosphamide plus allogeneic transplantation for the treatment of patients with leukemias. *Cancer* **97,** 1242–1247.

Drexler, H. G. (1996). Expression of FLT3 receptor and response to FLT3 ligand by leukemic cells. *Leukemia* **10,** 588–599.

Dudley, D. T., Pang, L., Decker, S. J., Bridges, A. J., and Saltiel, A. R. (1995). A synthetic inhibitor of the mitogen-activated protein kinase cascade. *Proc. Natl. Acad. Sci. USA* **92,** 7686–7689.

Estey, E. H. (2002). New drugs for therapy of AML. *Leukemia* **16,** 306–309.

Estey, E. H., Thall, P. F., Giles, F. J., Wang, X. M., Cortes, J. E., Beran, M., Pierce, S. A., Thomas, D. A., and Kantarjian, H. M. (2002). Gemtuzumab ozogamicin with or without interleukin 11 in patients 65 years of age or older with untreated acute myeloid leukemia and high-risk myelodysplastic syndrome: Comparison with idarubicin plus continuous-infusion, high-dose cytosine arabinoside. *Blood* **99,** 4343–4349.

Faretta, M., Di Croce, L., and Pelicci, P. G. (2001). Effects of the acute myeloid leukemia—associated fusion proteins on nuclear architecture. *Semin. Hematol.* **38,** 42–53.

Favata, M. F., Horiuchi, K. Y., Manos, E. J., Daulerio, A. J., Stradley, D. A., Feeser, W. S., Van Dyk, D. E., Pitts, W. J., Earl, R. A., Hobbs, F., Copeland, R. A., Magolda, R. L., Scherle, P. A., and Trzaskos, J. M. (1998). Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J. Biol. Chem.* **273,** 18623–18632.

Feldman, E., Kalaycio, M., Weiner, G., Frankel, S., Schulman, P., Schwartzberg, L., Jurcic, J., Velez-Garcia, E., Seiter, K., Scheinberg, D., Levitt, D., and Wedel, N. (2003). Treatment of relapsed or refractory acute myeloid leukemia with humanized anti-CD33 monoclonal antibody HuM195. *Leukemia* **17,** 314–318.

Feldman, E., Stone, R., Brandwein, M. E., Kalaycio, M., Moore, J. O., Chopra, R., Jurcic, J., Miller, C. B., Roboz, D., Levitt, D., Young, D. C., and O'Connor, J. (2002). Phase III randomized trial of an anti-CD33 monoclonal antibody (HuM195) in combination with chemotherapy compared to chemotherapy alone in adults with refractory or first-relapse acute myeloid leukemia (AML). *ASCO* **21,** 261a.

Ferrara, F. F., Fazi, F., Bianchini, A., Padula, F., Gelmetti, V., Minucci, S., Mancini, M., Pelicci, P. G., Lo Coco, F., and Nervi, C. (2001). Histone deacetylase-targeted treatment restores retinoic acid signaling and differentiation in acute myeloid leukemia. *Cancer Res.* **61,** 2–7.

Foran, J., O'Farrell, A. M., Fiedler, W., Serve, H., Paquette, R., Cooper, M., Heinrich, M. C., Kim, H., Yuen, H. A., Louie, S. G., Bello, C., Aoun, P., Hong, W. M., Jacobs, M., Scigalla, P., Manning, W. C., Kelsey, S., and Cherrington, J. M. (2002a). An innovative single dose clinical study shows potent inhibition of FLT3 phosphorylation by SU11248 *in vivo*: A clinical and pharmacodynamic study in AML patients. *Blood* **100,** 559a.

Foran, J., Paquette, R., Cooper, M., Jachobs, M., O'Farrell, A. M., Kim, H., Cherrington, J., and Scigalla, P. (2002b). A phase I study of repeated oral dosing with SU11248 for the treatment of patients with acute myeloid leukemia who have failed or are not eligible for conventional chemotherapy. *Blood* **100,** 558a.

Frank, D. A. (1999). STAT signaling in the pathogenesis and treatment of cancer. *Mol. Med.* **5,** 432–456.

Frankel, A. E., McCubrey, J. A., Miller, M. S., Delatte, S., Ramage, J., Kiser, M., Kucera, G. L., Alexander, R. L., Beran, M., Tagge, E. P., Kreitman, R. J., and Hogge, D. E. (2000). Diphtheria toxin fused to human interleukin-3 is toxic to blasts from patients with myeloid leukemias. *Leukemia* **14,** 576–585.

Frankel, A. E., Powell, B. L., Hall, P. D., Case, L. D., and Kreitman, R. J. (2002). Phase I trial of a novel diphtheria toxin/granulocyte macrophage colony-stimulating factor fusion protein (DT388GMCSF) for refractory or relapsed acute myeloid leukemia. *Clin. Cancer Res.* **8,** 1004–1013.

Gabrilovich, D. I., Ishida, T., Nadaf, S., Ohm, J. E., and Carbone, D. P. (1999). Antibodies to vascular endothelial growth factor enhance the efficacy of cancer immunotherapy by improving endogenous dendritic cell function. *Clin. Cancer Res.* **5,** 2963–2970.

Galmarini, C. M., Mackey, J. R., and Dumontet, C. (2001). Nucleoside analogues: Mechanisms of drug resistance and reversal strategies. *Leukemia* **15,** 875–890.

Gandhi, V., Plunkett, W., Du, M., Ayres, M., and Estey, E. H. (2002). Prolonged infusion of gemcitabine: Clinical and pharmacodynamic studies during a phase I trial in relapsed acute myelogenous leukemia. *J. Clin. Oncol.* **20,** 665–673.

Gardner, R. C., Assinder, S. J., Christie, G., Mason, G. G., Markwell, R., Wadsworth, H., McLaughlin, M., King, R., Chabot-Fletcher, M. C., Breton, J. J., Allsop, D., and Rivett, A. J. (2000). Characterization of peptidyl boronic acid inhibitors of mammalian 20 S and 26 S proteasomes and their inhibition of proteasomes in cultured cells. *Biochem. J.* **346**(Pt. 2), 447–454.

Gewirtz, A. M. (1998). Antisense oligonucleotide therapeutics for human leukemia. *Curr. Opin. Hematol.* **5,** 59–71.

Gibbs, J. B. (1991). Ras C-terminal processing enzymes—new drug targets? *Cell* **65,** 1–4.

Giles, F. J. (2002). Novel agents for the therapy of acute leukemia. *Curr. Opin. Oncol.* **14,** 3–9.

Giles, F. J., Cortes, J. E., Baker, S. D., Thomas, D. A., O'Brien, S., Smith, T. L., Beran, M., Bivins, C., Jolivet, J., and Kantarjian, H. M. (2001a). Troxacitabine, a novel dioxolane nucleoside analog, has activity in patients with advanced leukemia. *J. Clin. Oncol.* **19,** 762–771.

Giles, F. J., Faderl, S., Thomas, D. A., Cortes, J. E., Garcia-Manero, G., Douer, D., Levine, A. M., Koller, C. A., Jeha, S. S., O'Brien, S. M., Estey, E. H., and Kantarjian, H. M. (2003a). Randomized phase I/II study of troxacitabine combined with cytarabine, idarubicin, or topotecan in patients with refractory myeloid leukemias. *J. Clin. Oncol.* **21,** 1050–1056.

Giles, F. J., Garcia-Manero, G., Cortes, J. E., Baker, S. D., Miller, C. B., O'Brien, S. M., Thomas, D. A., Andreeff, M., Bivins, C., Jolivet, J., and Kantarjian, H. M. (2002). Phase II study of troxacitabine, a novel dioxolane nucleoside analog, in patients with refractory leukemia. *J. Clin Oncol.* **20,** 656–664.

Giles, F. J., Kantarjian, H. M., Cortes, J. E., Garcia-Manero, G., Verstovsek, S., Faderl, S., Thomas, D. A., Ferrajoli, A., O'Brien, S., Wathen, J. K., Xiao, L. C., Berry, D. A., and Estey, E. H. (2003b). Adaptive randomized study of idarubicin and cytarabine versus troxacitabine and cytarabine versus troxacitabine and idarubicin in untreated patients 50 years or older with adverse karyotype acute myeloid leukemia. *J. Clin. Oncol.* **21,** 1722–1727.

Giles, F. J., Kantarjian, H. M., Kornblau, S. M., Thomas, D. A., Garcia-Manero, G., Waddelow, T. A., David, C. L., Phan, A. T., Colburn, D. E., Rashid, A., and Estey, E. H. (2001b). Mylotarg (gemtuzumab ozogamicin) therapy is associated with hepatic venoocclusive disease in patients who have not received stem cell transplantation. *Cancer* **92,** 406–413.

Giles, F. J., Stopeck, A. T., Silverman, L. R., Lancet, J. E., Cooper, M. A., Hannah, A. L., Cherrington, J. M., O'Farrell, A. M., Yuen, H. A., Louie, S. G., Hong, W., Cortes, J. E., Verstovsek, S., Albitar, M., O'Brien, S. M., Kantarjian, H. M., and Karp, J. E. (2003c). SU5416, a small molecule tyrosine kinase receptor inhibitor, has biologic activity in

patients with refractory acute myeloid leukemia or myelodysplastic syndromes. *Blood* **102**(3), 795–801.

Gilliland, D. G., and Griffin, J. D. (2002a). Role of FLT3 in leukemia. *Curr. Opin. Hematol.* **9,** 274–281.

Gilliland, D. G., and Griffin, J. D. (2002b). The roles of FLT3 in hematopoiesis and leukemia. *Blood* **100,** 1532–1542.

Golub, T. R., Barker, G. F., Lovett, M., and Gilliland, D. G. (1994). Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell* **77,** 307–316.

Gotlib, J., Loh, M., Vattikuti, S., Dugan, K., Quesada, S., Katamneni, U., Sridhar, K., Wright, J., Thibault, A., Rybak, M. E., Shannon, K., and Greenberg, P. (2002). Phase I/II study of Zarnestra (farnesyl transferase inhibitor, R115777, Tipifarnib) in patients with myeloproliferative disorders: preliminary results. *Blood* **100,** 798a.

Greenberg, P., Advani, R., and Tallman, M. (1999). Treatment of refractory/relapsed AML with PSC833 plus mitoxantrone, etoposide, cytarabine (PSC-MEC) vs. MEC: Randomized phase III trial (E2995). *Blood* **94,** 383a.

Grove, K. L., and Cheng, Y. C. (1996). Uptake and metabolism of the new anticancer compound beta-L-(-)-dioxoane-cytidine in human prostate carcinoma DU-145 cells. *Cancer Res.* **56,** 4187–4191.

Grove, K. L., Guo, X., Liu, S. H., Gao, Z., Chu, C. K., and Cheng, Y. C. (1995). Anticancer activity of beta-L-dioxolane-cytidine, a novel nucleoside analogue with the unnatural L configuration. *Cancer Res.* **55,** 3008–3011.

Hall, P. D., Willingham, M. C., Kreitman, R. J., and Frankel, A. E. (1999). DT388-GM-CSF, a novel fusion toxin consisting of a truncated diphtheria toxin fused to human granulocyte-macrophage colony-stimulating factor, prolongs host survival in a SCID mouse model of acute myeloid leukemia. *Leukemia* **13,** 629–633.

He, L. Z., Tolentino, T., Grayson, P., Zhong, S., Warrell, R. P., Jr., Rifkind, R. A., Marks, P. A., Richon, V. M., and Pandolfi, P. P. (2001). Histone deacetylase inhibitors induce remission in transgenic models of therapy-resistant acute promyelocytic leukemia. *J. Clin. Invest.* **108,** 1321–1330.

Heinrich, M. C., Druker, B. J., Curtin, P., Paquette, R., Sawyers, C. L., DeAngelo, D. J., Gilliland, D. G., Stone, R. M., Caligiuri, M. A., Byrd, J. C., Heaney, M. L., Nimer, S., Romanko, K. P., Lambing, J. L., Lokker, N. A., Giese, N. A., and Gretler, D. D. (2002). A "first in man" study of the safety and PK/PD of an oral FLT3 inhibitor (MLN518) in patients with AML or high risk myelodysplasia. *Blood* **100,** 336a.

Hideshima, T., Chauhan, D., Richardson, P., Mitsiades, C., Mitsiades, N., Hayashi, T., Munshi, N., Dang, L., Castro, A., Palombella, V., Adams, J., and Anderson, K. C. (2002). NF-kappa B as a therapeutic target in multiple myeloma. *J. Biol. Chem.* **277,** 16639–16647.

Hideshima, T., Richardson, P., Chauhan, D., Palombella, V. J., Elliott, P. J., Adams, J., and Anderson, K. C. (2001). The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. *Cancer Res.* **61,** 3071–3076.

Hiebert, S. W., Lutterbach, B., and Amann, J. (2001). Role of co-repressors in transcriptional repression mediated by the t(8;21), t(16;21), t(12;21), and inv(16) fusion proteins. *Curr. Opin. Hematol.* **8,** 197–200.

Hogge, D. E., Willman, C. L., Kreitman, R. J., Berger, M., Hall, P. D., Kopecky, K. J., McLain, C., Tagge, E. P., Eaves, C. J., and Frankel, A. E. (1998). Malignant progenitors from patients with acute myelogenous leukemia are sensitive to a diphtheria toxin-granulocyte-macrophage colony-stimulating factor fusion protein. *Blood* **92,** 589–595.

Hussong, J. W., Rodgers, G. M., and Shami, P. J. (2000). Evidence of increased angiogenesis in patients with acute myeloid leukemia. *Blood* **95,** 309–313.

Issa, J. P., Baylin, S. B., and Herman, J. G. (1997). DNA methylation changes in hematologic malignancies: Biologic and clinical implications. *Leukemia* **11**(Suppl. 1), S7–S11.

Issa, J. P., Garcia-Manero, G., Mannari, R., Thomas, D., Giles, F., Cortes, J., Estey, E., and Kantarjian, H. (2001). Minimal effective dose of the hypomethylating agent decitabine in hematopoietic malignancies. *Blood* **98,** 594a–595a.

James, G. L., Goldstein, J. L., Brown, M. S., Rawson, T. E., Somers, T. C., McDowell, R. S., Crowley, C. W., Lucas, B. K., Levinson, A. D., and Marsters, J. C., Jr. (1993). Benzodiazepine peptidomimetics: Potent inhibitors of Ras farnesylation in animal cells [see comments]. *Science* **260,** 1937–1942.

Jones, P. A., and Laird, P. W. (1999). Cancer epigenetics comes of age. *Nat. Genet.* **21,** 163–167.

Jurcic, J. G., DeBlasio, T., Dumont, L., Yao, T. J., and Scheinberg, D. A. (2000). Molecular remission induction with retinoic acid and anti-CD33 monoclonal antibody HuM195 in acute promyelocytic leukemia. *Clin Cancer Res.* **6,** 372–380.

Jurcic, J. G., Larson, S. M., Sgouros, G., McDevitt, M. R., Finn, R. D., Divgi, C. R., Ballangrud, A. M., Hamacher, K. A., Ma, D., Humm, J. L., Brechbiel, M. W., Molinet, R., and Scheinberg, D. A. (2002). Targeted alpha particle immunotherapy for myeloid leukemia. *Blood* **100,** 1233–1239.

Kang, C. D., Yoo, S. D., Hwang, B. W., Kim, K. W., Kim, D. W., Kim, C. M., Kim, S. H., and Chung, B. S. (2000). The inhibition of ERK/MAPK not the activation of JNK/SAPK is primarily required to induce apoptosis in chronic myelogenous leukemic K562 cells. *Leuk. Res.* **24,** 527–534.

Kantarjian, H. M., Gandhi, V., Cortes, J., Verstovsek, S., Du, M., Garcia-Manero, G., Giles, F., Faderl, S., O'Brien, S., Jeha, S., Davis, J., Shaked, Z., Craig, A., Keating, M., Plunkett, W., and Freireich, E. J. (2003a). Phase II clinical and pharmacology study of clofarabine in patients with refractory or relapsed acute leukemia. *Blood* **102,** 2379–2386.

Kantarjian, H. M., Gandhi, V., Kozuch, P., Faderl, S., Giles, F., Cortes, J., O'Brien, S., Ibrahim, N., Khuri, F., Du, M., Rios, M. B., Jeha, S., McLaughlin, P., Plunkett, W., and Keating, M. (2003b). Phase I clinical and pharmacology study of clofarabine in patients with solid and hematologic cancers. *J. Clin. Oncol.* **21,** 1167–1173.

Kantarjian, H. M., O'Brien, S. M., Estey, E., Giralt, S., Beran, M., Rios, M. B., Keating, M., de Vos, D., and Talpaz, M. (1997a). Decitabine studies in chronic and acute myelogenous leukemia. *Leukemia* **11**(Suppl. 1), S35–S36.

Kantarjian, H. M., O'Brien, S. M., Keating, M., Beran, M., Estey, E., Giralt, S., Kornblau, S., Rios, M. B., de Vos, D., and Talpaz, M. (1997b). Results of decitabine therapy in the accelerated and blastic phases of chronic myelogenous leukemia. *Leukemia* **11,** 1617–1620.

Karin, M., and Ben-Neriah, Y. (2000). Phosphorylation meets ubiquitination: The control of NF-[kappa]B activity. *Annu. Rev. Immunol.* **18,** 621–663.

Karp, J. E. (2001). Farnesyl protein transferase inhibitors as targeted therapies for hematologic malignancies. *Semin. Hematol.* **38,** 16–23.

Karp, J. E., Gojo, I., Gocke, C., Greer, J., Tidwell, M., Sarkodee-Adoo, C., Passaniti, A., Chen, H., and Zwiebel, J. A. (2002). Timed sequential therapy (TST) of relapsed and refractory adult acute myeloid leukemia (AML) with the anti-vascular endothelial growth factor (VEGF) monoclonal antibody Bevacizumab. *Blood* **100,** 198a.

Karp, J. E., Lancet, J. E., Kaufmann, S. H., End, D. W., Wright, J. J., Bol, K., Horak, I., Tidwell, M. L., Liesveld, J., Kottke, T. J., Ange, D., Buddharaju, L., Gojo, I., Highsmith, W. E., Belly, R. T., Hohl, R. J., Rybak, M. E., Thibault, A., and Rosenblatt, J. (2001). Clinical and biologic activity of the farnesyltransferase inhibitor R115777 in adults with refractory and relapsed acute leukemias: A phase 1 clinical-laboratory correlative trial. *Blood* **97,** 3361–3369.

Kell, J. W., Burnett, A. K., Chopra, R., Yin, J., Culligan, D., Clark, R., Hunter, A., Rohatiner, A., Milligan, D. W., Russell, N., and Prentice, A. (2002). Mylotarg (gemtuzumab

ozogamicin) given simultaneously with intensive induction and/or consolidation therapy for AML is feasible and may improve response rate. *Blood* **100,** 199a.

Kelly, L. M., Liu, Q., Kutok, J. L., Williams, I. R., Boulton, C. L., and Gilliland, D. G. (2002). FLT3 internal tandem duplication mutations associated with human acute myeloid leukemias induce myeloproliferative disease in a murine bone marrow transplant model. *Blood* **99,** 310–318.

Kim, S. C., Hahn, J. S., Min, Y. H., Yoo, N. C., Ko, Y. W., and Lee, W. J. (1999). Constitutive activation of extracellular signal-regulated kinase in human acute leukemias: Combined role of activation of MEK, hyperexpression of extracellular signal-regulated kinase, and downregulation of a phosphatase, PAC1. *Blood* **93,** 3893–3899.

Kindler, T., Breitenbuecher, F., Marx, A., Hess, G., Gschaidmeier, H., Gamm, H., Kirkpatrick, C. J., Huber, C., and Fischer, T. (2003). Sustained complete hematologic remission after administration of the tyrosine kinase inhibitor imatinib mesylate in a patient with refractory, secondary AML. *Blood* **101,** 2960–2962.

King, R. W., Deshaies, R. J., Peters, J. M., and Kirschner, M. W. (1996). How proteolysis drives the cell cycle. *Science* **274,** 1652–1659.

Kiyoi, H., Ohno, R., Ueda, R., Saito, H., and Naoe, T. (2002). Mechanism of constitutive activation of FLT3 with internal tandem duplication in the juxtamembrane domain. *Oncogene* **21,** 2555–2563.

Klisovic, M. I., Maghraby, E. A., Parthun, M. R., Guimond, M., Sklenar, A. R., Whitman, S. P., Chan, K. K., Murphy, T., Anon, J., Archer, K. J., Rush, L. J., Plass, C., Grever, M. R., Byrd, J. C., and Marcucci, G. (2003). Depsipeptide (FR 901228) promotes histone acetylation, gene transcription, apoptosis and its activity is enhanced by DNA methyltransferase inhibitors in AML1/ETO-positive leukemic cells. *Leukemia* **17,** 350–358.

Kohl, N. E., Mosser, S. D., deSolms, S. J., Giuliani, E. A., Pompliano, D. L., Graham, S. L., Smith, R. L., Scolnick, E. M., Oliff, A., and Gibbs, J. B. (1993). Selective inhibition of Ras-dependent transformation by a farnesyltransferase inhibitor [see comments]. *Science* **260,** 1934–1937.

Kornblith, A. B., Herndon, J. E., 2nd, Silverman, L. R., Demakos, E. P., Odchimar-Reissig, R., Holland, J. F., Powell, B. L., DeCastro, C., Ellerton, J., Larson, R. A., Schiffer, C. A., and Holland, J. C. (2002). Impact of azacytidine on the quality of life of patients with myelodysplastic syndrome treated in a randomized phase III trial: A Cancer and Leukemia Group B study. *J. Clin. Oncol.* **20,** 2441–2452.

Kottaridis, P. D., Gale, R. E., Frew, M. E., Harrison, G., Langabeer, S. E., Belton, A. A., Walker, H., Wheatley, K., Bowen, D. T., Burnett, A. K., Goldstone, A. H., and Linch, D. C. (2001). The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: Analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood* **98,** 1752–1759.

Kramer, O. H., Zhu, P., Ostendorff, H. P., Golebiewski, M., Tiefenbach, J., Peters, M. A., Brill, B., Groner, B., Bach, I., Heinzel, T., and Gottlicher, M. (2003). The histone deacetylase inhibitor valproic acid selectively induces proteasomal degradation of HDAC2. *EMBO J.* **22,** 3411–3420.

Kulkarni, S., Health, C., Parker, S., Chase, A., Iqbal, S., Pocock, C. F., Kaeda, J., Cwynarski, K., Goldman, J. M., and Cross, N. C. (2000). Fusion of H4/D10S170 to the platelet-derived growth factor receptor beta in BCR-ABL-negative myeloproliferative disorders with a t(5;10)(q33;q21). *Cancer Res.* **60,** 3592–3598.

Kurzrock, R., Sebti, S. M., Kantarjian, H. M., Wright, J., Cortes, J. E., Thomas, D. A., Wilson, E., Beran, M., Koller, C. A., O'Brien, S., Freireich, E. J., and Talpaz, M. (2001). Phase I study of a farnesyl transferase inhibitor, R115777, in patients with myelodysplastic syndrome. *Blood* **98,** 623a.

Laird, A. D., Vajkoczy, P., Shawver, L. K., Thurnher, A., Liang, C., Mohammadi, M., Schlessinger, J., Ullrich, A., Hubbard, S. R., Blake, R. A., Fong, T. A., Strawn, L. M., Sun, L., Tang, C., Hawtin, R., Tang, F., Shenoy, N., Hirth, K. P., McMahon, G., and Cherrington (2000). SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. *Cancer Res.* **60,** 4152–4160.

Lancet, J. E., Karp, J. E., Gotlib, J., Liesveld, J. L., Kaufmann, S. H., Gojo, I., Greenberg, P., Meschinchi, S., Adjei, A., Tidwell, M., Messina, P., Greer, J., Dugan, K., Bruzek, L., Radich, J. P., and Wright, J. (2002). Zarnestra (R115777) in previously untreated poor-risk AML and MDS: Preliminary results of a phase II trial. *Blood* **100,** 560a.

Lee, J. T., Jr., and McCubrey, J. A. (2002). The Raf/MEK/ERK signal transduction cascade as a target for chemotherapeutic intervention in leukemia. *Leukemia* **16,** 486–507.

Leith, C. P., Kopecky, K. J., Godwin, J., McConnell, T., Slovak, M. L., Chen, I. M., Head, D. R., Appelbaum, F. R., and Willman, C. L. (1997). Acute myeloid leukemia in the elderly: Assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. *Blood* **89,** 3323–3329.

Levis, M., Allebach, J., Tse, K. F., Zheng, R., Baldwin, B. R., Smith, B. D., Jones-Bolin, S., Ruggeri, B., Dionne, C., and Small, D. (2002). A FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukemia cells *in vitro* and *in vivo*. *Blood* **99,** 3885–3891.

Levis, M., Tse, K. F., Smith, B. D., Garrett, E., and Small, D. (2001). A FLT3 tyrosine kinase inhibitor is selectively cytotoxic to acute myeloid leukemia blasts harboring FLT3 internal tandem duplication mutations. *Blood* **98,** 885–887.

Li, B., and Dou, Q. P. (2000). Bax degradation by the ubiquitin/proteasome-dependent pathway: Involvement in tumor survival and progression. *Proc. Natl. Acad. Sci. USA* **97,** 3850–3855.

List, A. F., Kopecky, K. J., Willman, C. L., Head, D. R., Persons, D. L., Slovak, M. L., Dorr, R., Karanes, C., Hynes, H. E., Doroshow, J. H., Shurafa, M., and Appelbaum, F. R. (2001). Benefit of cyclosporine modulation of drug resistance in patients with poor-risk acute myeloid leukemia. A Southwest Oncology Group study. *Blood* **98,** 3212–3220.

Lubbert, M., Wijermans, P., Kunzmann, R., Verhoef, G., Bosly, A., Ravoet, C., Andre, M., and Ferrant, A. (2001). Cytogenetic responses in high-risk myelodysplastic syndrome following low-dose treatment with the DNA methylation inhibitor 5-aza-2′-deoxycytidine. *Br. J. Haematol.* **114,** 349–357.

Magnusson, M. K., Meade, K. E., Brown, K. E., Arthur, D. C., Krueger, L. A., Barrett, A. J., and Dunbar, C. E. (2001). Rabaptin-5 is a novel fusion partner to platelet-derived growth factor beta receptor in chronic myelomonocytic leukemia. *Blood* **98,** 2518–2525.

Magnusson, M. K., Meade, K. E., Nakamura, R., Barrett, J., and Dunbar, C. E. (2002). Activity of STI571 in chronic myelomonocytic leukemia with a platelet-derived growth factor beta receptor fusion oncogene. *Blood* **100,** 1088–1091.

Mansour, T. S., Jin, H., Wang, W., Hooker, E. U., Ashman, C., Cammack, N., Salomon, H., Belmonte, A. R., and Wainberg, M. A. (1995). Anti-human immunodeficiency virus and anti-hepatitis-B virus activities and toxicities of the enantiomers of 2′-deoxy-3′-oxa-4′-thiocytidine and their 5-fluoro analogues *in vitro*. *J. Med. Chem.* **38,** 1–4.

Marcucci, G., Bruner, R. J., Binkley, P. E., Xian, J., Chan, K. K., Parthun, M. R., Davis, M., Fischer, B., Shank, R., Moran, M., Byrd, J. C., and Grever, M. R. (2002). Phase I trial of the histone deacetylase inhibitor depsipeptide (FR901228) in acute myeloid leukemia (AMI). *Blood* **100,** 86a.

Marcucci, G., Byrd, J. C., Dai, G., Klisovic, M. J., Kourlas, P. J., Young, D. C., Cataland, S. R., Fisher, D. B., Lucas, D., Chan, K. K., Porcu, P., Lin, Z. P., Farag, S. F., Frankel, S. R., Zwiebel, J. A., Kraut, E. H., Balcerzak, S. P., Bloomfield, C. D., Grever, M. R., and Caligiuri, M. A. (2003). Phase 1 and pharmacodynamic studies of G3139, a Bcl-2 antisense oligonucleotide, in combination with chemotherapy in refractory or relapsed acute leukemia. *Blood* **101,** 425–432.

Marshansky, V., Wang, X., Bertrand, R., Luo, H., Duguid, W., Chinnadurai, G., Kanaan, N., Vu, M. D., and Wu, J. (2001). Proteasomes modulate balance among proapoptotic and antiapoptotic Bcl-2 family members and compromise functioning of the electron transport chain in leukemic cells. *J. Immunol.* **166,** 3130–3142.

Masdehors, P., Merle-Beral, H., Maloum, K., Omura, S., Magdelenat, H., and Delic, J. (2000). Deregulation of the ubiquitin system and p53 proteolysis modify the apoptotic response in B-CLL lymphocytes. *Blood* **96,** 269–274.

Masdehors, P., Omura, S., Merle-Beral, H., Mentz, F., Cosset, J. M., Dumont, J., Magdelenat, H., and Delic, J. (1999). Increased sensitivity of CLL-derived lymphocytes to apoptotic death activation by the proteasome-specific inhibitor lactacystin. *Br. J. Haematol.* **105,** 752–757.

Matthews, D. C., Appelbaum, F. R., Eary, J. E., Fisher, D. R., Durack, L. D., Hui, T. E., Martin, P. J., Mitchell, D., Press, O. W., Storb, R., and Bernstein, I. D. (1999). Phase I study of (131)I-anti-CD45 antibody plus cyclophosphamide and total body irradiation for advanced acute leukemia and myelodysplastic syndrome. *Blood* **94,** 1237–1247.

Matthews, W., Jordan, C. T., Wiegand, G. W., Pardoll, D., and Lemischka, I. R. (1991). A receptor tyrosine kinase specific to hematopoietic stem and progenitor cell-enriched populations. *Cell* **65,** 1143–1152.

McDevitt, M. R., Ma, D., Lai, L. T., Simon, J., Borchardt, P., Frank, R. K., Wu, K., Pellegrini, V., Curcio, M. J., Miederer, M., Bander, N. H., and Scheinberg, D. A. (2001). Tumor therapy with targeted atomic nanogenerators. *Science* **294,** 1537–1540.

Mesters, R. M., Padro, T., Bieker, R., Steins, M., Kreuter, M., Goner, M., Kelsey, S., Scigalla, P., Fiedler, W., Buchner, T., and Berdel, W. E. (2001). Stable remission after administration of the receptor tyrosine kinase inhibitor SU5416 in a patient with refractory acute myeloid leukemia. *Blood* **98,** 241–243.

Mitsiades, N., Mitsiades, C. S., Poulaki, V., Chauhan, D., Richardson, P. G., Hideshima, T., Munshi, N., Treon, S. P., and Anderson, K. C. (2002). Biologic sequelae of nuclear factor-kappaB blockade in multiple myeloma: Therapeutic applications. *Blood* **99,** 4079–4086.

Momparler, R. L., Bouchard, J., Onetto, N., and Rivard, G. E. (1984). 5-aza-2′-deoxycytidine therapy in patients with acute leukemia inhibits DNA methylation. *Leuk. Res.* **8,** 181–185.

Momparler, R. L., Rivard, G. E., and Gyger, M. (1985). Clinical trial on 5-aza-2′-deoxycytidine in patients with acute leukemia. *Pharmacol. Ther.* **30,** 277–286.

Morgan, M. A., Dolp, O., and Reuter, C. W. (2001). Cell-cycle-dependent activation of mitogen-activated protein kinase (MEK-1/2) in myeloid leukemia cell lines and induction of growth inhibition and apoptosis by inhibitors of RAS signaling. *Blood* **97,** 1823–1834.

Nooter, K., Sonneveld, P., Oostrum, R., Herweijer, H., Hagenbeek, T., and Valerio, D. (1990). Overexpression of the mdr1 gene in blast cells from patients with acute myelocytic leukemia is associated with decreased anthracycline accumulation that can be restored by cyclosporin-A. *Int. J. Cancer* **45,** 263–268.

Okuda, K., Matulonis, U., Salgia, R., Kanakura, Y., Druker, B., and Griffin, J. D. (1994). Factor independence of human myeloid leukemia cell lines is associated with increased phosphorylation of the proto-oncogene Raf-1. *Exp. Hematol.* **22,** 1111–1117.

Parker, W. B., Shaddix, S. C., Chang, C. H., White, E. L., Rose, L. M., Brockman, R. W., Shortnacy, A. T., Montgomery, J. A., Secrist, J. A., 3rd, and Bennett, L. L., Jr. (1991). Effects of 2-chloro-9-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl)adenine on K562 cellular metabolism and the inhibition of human ribonucleotide reductase and DNA polymerases by its 5′-triphosphate. *Cancer Res.* **51,** 2386–2394.

Perez-Manga, G., Lluch, A., Alba, E., Moreno-Nogueira, J. A., Palomero, M., Garcia-Conde, J., Khayat, D., and Rivelles, N. (2000). Gemcitabine in combination with doxorubicin in advanced breast cancer: Final results of a phase II pharmacokinetic trial. *J. Clin. Oncol.* **18,** 2545–2552.

Petti, M. C., Mandelli, F., Zagonel, V., De Gregoris, C., Merola, M. C., Latagliata, R., Gattei, V., Fazi, P., Monfardini, S., and Pinto, A. (1993). Pilot study of 5-aza-2′-deoxycytidine (Decitabine) in the treatment of poor prognosis acute myelogenous leukemia patients: Preliminary results. *Leukemia* **7**(Suppl. 1), 36–41.

Pinto, A., and Zagonel, V. (1993). 5-Aza-2′-deoxycytidine (Decitabine) and 5-azacytidine in the treatment of acute myeloid leukemias and myelodysplastic syndromes: Past, present and future trends. *Leukemia* **7**(Suppl. 1), 51–60.

Porter, A. C., and Vaillancourt, R. R. (1998). Tyrosine kinase receptor-activated signal transduction pathways which lead to oncogenesis. *Oncogene* **17,** 1343–1352.

Powis, G., Bonjouklian, R., Berggren, M. M., Gallegos, A., Abraham, R., Ashendel, C., Zalkow, L., Matter, W. F., Dodge, J., and Grindey, G. (1994). Wortmannin, a potent and selective inhibitor of phosphatidylinositol-3-kinase. *Cancer Res.* **54,** 2419–2423.

Propper, D. J., McDonald, A. C., Man, A., Thavasu, P., Balkwill, F., Braybrooke, J. P., Caponigro, F., Graf, P., Dutreix, C., Blackie, R., Kaye, S. B., Ganesan, T. S., Talbot, D. C., Harris, A. L., and Twelves, C. (2001). Phase I and pharmacokinetic study of PKC412, an inhibitor of protein kinase C. *J. Clin. Oncol.* **19,** 1485–1492.

Rajvanshi, P., Shulman, H. M., Sievers, E. L., and McDonald, G. B. (2002). Hepatic sinusoidal obstruction after gemtuzumab ozogamicin (Mylotarg) therapy. *Blood* **99,** 2310–2314.

Ratajczak, M. Z., Ratajczak, J., Machalinski, B., Majka, M., Marlicz, W., Carter, A., Pietrzkowski, Z., and Gewirtz, A. M. (1998). Role of vascular endothelial growth factor (VEGF) and placenta-derived growth factor (PIGF) in regulating human hematopoeitic cell growth. *Br. J. Haematol.* **103,** 969–979.

Ravandi, F., Kantarjian, H., Cohen, A., Davis, M., O'Brien, S., Anderlini, P., Andersson, B., Claxton, D., Donato, M., Gajewski, J., Khouri, I., Korbling, M., Ueno, N., deVos, D., Champlin, R., and Giralt, S. (2001). Decitabine with allogeneic peripheral blood stem cell transplantation in the therapy of leukemia relapse following a prior transplant: Results of a phase I study. *Bone Marrow Transplant.* **27,** 1221–1225.

Ravandi, F., Talpaz, M., and Estrov, Z. (2003). Modulation of cellular signaling pathways: Prospects for targeted therapy in hematological malignancies. *Clin. Cancer Res.* **9,** 535–550.

Ravoet, C., Mineur, P., Robin, V., Debusscher, L., Bosly, A., Andre, M., El Housni, H., Soree, A., and Martiat, P. (2002). Phase I-II study of a farnesyl transferase inhibitor (FTI), SCH66336, in patients with myelodysplastic syndrome (MDS) or secondary acute myeloid leukemia (sAML). *Blood* **100,** 794a.

Reed, J. C. (1997). Bcl-2 family proteins: Regulators of apoptosis and chemoresistance in hematologic malignancies. *Semin. Hematol.* **34,** 9–19.

Reed, J. C. (1999). Dysregulation of apoptosis in cancer. *J. Clin. Oncol.* **17,** 2941–2953.

Richel, D. J., Colly, L. P., Kluin-Nelemans, J. C., and Willemze, R. (1991). The antileukaemic activity of 5-Aza-2 deoxycytidine (Aza-dC) in patients with relapsed and resistant leukaemia. *Br. J. Cancer* **64,** 144–148.

Rivard, G. E., Momparler, R. L., Demers, J., Benoit, P., Raymond, R., Lin, K., and Momparler, L. F. (1981). Phase I study on 5-aza-2′-deoxycytidine in children with acute leukemia. *Leuk. Res.* **5,** 453–462.

Rizzieri, D. A., Bass, A. J., Rosner, G. L., Gockerman, J. P., DeCastro, C. M., Petros, W. P., Adams, D. J., Laughlin, M. J., Davis, P., Foster, T., Jacobson, R., Hurwitz, H., and Moore, J. O. (2002). Phase I evaluation of prolonged-infusion gemcitabine with mitoxantrone for relapsed or refractory acute leukemia. *J. Clin. Oncol.* **20,** 674–679.

Rizzieri, D. A., Ibom, V. K., Moore, J. O., DeCastro, C. M., Rosner, G. L., Adams, D. J., Foster, T., Payne, N., Thompson, M., Vredenburgh, J. J., Gasparetto, C., Long, G. D., Chao, N. J., and Gockerman, J. P. (2003). Phase I evaluation of prolonged-infusion gemcitabine with fludarabine for relapsed or refractory acute myelogenous leukemia. *Clin. Cancer Res.* **9,** 663–668.

Ross, T. S., Bernard, O. A., Berger, R., and Gilliland, D. G. (1998). Fusion of Huntingtin interacting protein 1 to platelet-derived growth factor beta receptor (PDGFbetaR) in chronic myelomonocytic leukemia with t(5;7)(q33;q11.2). *Blood* **91,** 4419–4426.

Santi, D. V., Garrett, C. E., and Barr, P. J. (1983). On the mechanism of inhibition of DNA-cytosine methyltransferases by cytosine analogs. *Cell* **33,** 9–10.

Santini, V., Kantarjian, H. M., and Issa, J. P. (2001). Changes in DNA methylation in neoplasia: Pathophysiology and therapeutic implications. *Ann. Intern. Med.* **134,** 573–586.

Santoro, A., Bredenfeld, H., Devizzi, L., Tesch, H., Bonfante, V., Viviani, S., Fiedler, F., Parra, H. S., Benoehr, C., Pacini, M., Bonadonna, G., and Diehl, V. (2000). Gemcitabine in the treatment of refractory Hodgkin's disease: Results of a multicenter phase II study. *J. Clin. Oncol.* **18,** 2615–2619.

Scheijen, B., and Griffin, J. D. (2002). Tyrosine kinase oncogenes in normal hematopoiesis and hematological disease. *Oncogene* **21,** 3314–3333.

Scheinberg, D. A., Tanimoto, M., McKenzie, S., Strife, A., Old, L. J., and Clarkson, B. D. (1989). Monoclonal antibody M195: A diagnostic marker for acute myelogenous leukemia. *Leukemia* **3,** 440–445.

Schwartsmann, G., Fernandes, M. S., Schaan, M. D., Moschen, M., Gerhardt, L. M., Di Leone, L., Loitzembauer, B., and Kalakun, L. (1997). Decitabine (5-aza-2′-deoxycytidine; DAC) plus daunorubicin as a first line treatment in patients with acute myeloid leukemia: Preliminary observations. *Leukemia* **11**(Suppl. 1), S28–31.

Schwartz, M. A., Lovett, D. R., Redner, A., Finn, R. D., Graham, M. C., Divgi, C. R., Dantis, L., Gee, T. S., Andreeff, M., Old, L. J. *et al.* (1993). Dose-escalation trial of M195 labeled with iodine 131 for cytoreduction and marrow ablation in relapsed or refractory myeloid leukemias. *J. Clin. Oncol.* **11,** 294–303.

Sebolt-Leopold, J. S. (2000). Development of anticancer drugs targeting the MAP kinase pathway. *Oncogene* **19,** 6594–6599.

Seynaeve, C. M., Kazanietz, M. G., Blumberg, P. M., Sausville, E. A., and Worland, P. J. (1994). Differential inhibition of protein kinase C isozymes by UCN-01, a staurosporine analogue. *Mol. Pharmacol.* **45,** 1207–1214.

Sherr, C. J. (1990). Colony-stimulating factor-1 receptor. *Blood* **75,** 1–12.

Shiotsu, Y., Neckers, L. M., Wortman, I., An, W. G., Schulte, T. W., Soga, S., Murakata, C., Tamaoki, T., and Akinaga, S. (2000). Novel oxime derivatives of radicicol induce erythroid differentiation associated with preferential G(1) phase accumulation against chronic myelogenous leukemia cells through destabilization of Bcr-Abl with Hsp90 complex. *Blood* **96,** 2284–2291.

Sievers, E. L., Appelbaum, F. R., Spielberger, R. T., Forman, S. J., Flowers, D., Smith, F. O., Shannon-Dorcy, K., Berger, M. S., and Bernstein, I. D. (1999). Selective ablation of acute myeloid leukemia using antibody-targeted chemotherapy: A phase I study of an anti-CD33 calicheamicin immunoconjugate. *Blood* **93,** 3678–3684.

Sievers, E. L., Larson, R. A., Stadtmauer, E. A., Estey, E., Lowenberg, B., Dombret, H., Karanes, C., Theobald, M., Bennett, J. M., Sherman, M. L., Berger, M. S., Eten, C. B., Loken, M. R., van Dongen, J. J., Bernstein, I. D., and Appelbaum, F. R. (2001). Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J. Clin. Oncol.* **19,** 3244–3254.

Sigalotti, L., Altomonte, M., Colizzi, F., Degan, M., Rupolo, M., Zagonel, V., Pinto, A., Gattei, V., Maio, M., Lubbert, M., Wijermans, P. W., Jones, P. A., and Hellstrom-Lindberg, E. (2003). 5 Aza-2′-deoxycytidine (decitabine) treatment of hematopoietic malignancies: A multimechanism therapeutic approach? *Blood* **101,** 4644–4646.

Silverman, L. R., Demakos, E. P., Peterson, B. L., Kornblith, A. B., Holland, J. C., Odchimar-Reissig, R., Stone, R. M., Nelson, D., Powell, B. L., DeCastro, C. M., Ellerton, J., Larson, R. A., Schiffer, C. A., and Holland, J. F. (2002). Randomized controlled trial of

azacitidine in patients with the myelodysplastic syndrome: A study of the cancer and leukemia group B. *J. Clin. Oncol.* **20,** 2429–2440.

Singal, R., and Ginder, G. D. (1999). DNA methylation. *Blood* **93,** 4059–4070.

Smith, B. D., Levis, M., Brown, P., Russell, L., Hellriegel, E., Dauses, T., Allebach, J., and Small, D. (2002). Single agent CEP-701, a novel FLT3 inhibitor, shows initial responses in patients with refractory acute myeloid leukemia. *Blood* **100,** 85a.

Soga, S., Neckers, L. M., Schulte, T. W., Shiotsu, Y., Akasaka, K., Narumi, H., Agatsuma, T., Ikuina, Y., Murakata, C., Tamaoki, T., and Akinaga, S. (1999). KF25706, a novel oxime derivative of radicicol, exhibits *in vivo* antitumor activity via selective depletion of Hsp90 binding signaling molecules. *Cancer Res.* **59,** 2931–2938.

Sonneveld, P. (2000). Multidrug resistance in haematological malignancies. *J. Intern. Med.* **247,** 521–534.

Steins, M., Padro, T., Bieker, R., Ruiz, S., Kropff, M., Kienast, J., Kessler, T., Buechner, T., Berdel, W. E., and Mesters, R. M. (2002). Efficacy and safety of thalidomide in patients with acute myeloid leukemia. *Blood* **99,** 834–839.

Stone, R., Klimek, V., DeAngelo, D. J., Nimer, S., Estey, E., Galinsky, I., Neuberg, D., Yap, A., Fox, E. A., Gilliland, D. G., and Griffin, J. D. (2002). PKC412, an oral FLT3 inhibitor, has activity in mutant FLT3 acute myeloid leukemia (AML): A phase II clinical trial. *Blood* **100,** 86a.

Tanimoto, M., Scheinberg, D. A., Cordon-Cardo, C., Huie, D., Clarkson, B. D., and Old, L. J. (1989). Restricted expression of an early myeloid and monocytic cell surface antigen defined by monoclonal antibody M195. *Leukemia* **3,** 339–348.

Teofili, L., Martini, M., Luongo, M., Diverio, D., Capelli, G., Breccia, M., Lo Coco, F., Leone, G., and Larocca, L. M. (2003). Hypermethylation of GpG islands in the promoter region of p15(INK4b) in acute promyelocytic leukemia represses p15(INK4b) expression and correlates with poor prognosis. *Leukemia* **17,** 919–924.

Thavasu, P., Propper, D., McDonald, A., Dobbs, N., Ganesan, T., Talbot, D., Braybrook, J., Caponigro, F., Hutchison, C., Twelves, C., Man, A., Fabbro, D., Harris, A., and Balkwill, F. (1999). The protein kinase C inhibitor CGP41251 suppresses cytokine release and extracellular signal-regulated kinase 2 expression in cancer patients. *Cancer Res.* **59,** 3980–3984.

Thiede, C., Steudel, C., Mohr, B., Schaich, M., Schakel, U., Platzbecker, U., Wermke, M., Bornhauser, M., Ritter, M., Neubauer, A., Ehninger, G., and Illmer, T. (2002). Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: Association with FAB subtypes and identification of subgroups with poor prognosis. *Blood* **99,** 4326–4335.

Tidefelt, U., Liliemark, J., Gruber, A., Liliemark, E., Sundman-Engberg, B., Juliusson, G., Stenke, L., Elmhorn-Rosenborg, A., Mollgard, L., Lehman, S., Xu, D., Covelli, A., Gustavsson, B., and Paul, C. (2000). P-Glycoprotein inhibitor valspodar (PSC 833) increases the intracellular concentrations of daunorubicin *in vivo* in patients with P-glycoprotein-positive acute myeloid leukemia. *J. Clin. Oncol.* **18,** 1837–1844.

Toyota, M., Kopecky, K. J., Toyota, M. O., Jair, K. W., Willman, C. L., and Issa, J. P. (2001). Methylation profiling in acute myeloid leukemia. *Blood* **97,** 2823–2829.

Tse, K. F., Mukherjee, G., and Small, D. (2000). Constitutive activation of FLT3 stimulates multiple intracellular signal transducers and results in transformation. *Leukemia* **14,** 1766–1776.

Tsimberidou, A., Cortes, J., Thomas, D., Garcia-Manero, G., Verstovsek, S., Faderl, S., Albitar, M., Kantarjian, H., Estey, E., and Giles, F. J. (2003a). Gemtuzumab ozogamicin, fludarabine, cytarabine and cyclosporine combination regimen in patients with CD33+ primary resistant or relapsed acute myeloid leukemia. *Leuk. Res.* **27,** 893–897.

Tsimberidou, A., Estey, E., Cortes, J., Thomas, D., Faderl, S., Verstovsek, S., Garcia-Manero, G., Keating, M., Albitar, M., O'Brien, S., Kantarjian, H., and Giles, F. (2003b).

Gemtuzumab, fludarabine, cytarabine, and cyclosporine in patients with newly diagnosed acute myelogenous leukemia or high-risk myelodysplastic syndromes. *Cancer* **97,** 1481–1487.

Ueda, K., Cardarelli, C., Gottesman, M. M., and Pastan, I. (1987). Expression of a full-length cDNA for the human "MDR1" gene confers resistance to colchicine, doxorubicin, and vinblastine. *Proc. Natl. Acad. Sci. USA* **84,** 3004–3008.

Villar-Garea, A., and Esteller, M. (2003). DNA demethylating agents and chromatin-remodelling drugs: Which, how and why? *Curr. Drug. Metab.* **4,** 11–31.

Vlahos, C. J., Matter, W. F., Hui, K. Y., and Brown, R. F. (1994). A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J. Biol. Chem.* **269,** 5241–5248.

Wadleigh, M., Richardson, P. G., Zahrieh, D., Lee, S. J., Cutler, C., Ho, V., Alyea, E. P., Antin, J. H., Stone, R. M., Soiffer, R. J., and DeAngelo, D. J. (2003). Prior gemtuzumab ozogamicin exposure significantly increases the risk of veno-occlusive disease in patients who undergo myeloablative allogeneic stem cell transplantation. *Blood* **102,** 1578–1582.

Warrell, R. P., Jr., He, L. Z., Richon, V., Calleja, E., and Pandolfi, P. P. (1998). Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase. *J. Natl. Cancer Inst.* **90,** 1621–1625.

Weiss, A., and Schlessinger, J. (1998). Switching signals on or off by receptor dimerization. *Cell* **94,** 277–280.

Weiss, F. U., Daub, H., and Ullrich, A. (1997). Novel mechanisms of RTK signal generation. *Curr. Opin. Genet. Develop.* **7,** 80–86.

Whitman, S. P., Archer, K. J., Feng, L., Baldus, C., Becknell, B., Carlson, B. D., Carroll, A. J., Mrozek, K., Vardiman, J. W., George, S. L., Kolitz, J. E., Larson, R. A., Bloomfield, C. D., and Caligiuri, M. A. (2001). Absence of the wild-type allele predicts poor prognosis in adult *de novo* acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: A cancer and leukemia group B study. *Cancer Res.* **61,** 7233–7239.

Wijermans, P., Lubbert, M., Verhoef, G., Bosly, A., Ravoet, C., Andre, M., and Ferrant, A. (2000). Low-dose 5-aza-2′-deoxycytidine, a DNA hypomethylating agent, for the treatment of high-risk myelodysplastic syndrome: A multicenter phase II study in elderly patients. *J. Clin. Oncol.* **18,** 956–962.

Wijermans, P. W., Krulder, J. W., Huijgens, P. C., and Neve, P. (1997). Continuous infusion of low-dose 5-aza-2′-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome. *Leukemia* **11**(Suppl. 1), S19–S23.

Willemze, R., Archimbaud, E., and Muus, P. (1993). Preliminary results with 5-aza-2′-deoxycytidine (DAC)-containing chemotherapy in patients with relapsed or refractory acute leukemia. The EORTC Leukemia Cooperative Group. *Leukemia* **7**(Suppl. 1), 49–50.

Willemze, R., Suciu, S., Archimbaud, E., Muus, P., Stryckmans, P., Louwagie, E. A., Berneman, Z., Tjean, M., Wijermans, P., Dohner, H., Jehn, U., Labar, B., Jaksic, B., Dardenne, M., and Zittoun, R. (1997). A randomized phase II study on the effects of 5-aza-2′-deoxycytidine combined with either amsacrine or idarubicin in patients with relapsed acute leukemia. An EORTC Leukemia Cooperative Group phase II study (06893). *Leukemia* **11**(Suppl. 1), S24–S27.

Winter, G., and Milstein, C. (1991). Man-made antibodies. *Nature* **349,** 293–299.

Yamamoto, Y., Kiyoi, H., Nakano, Y., Suzuki, R., Kodera, Y., Miyawaki, S., Asou, N., Kuriyama, K., Yagasaki, F., Shimazaki, C., Akiyama, H., Saito, K., Nishimura, M., Motoji, T., Shinagawa, K., Takeshita, A., Saito, H., Ueda, R., Ohno, R., and Naoe, T. (2001). Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood* **97,** 2434–2439.

Zagonel, V., Lo Re, G., Marotta, G., Babare, R., Sardeo, G., Gattei, V., De Angelis, V., Monfardini, S., and Pinto, A. (1993). 5-aza-2′-deoxycytidine (Decitabine) induces trilineage response in unfavourable myelodysplastic syndromes. *Leukemia* 7(Suppl. 1), 30–35.

Zinzani, P. L., Baliya, G., Magagnoli, M., Bendandi, M., Modugno, G., Gherlinzoni, F., Orcioni, G. F., Ascani, S., Simoni, R., Pileri, S. A., and Tura, S. (2000). Gemcitabine treatment in pretreated cutaneous T-cell lymphoma: experience in 44 patients. *J. Clin. Oncol.* **18,** 2603–2606.

Elihu Estey

Department of Leukemia
The University of Texas M. D. Anderson Cancer Center
Houston, Texas 77030

Methodologic Issues in Investigation of Targeted Therapies in Acute Myeloid Leukemia

I. Chapter Overview

The treatment of acute myeloid leukemia (AML) is undergoing change due to the introduction of various "targeted therapies." These are qualitatively different from prior investigational regimens in that they are less toxic but may also have less chance of producing responses as traditionally defined. This chapter considers methodologic issues relevant to investigation of targeted therapies.

II. Introduction

Throughout this section, targeted therapies (TTs) are defined as those likely associated with much lower rates of treatment-related mortality than more conventional chemotherapy (CT) typically used for AML. TTs are

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

frequently administered orally and do not require initial hospitalization. Thus, TTs have great intuitive appeal to both patients and physicians; in contrast, CT is currently viewed as a much less attractive option. This can be appreciated by contrasting the proportion of patients over the age of 60 years with untreated AML or high-risk myelodysplastic syndrome (MDS; >10% blasts in the bone marrow, >5% in the peripheral blood) who received CT, both on- and off-protocol, during the last 6 months of two CT protocols: June 1997–December 1997 vs. September 2002–March 2003. These proportions were 85% (of 55 patients) during the former and 75% (of 79 patients) during the latter. Despite a nonsignificant p-value (0.11), the 95% confidence limits for the true difference in these rates is [−0.03, 24], suggesting as much evidence for a true 20% difference as for no difference.

Leaving aside the significance of this trend for interpretation of results of CT studies, the trend makes this an appropriate time to explore certain issues relevant to investigation of TTs, specifically (1) selection of appropriate candidates for these therapies, (2) selection of endpoints to measure the success of these therapies, and (3) the need for comparative studies, both of various TTs with each other and of TT with CT. These topics, which are clearly interrelated, have received insufficient attention. This belief motivates this section.

III. Patient Selection for Targeted Therapy Trials in Acute Myeloid Leukemia

Not infrequently, protocols governing conduct of trials of TTs, at least in AML, allow enrollment of all patients who are not suitable candidates for myelosuppressive therapy; more specific eligibility criteria often permit enrollment of a wide variety of patients, both untreated and relapsed or refractory. The total sample size is however, typically, small. Although it is likely that results in one group are not totally irrelevant to results in another, statistical methods that allow "borrowing strength" across prognostic groups (Berry, 2003) are not yet in vogue. As a consequence, such protocols are often faced with the equally undesirable alternatives of considering all patients as comprising one group or considering them as several groups, results in each of which are surrounded by very wide confidence limits.

At the M. D. Anderson Cancer Center, given the sample size constraint noted previously, two considerations have dictated selection of which patients with AML are reasonable candidates for TT. The first is implicit, that is, the benefit:risk ratio associated with TT relative to the ratio associated with CT must plausibly be high. Second, and subject to the previous principle, the prime candidates for TTs should be untreated patients. This follows from the possibility that untreated AML is more likely to respond to

TTs than relapsed or refractory AML and from the desire to avoid falsely negative results with TT, recalling that the sample size limitations make it impractical to enter large numbers of both untreated and relapsed patients. It can be argued that, although the negative prognosis associated with prior CT is indisputable with CT (Estey *et al*., 1996), CT and TT are qualitatively different; accordingly, the same relation might not pertain with TT. I accede to this possibility (and to the point that allocating more untreated patients to TT means fewer remain to receive investigational CT), but believe that the burden of proof rests on those who believe, unconservatively, that results of TT in relapsed AML can be generalized to untreated AML.

Perhaps the ideal candidates for TT are patients in first complete remission (CR). There are three reasons. First, the AML is in the state of minimal residual disease (MRD), in which TTs are often judged to be particularly effective (although the difference between the number of cells present in this state and in the active disease state is far less than the comparable difference between cured and MRD states). Second, the AML has proven at least minimally responsive to CT (although the relationship between responsiveness to TT and to CT remains unknown, as noted previously). Third, with continued CT, the expected interval between CR and relapse is often very short, for example, 3–6 months in patients with chromosome 5 or 7 abnormalities, or both, or complex karyotypes, thus allowing results of TT to be estimated relatively quickly (Estey, 2000).

Nonetheless, in general, such patients have been ineligible for TT trials. (An exception is a trial of the farnesyl transferase inhibitor R115777.) Hence, patients with untreated disease are the most appropriate candidates for studies of TT in AML or high-risk MDS. Comparative benefit:risk ratios have led us to select patients aged 65 years or more or patients aged 60–64 years with abnormal karyotypes as those in whom TT could be plausibly substituted for CT as initial therapy. Any such groupings are arbitrary; however, with CTs used at the M. D. Anderson Cancer Center from 1997 to 2001, such patients ($n = 344$) had a CR rate of 43%, a 50-day mortality rate of 20% (the source paper details the selection of the 50 days), a median relapse-free survival (RFS) time of 35 weeks, and a probability of remaining alive in CR at 1 year of 36%. Thus, 15% of all the patients are predicted to be alive in CR at 1 year. Some patients under 60 years, for example, those with chromosome 5 or 7 abnormalities, or both, or complex karyotypes or whose blasts show homozygous FLT3 internal tandem duplications (ITDs), might have similar 15% 1-year rates, but are unlikely to incur the 20% 50-day mortality rates. The low level of risk at least previously associated with use of CT in these patients mitigates against employment of TTs.

Administration of TTs should not be limited to patients in whom the therapeutic target can be identified. To do so risks overestimating our knowledge of what these targets are. For example, imatinib mesylate, widely viewed as the exemplar par excellence of a targeted therapy, is now

known to inhibit tyrosine kinases (TKs) other than those resulting from the BCR/ABL rearrangement. As another example, at M.D. Anderson Cancer Center, PKC412, which presumably targets the TK arising consequent to FLT3 ITDs, has affected the circulating blast count in not only the two patients with these ITDs but also in three of six patients in whom neither FLT3 ITDs nor mutations have been detectable by conventional techniques (Estey *et al*., in press).

IV. Endpoints for Clinical Trials of TTs in AML

Two types of response to TTs should be distinguished. The first is one that indicates that the TT is not inert. An example is a fall in circulating blast count. Because this type of response can also be produced by agents such as hydroxyurea that are acknowledged to be of only cosmetic value, it is important to note the second type of response, that is, one that improves survival or improves quality of life. Although this type distinction appears elementary, experience suggests that the two types of responses are frequently confused when protocols are written. For example, the statistical section of a protocol investigating the antiangiogenesis agent SU5416 in AML stated that "because this agent has a unique mechanism of action, a response rate as low as 10% is of interest." Left unsaid was that in several groups eligible for the trial, namely patients who developed AML after a period of MDS or even untreated patients with abnormal karyotypes, the CR rate with CT has been 30–40%. Because of the simple inverse relation between the target response rate and the number of patients needed to effectively rule out such a rate, such a protocol could result in accrual of patients even after it was known to be highly unlikely that the proposed treatment would produce CR rates even as high as those seen with other available treatments. In their defense, the investigators (who included the author) point out that the CT-induced CRs would likely be transient and obtained only with considerable mortality or morbidity. Nonetheless, the stopping rule in the protocol was formally predicated only on response rate and not on these other outcomes.

Many trials of TT in AML focus on response broadly defined to include CR + major response (MR) [or hematologic improvement (HI)]. Thus, patients continue to be accrued unless it becomes likely that the CR + MR rate falls below a certain level, often taken as 20–30%, without any requirement for a minimum CR rate. This practice is based on the possibility that CR, as defined for CT, will occur infrequently with CT and on the hypothesis that MR will translate into improvement in either survival time or quality of life. A problem with this formulation is that, at least as applied to CT, this hypothesis appears incorrect. In particular, we examined whether the subtype of resistance affected survival among all 314 M. D. Anderson Cancer

Center patients declared resistant to CT between 1991 and 2001, with resistance defined as the complement of CR and induction death (Lopez *et al.*, 2001). The resistance date was taken as whichever came first among the date a second course of the initial CT was begun (with this second course not producing CR), the date alternative therapy was begun, or 50 days from the start of the initial course of chemotherapy. Patients with marrow CR ($<5\%$ blasts but $<100{,}000$ platelets/μl, or <1000 neutrophils/μl, at resistance date, or both) typically lived 2–4 months longer than patients without marrow CR ($>5\%$ blasts at resistance date), with this distinction reaching statistical significance. There was no indication that platelet count at resistance date, at least when grouped as $<50{,}000/\mu$l vs. 50,000–100,000/μl vs. $>100{,}000$ μl, or neutrophil count (grouped as $<500/\mu$l vs. 500–1000/μl vs. $>1000/\mu$l) affected survival time. The rare patients (6% of all resistant patients) with CRp, that is, $<5\%$ marrow blasts, >1000 neutrophils/μl, and $>50{,}000$ platelets/μl at resistance date, lived longer than those in the marrow CR group. Most strikingly, however, the difference in survival between the 946 patients who entered CR in one course during the same 1991–2001 period and the CR patients was much greater than the difference in survival between the CRp group and the other subtypes of resistant patients. This difference did not result from differences in time to achieve CR vs. time to resistance date. The results suggest that only CR lengthens survival in AML. Given that many CRs, because of their transiency, do not prolong survival, it is perhaps not surprising that responses short of CR do not in general increase survival. It can be contended that these results might not be relevant for TTs; specifically, these therapies might improve survival without producing CR. This hypothesis remains unproven. It follows that endpoints such as survival time or quality of life must be included in TT trials if these trials are to be plausibly seen as potentially benefiting patients, with stopping rules explicitly based on achieving minimal goals for these outcomes. The short survival time of untreated AML in patients over 59 years makes it feasible to monitor survival for purposes of early stopping.

V. Need for Randomized Trials of TTs and a Proposed Statistical Design

The great majority of TTs are tested in single-agent Phase II trials. A problem with this approach is that therapeutic trials in AML are inherently comparative (Estey and Thall, 2003). Thus, patients are fundamentally interested in whether a particular TT (TT1) is superior to another TT (TT2) and whether TT1, TT2, TT3, etc., are superior to CT. The comparative nature of Phase II trials is implicit in designs, such as the Simon two-stage design that governs conduct of such trials. For example, the Simon design specifies p_0 and p_1, the former corresponding to a response rate of no interest

and the latter to a minimum target response rate. If, after the initial stage of the trial, it appears that p_1 is unlikely to be achieved, accrual is terminated. Clearly, p_0 and p_1 can only be derived from historical data.

Use of single-arm Phase II trials to compare TTs with each other or with CT is complicated, however, given the limited sample size of Phase II trials in the face of the prognostic diversity of AML. In particular, the current trial might accrue more poor-prognosis patients than the previous trial did and vice versa; thus, the effects of treatment are confounded with the effects of prognosis. Previously, we have tried to address this problem by using multivariate regression to examine whether treatment (e.g., TT1 rather than TT2) affected outcome after accounting for prognostic covariates such as age or cytogenetics. The limitations of such an approach were recently demonstrated (Estey and Thall, 2003). A single-arm trial of fludarabine + Ara-C + idarubicin + G-CSF + all-*trans* retinoic acid (FAIGA) for treatment of AML/MDS was conducted at the M. D. Anderson Cancer Center (in 1995). Subsequently (from 1996 to 1998), the FAIGA combination was studied as one arm of a randomized Phase II trial. Because FAIGA was studied in two separate trials, the resulting data provide a basis for estimating the between-trial effect. We analyzed the data from the two trials by using a Bayesian survival time regression model accounting for the between-trial effect and also the effects of prognostic covariates, including performance status, type of cytogenetic abnormality, and whether the patient was treated in a laminar airflow room. Patients in the second trial had a much lower death rate. Such between-trial effects are due to the composite effects of latent (unobserved) variables and the play of chance. The salient point here is that, in separate trials of two different treatments A and B, the trial effect and the A vs. B treatment effect cannot be estimated separately, but rather are completely confounded by each other. Given the conventional widespread practice of conducting sequences of single-arm Phase II trials of different treatments and then comparing their results, it appears that many of the so-called treatment effects reported in the medical literature are nothing more than trial effects and that accounting for the effects of patient prognostic covariates cannot solve this problem. This example provides a strong motivation for randomizing in Phase II, because this is the only method that can effectively do away with treatment-trial effect confounding.

An example of how such randomization might be conducted is provided by a planned M. D. Anderson Cancer Center trial comparing TT and CT in patients more than 64 years old (or age 60–64 years but with abnormal cytogenetics) with untreated AML or high-risk MDS. A simple trial of TT vs. CT ignores the effect of subsequent treatment on survival; in common with many statistical designs, it thus wastes information. Thus, we decided to compare strategies as well as treatments; in particular, we compared the strategy of TT followed at failure by CT vs. the alternative strategy

of CT followed by TT. Response is defined as CR + partial remission (PR) + major HI, the latter as specified by National Cancer Institute (NCI) MDS criteria (Cheson *et al.*, 2000). To avoid the criticism raised that responses such as PR or HI are artificial constructs without proven benefits to patients, we monitor survival as well as response. Accounting for both response and death is accomplished by use of an objective function (a utility) that quantifies the clinically acceptable trade-off between these two outcomes, as specified by the physician based on prior experience. In this trial, we assign a utility of 0 to the average historical probabilities of response and death, (0.50, 0.20), with the utility 0 also given to the pairs (0, 0), (0.15, 0.10), and (0.60, 0.35), so that these pairs are considered equivalent. A utility of 1 denotes a desirable outcome and corresponds to the following, equally desirable, pairs of probabilities of response and death: (0.30, 0), (0.50, 0.10), (0.70, 0.30). A utility less than 0 corresponds to a situation worse than the historical. This utility function is used as the basis for the interim decisions and the final selection.

The trial is conducted as follows. Patients are randomized among CT (clofarabine + cytarabine), TT1 (PKC412 + low-dose cytarabine), TT2 (R115777 + low-dose cytarabine), and TT3 (decitabine). PKC421 inhibits the constitutively activated TK formed as a result of ITDs of the FLT3 gene, and R115777 is a farnesyl transferase inhibitor. The combination of PKC412 and of R155777 with chemotherapy bespeaks the likelihood that TT will be more effective when thus combined. On the other hand, the CT, that is, low-dose cytarabine, while unlikely to be effective per se, is also unlikely to produce the mortality rates associated with higher-dose regimens, for example, an anthracycline with standard-dose cytarabine given in a 3 + 7 fashion. Patients who fail CT are randomized among TT1, TT2, and TT3. Patients who fail TT1, TT2, or TT3 receive CT. The strategies TT1→TT2, TT1→TT3, TT2→TT1, TT2→TT3, TT3→TT1, and TT3→TT2 are disallowed in deference to the notion that CT is the standard therapy whereas TT is the experimental therapy. After a first stage of randomizing approximately 60 patients, inferior arms (CT, TT1, TT2, TT3) and strategies are dropped and another 60 patients randomized among the remaining arms and strategies. At the end of this second stage, the superior arms and strategies are selected for further investigation. The design allows for the possibility that the best arms or strategy might differ across prognostic groups as defined by cytogenetics (normal vs. abnormal) and age. Thall *et al.* (2002) give the operating characteristics (i.e., probabilities of correctly selecting superior arms/strategies). The design's ability to efficiently evaluate each of CT, TT1, TT2, and TT3 as initial therapy and as first salvage therapy and to evaluate the six two-course strategies (CT→TT1, CT→TT2, CT→TT3, TT1→CT, TT2→CT, and TT3→CT) follows from its statistical framework, which is based on a family of models that incorporates historical data, while accommodating multiple treatment courses, the

trinary outcome (response, failure, death) in each course, and prognostic covariates.

References

Berry, D. A. (2003). Statistical innovations in cancer research. *In* "Cancer Medicine" (D. W. Kufe, R. E. Pollock, R. R. Weischselbaum, R. C. Bast, T. S. Gansler, J. F. Holland, and E. Frei, III, Eds.), 6th ed., pp. 465–478. BC Decker, London.

Cheson, B. D., Bennett, J. M., Kantarjian, H., Pinto, A., Schiffer, C. A., Nimer, S. D., Löwenberg, B., Beran, M., de Witte, T., Stone, R. M., Mittelman, M., Sanz, G. F., Wijermans, P. W., Gore, S., and Greenberg, P. L. (2000). Report of an international working group to standardize response criteria for myelodysplastic syndromes. *Blood* **96,** 3671–3674.

Estey, E. (2000). How I treat older patients with AML. *Blood* **96,** 1670–1673.

Estey, E., Fischer, T., Giles, F., Feldman, E., Ehninger, G., Schiller, G., Klimek, V., Nimer, S., DeAngelo, D., Gilliland, G., Fox, E., Wang, Y. F., Rosamilia, M., Resta, D., Cohen, P., and Stone, R. (in press). A randomized phase II trial of the tyrosine kinase inhibitor PKC412 in patients with acute myeloid leukemia (AML)/high-risk myelodysplastic syndromes (MDS) characterized by wild-type (WT) or mutated FLT3 [abstract]. *Blood* **102**(11) 614a (abs).

Estey, E., Kornblau, S., Pierce, S., Kantarjian, H., Beran, M., and Keating, M. (1996). A stratification system for evaluating and selecting therapies in patients with relapsed or refractory AML [letter]. *Blood* **88,** 756.

Estey, E., and Thall, P. (2003). New designs for phase II clinical trials. *Blood* **102,** 442–448.

Lopez, G., Giles, F., Cortes, J., Pierce, S., O'Brien, S., Kantarjian, H., and Estey, E. (2001). Subtypes of resistant disease in patients with AML, RAEB-t, or RAEB who fail initial induction chemotherapy [abstract]. *Blood* **98,** 329a.

Thall, P., Sung, H.-G., and Estey, E. (2002). Selecting therapeutic strategies based on efficacy and death in multicourse clinical trials. *J. Am. Stat. Assoc.* **97,** 29–39.

Nicole Lamanna* and Mark Weiss†

*Instructor
Leukemia Service, Department of Medicine
Memorial Sloan-Kettering Cancer Center
New York, New York 10021

†Associate Attending
Leukemia Service, Department of Medicine
Memorial Sloan-Kettering Cancer Center
New York, New York 10021

Purine Analogs in Leukemia

I. Chapter Overview

The mechanism of action and the use of purine analogs in leukemia will be reviewed in this chapter.

II. Introduction

Three closely related purine analogs (fludarabine, cladribine, and pentostatin) were introduced in the early 1980s, and their role as important chemotherapeutic agents in hematologic malignancies was quickly established. Mechanistically, these agents inhibit DNA synthesis, which initiates drug-induced programmed cell death. These drugs were initially used as single agents in low-grade lymphoid malignancies. However, as more data emerge about other potential actions of this class of agents, they have been

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

shown to have activity in other settings, including myeloid leukemias and reduced-intensity allogeneic stem cell transplants. More recently, there has been growing clinical evidence regarding the synergy between purine analogs and other cytotoxic agents in the treatment of hematologic malignancies, leading to the development of several new treatment regimens.

III. Mechanisms of Action

Fludarabine, pentostatin, and cladribine are structurally similar analogs of adenosine, but differ in their interaction with the purine salvage enzyme adenosine deaminase (ADA). This enzyme normally regulates intracellular adenosine levels through irreversible deamination of adenosine to inosine and ultimately serves to degrade purine and deoxypurine nucleotides. Arabinosyladenine (Ara-A) can be viewed as the progenitor of purine analogs. Its rapid deamination limited its further development as a cancer therapeutic. However, the presence of a halogen atom on the nucleobase produces a congener of Ara-A that is resistant to deamination (Montgomery and Hewson, 1957). Montgomery synthesized fludarabine phosphate [2-fluoro, 5′-phosphate derivative of 9-β-D-arabinofuranosyl adenine (Ara-A)], which was more water soluble and resistant to deamination than was Ara-A. The active metabolite of fludarabine, F-Ara-A (9-β-D-arabinofuranosyl-2-fluoroadenine) triphosphate, is incorporated into elongating nucleic acid chains, resulting in the termination of DNA or RNA synthesis (Huang *et al.*, 1990; Seto *et al.*, 1985). It also inhibits several intracellular enzymes, including DNA and RNA polymerases, DNA primase, DNA ligase, and ribonucleotide reductase, and potentiates deoxycytidine kinase activity (Adkins *et al.*, 1997; Plunkett *et al.*, 1990). As a result of these actions, there is a lowering of cellular deoxynucleotide pools that are normally maintained by ribonucleotide reductase and thus a change in the ratio of F-Ara-ATP to dATP, resulting in the self-potentiation of the DNA-synthesis-directed actions of fludarabine (Gandhi and Plunkett, 2002; Seymour *et al.*, 1996). The exact mechanism by which fludarabine-induced programmed cell death or apoptosis occurs is not fully established, although it is believed that the incorporation of this agent into DNA might play a role (Robertson *et al.*, 1993). Alternative mechanisms including the depletion of adenosine, including ATP and NAD, might lead to a devitalized state and might also be involved. Fludarabine is extensively bound to body tissues and is eliminated primarily by the kidneys. After termination of a fludarabine infusion, the peak intracellular level of F-Ara-A after it has been phosphorylated to its active metabolite occurs within 3–4 h, with a median half-life of approximately 23 h (Gutheil and Kearns, 1997). Fludarabine is available

orally, with a bioavailability of approximately 50% that is unaffected by food.

Another halogenated congener, cladribine (2-chlorodeoxyadenosine) was soon thereafter synthesized by Carson. Cladribine is resistant to deamination of adenosine deaminase. It is also a prodrug and its intracellular phosphorylation by deoxycytidine kinase is necessary for cytostatic effect to occur. It accumulates as chlorodeoxyadenosine triphosphate (2-CdA ATP), and this metabolite disrupts cell metabolism by incorporating into the DNA of actively dividing cells, including DNA single-strand breaks, and inhibiting DNA synthesis (Griffig *et al*., 1989; Hirota *et al*., 1989). It also works by inhibiting ribonucleotide reductase, resulting in disturbances of intracellular deoxynucleotide triphosphate pools and interference with the formation of NAD. Cladribine also induces apoptosis, but laboratory studies suggest that it can induce cell death by direct mitochondrial injury as well (Genini *et al*., 2000). Cladribine is approximately 25% protein bound. The oral bioavailability is approximately 50% of the administered dose. It is 100% bioavailable after subcutaneous administration. Cladribine also enters the cerebral spinal fluid with concentrations reaching approximately 12–38% of the concurrent plasma concentrations during continuous intravenous infusions. Renal elimination of cladribine accounts for approximately 50% of the dose, and it has a linear biphasic pharmacokinetic profile with an α half-life of approximately 3–35 min and a β half-life of approximately 6–20 h (Gutheil and Kearns, 1997).

Unlike fludarabine and cladribine, pentostatin (deoxycoformycin) is an irreversible inhibitor of adenosine deaminase. As a result of the high ratio of the phosphorylating enzyme deoxycytidine kinase (dCK) to the dephosphorylating enzyme 5′-nucleotidase in lymphocytes, adenosine and deoxyadenosine are converted to triphosphate metabolites. Accumulation of these metabolites inhibits ribonucleotide reductase, which in turn inhibits DNA synthesis (Grever *et al*., 1981). Cells most susceptible to the effects of ADA inhibition are those with a relatively high dCK ratio, such as T lymphocytes. Again, the exact mechanism by which cytotoxicity occurs is not entirely clear and might also include incorporation of triphosphate derivatives of deoxycoformycin into DNA (Siaw and Coleman, 1984) and interference with NAD formation (Carson *et al*., 1983). Congenital adenosine deaminase deficiency has been identified as one of the causes of severe combined immunodeficiency (SCID), a disease characterized by severe T-lymphocyte deficiency and impaired B-lymphocyte function. Pentostatin is minimally protein bound and, like cladribine, displays a biphasic pharmacokinetic profile. The α half-life is approximately 10 min, followed by a more prolonged β half-life of approximately 5–6 h (Gutheil and Kearns, 1997). Pentostatin is mainly renally eliminated, and thus dose reduction is necessary in patients with renal dysfunction.

IV. Purine Analogs in Chronic Lymphocytic Leukemia

Purine analogs have been most extensively studied in chronic lymphocytic leukemia (CLL). The first report of these agents having activity in CLL were the descriptions of Grever and colleagues that pentostatin (Grever *et al.*, 1985) and fludarabine (Grever *et al.*, 1988) induced responses in heavily pretreated patients with CLL. Keating *et al.* (1989), in a seminal series of papers, confirmed the utility of fludarabine in previously treated patients, and, in subsequent work, reported that in previously untreated patients fludarabine induced complete remissions (CRs) in 33%, a finding that was unheard of with alkylating-agent-based treatments. To build on the success of single-agent fludarabine, a few groups in the early 1990s treated patients with fludarabine in combination with other active agents. The group at the M. D. Anderson Cancer Center combined fludarabine 30 mg/m^2/day with prednisone 30 mg/m^2/day orally for 5 days and repeated cycles every 28 days. The patients were a mixed population consisting of both previously untreated and treated patients. An overall response rate of 60% was obtained (Robertson *et al.*, 1992). Despite early enthusiasm for the high frequency of response, subsequent reports of severe opportunistic infections, including *Pneumocystis carinii* pneumonia and *Listeria meningitis*, led to the abandonment of this combination (Anaissie *et al.*, 1992). A more recent analysis (nonrandomized) revealed that patients receiving single-agent fludarabine as initial therapy had comparable or superior survival (Keating *et al.*, 1988) compared to patients receiving fludarabine and prednisone as initial therapy. In the early 1990s, two other groups investigated fludarabine in combination. Both the Southwest Oncology Group (SWOG) (Elias *et al.*, 1993) and Memorial Sloan-Kettering Cancer Center (MSKCC) (Weiss *et al.*, 1994) trials investigated combinations of fludarabine and chlorambucil. Both trials had similar results. Because of overlapping synergistic toxicities, the initial dose level of these Phase I trials were too toxic to administer, and the dose intensity of chlorambucil was reduced by 50%. Even with this dose reduction, the overlapping myelosuppression and immunosuppression required compromised dosing of both agents, with generally increased toxicity and no obvious benefit in terms of response. The results of these three early combination trials led to abandoning of combination therapy with prednisone or chlorambucil.

In previously untreated patients, single-agent fludarabine induced responses in a majority of patients and CRs in a significant minority (Keating *et al.*, 1991). These results led to the initiation of a large randomized intergroup trial (Rai *et al.*, 1996). A total of 509 patients were randomized between 1990 and 1994 to receive fludarabine, chlorambucil, or combination chemotherapy with fludarabine and chlorambucil. Treatments were administered every 4 weeks for up to 12 months. The frequency

of response in the fludarabine arm was 63% [20% CR, 43% PR (partial response)], which was significantly better than the response rate of 37% in the chlorambucil arm (4% CR, 33% PR). Median response duration was superior for patients in the fludarabine group at 25 months compared with those in the chlorambucil group at 14 months. Despite the improvement in frequency and duration of response, patients initially randomized to fludarabine did not have improved survival compared with those patients receiving chlorambucil as initial therapy. Another important finding was confirmation of the results reported by Elias and Weiss: the combined fludarabine and chlorambucil arm was closed early as too toxic to administer even to previously untreated patients. Similar results have been obtained in other studies that also demonstrate that fludarabine is more active than alkylator-based therapy, but despite improvement in response frequency and duration, there is no benefit in terms of overall survival (Rai *et al.*, 2000).

The failure of early attempts to combine fludarabine with chlorambucil is thought to be because of overlapping synergistic myelosuppression and immunosuppression. In an attempt to maximize therapy with fludarabine and alkylators, we at MSKCC have studied a sequential treatment approach (Weiss *et al.*, 2000). Patients received fludarabine (25 mg/m^2/day for 5 days every 4 weeks for six cycles) as induction. Subsequently, patients received consolidation with high-dose cyclophosphamide (3000 mg/m^2 every 3 weeks for three cycles). Such consolidation improved the quality of response in 48% of patients and substantially increased the frequency of CR from 8% to 32%. Other groups have chosen to combine fludarabine with cyclophosphamide in a concomitant fashion. The best known of these studies reported by O'Brien *et al.* (2001) details the results of combination fludarabine and cyclophosphamide in 128 patients. The doses initially chosen (fludarabine 30 mg/m^2/day and cyclophosphamide 500 mg/m^2/day both administered on three consecutive days) proved too toxic to administer. The cyclophosphamide dose was then decreased initially to 350 mg/m^2/day but, as this was still too toxic, a final dose reduction of cyclophosphamide to 300 mg/m^2/day was chosen for further study. Patients were subdivided into four groups: (1) previously untreated, (2) previously treated with alkylating agents, (3) previously treated with alkylating agents and fludarabine but relapsing, and (4) refractory to fludarabine with or without alkylating agents. This combination of fludarabine and cyclophosphamide produced responses in 88% of previously untreated patients and in 82% of previously treated fludarabine (naïve or) sensitive patients. In patients known to be refractory to fludarabine, responses were seen in 39%. The main complication of therapy was related to myelosuppression with neutropenia in 48% of patients, even at the reduced cyclophosphamide dose of 300 mg/m^2. Further cyclophosphamide dose reductions (to 250 mg/m^2/day) were required in 29% of patients. Documented pneumonia or sepsis occurred in 25% of patients, and fever of unknown origin occurred in another 25%. Subsequently, the group

at M. D. Anderson Cancer Center has added rituximab to this regimen, with preliminary reports indicating that it is well tolerated and very active. Other groups have also reported the significant activity of the fludarabine and cyclophosphamide combination with modifications of dose and schedule (Flinn *et al*., 2000; Hallek *et al*., 2001).

Most recently, we have further modified the sequential approach studied at MSKCC include a third treatment with rituximab (Lamanna *et al*., 2003). In this study, patients receive induction therapy with fludarabine 25 mg/m^2 for 5 days every 4 weeks for six cycles. This is followed by consolidation with high-dose cyclophosphamide (3000 mg/m^2 every 3 weeks for three cycles) and then by a second consolidation with rituximab (375 mg/m^2 weekly for 4 weeks). Our preliminary results confirm that this regimen has acceptable toxicity and that each phase of consolidation therapy leads to an improvement in response.

Several investigators have also looked at combination therapy with fludarabine and rituximab (Byrd *et al*., 2003; Czuczman *et al*., 2002). In the study by Byrd *et al*. (2003), patients with previously untreated CLL were randomized to receive either six monthly courses of fludarabine (25 mg/m^2 on Days 1–5) concurrently with rituximab (375 mg/m^2 on Days 1 and 4 of Cycle 1, and then on Day 1 of Cycles 2–6), followed 2 months later by four weekly doses of rituximab (375 mg/m^2) for consolidation therapy or sequential fludarabine alone (25 mg/m^2 on Days 1–5) followed 2 months later by four weekly doses of rituximab (375 mg/m^2). A total of 104 patients were randomized to the concurrent ($n = 51$) and sequential regimens ($n = 53$). Overall response rate with the concurrent regimen was 90% (47% CR, 43% PR) compared with 77% (28% CR, 49% PR) with the sequential regimen. However, patients in the concurrent regimen received a substantially higher dose of rituximab (11 doses) than those in the sequential arm (4 doses). This imbalance in the treatment between the two arms confounds the analysis as to whether concurrent or sequential therapy is preferred.

Although fludarabine is the most extensively tested purine analog in CLL, both cladribine and pentostatin have also been studied in combination therapy. A prospective randomized trial of cladribine and prednisone compared with chlorambucil and prednisone in previously untreated patients with CLL was performed at nine centers in Poland between 1995 and 1998 (Robak *et al*., 2000). The cladribine group achieved both a superior frequency of response (87% vs. 57%) and a complete response (47% vs. 12%) compared with the chlorambucil group. This was offset by increased toxicity in the cladribine arm, and ultimately survival was comparable for the two groups (78% vs. 82% at 2 years). Cladribine has also been combined with cyclophosphamide in a study of 29 patients with refractory or recurrent CLL/PLL (Montillo *et al*., 2003). This combination had activity but significant toxicity, and the authors concluded that this regimen was inferior to fludarabine-containing combinations.

Given our prior experience that fludarabine combinations are limited by severe combined myelosuppression and immunosuppression, we have chosen to study combination therapy with pentostatin, the least myelosuppressive of these purine analogs. We hypothesized that combining pentostatin with cyclophosphamide would result in less myelosuppression than would combinations incorporating either fludarabine or cladribine and that the improved therapeutic index would permit us to exploit the antileukemic effect of this synergistic combination. Initially, we studied combination pentostatin (4 mg/m^2) and cyclophosphamide (600 mg/m^2) in heavily pretreated patients with CLL (Weiss *et al.*, 2003b). This regimen was quite active (74% overall response, 17% CR) and had relatively little myelosuppression. Subsequently, we have added rituximab to this combination regimen and again noted a highly active, well-tolerated regimen (Weiss *et al.*, 2003a). The three-drug PCR regimen combines pentostatin (4 mg/m^2), cyclophosphamide (600 mg/m^2), and rituximab (375 mg/m^2). For Cycle 1, patients received only pentostatin and cyclophosphamide. For Cycles 2–6, all three drugs were administered on Day 1. Cycles were repeated every 3 weeks for six treatments. Supportive measures included hydration with each treatment (and monitoring of renal function) and prophylactic administration of filgrastim, sulfamethoxazole/trimethoprim, acyclovir, and antiemetics.

Another purine analog that might show some promise in the treatment of indolent as well as other leukemias is nelarabine, an Ara-G prodrug. Ara-G (9-β-D-arabinosylguanine) was synthesized in 1964 (Reist and Goodman, 1964); however, its low solubility and difficulty in synthesis did not allow for further clinical development until much later. Nelarabine is a water-soluble prodrug of Ara-G (2-amino-6-methoxypurine arabinoside) synthesized enzymatically from diaminopurine arabinoside. Demethoxylation of nelarabine by adenosine deaminase converts it to biologically active Ara-G. (Krenitsky *et al.*, 1981; Lambe *et al.*, 1995). Cohen *et al.* (1983) reported that the nucleobase arabinosyl guanine was resistant to cleavage by purine nucleoside phosphorylase and was toxic to T lymphocytes. Further data indicate that accumulation of arabinosyl GTP in leukemic blasts has been associated with cytotoxic activity against malignant cells (Kurtzberg *et al.*, 1999). Phase I studies by Kurtzberg *et al.* (1999) in both pediatric and adult patients with refractory hematological malignancies confirmed the high rate of response in patients with T-cell malignancies. In this study, 54% of patients with T-cell acute lymphoblastic leukemia (ALL) achieved a complete or partial response after one or two courses. The maximum tolerated dose was defined at 60 mg/kg/day for 5 days in children and 40 mg/kg/day for 5 days in adults. Relatively little myelosuppression was observed with nelarabine, and this prompted combination with other active nucleoside analogs such as fludarabine. Gandhi *et al.* (2001) reported a combination study with nelarabine and fludarabine in 13 patients (7 with CLL, 2 with PLL, 2 with T-ALL, 1 with chronic myeloid leukemia (CML), 1 with mycosis

fungoides). Nelarabine (1.2 g/m^2) was infused on Days 1, 3, and 5, and fludarabine (30 mg/m^2) was given on Days 3 and 5 prior to nelarabine infusion. Seven patients had a partial or complete response, six of whom had indolent leukemias. Four of the responders had failed prior fludarabine therapy. One of the two patients with T-ALL had a complete response. There was modest grade 3 and 4 hematologic toxicity in 31% and 13%, respectively. Grade 3 and 4 nonhematologic toxicity consisted of muscle weakness in two patients. These findings suggest that nelarabine has activity in indolent and T-cell leukemias and warrants further investigation.

V. Purine Analogs in Hairy Cell Leukemia

One of the great ironies of medical oncology is that the biggest advances are made in the most uncommon diseases. Hairy cell leukemia is the paradigm, and three drugs (interferon, pentostatin, and cladribine) have received their initial Food and Drug Administration (FDA) approval for this rare disease. The introduction of interferon-α in 1984 altered the management of hairy cell leukemia with a high overall response rate, though few patients achieved a complete response. The purine analogs (pentostatin and cladribine, but not fludarabine), however, are much more active, with complete responses noted in the majority of patients. In a Phase III intergroup study sponsored by the National Cancer Institute (NCI), pentostatin produced significantly more complete responses than did interferon-β (76% vs. 11%) (Grever *et al.*, 1995). Long-term follow-up of this study reveals that remarkably only 2 patients (1%) of the 241 who received pentostatin died because of hairy cell leukemia (Flinn *et al.*, 2000).

Cladribine was next introduced to treat hairy cell leukemia, and, most remarkably, often produces complete responses after only a single cycle (Piro *et al.*, 1990). In addition, relapse rates with cladribine also appear to be infrequent and in the same range as those for pentostatin (Hoffman *et al.*, 1997; Seymour *et al.*, 1994; Tallman *et al.*, 1996). In one long-term follow-up study of 358 patients treated with a single course of cladribine, 26% of the 349 evaluated patients had relapsed at a median of 29 months. Although a randomized comparison between cladribine and pentostatin has never been performed, most clinicians favor cladribine because of the need for only a single course of therapy for most patients (Saven *et al.*, 1998).

VI. Purine Analogs in Acute Myeloid Leukemia

Purine analogs not only demonstrate significant activity in lymphoid malignancies, but, as shown by several investigators, are active in myeloid malignancies as well. In fact, fludarabine was initially evaluated in acute

leukemia; however, the high doses required for activity in those patients led to severe unacceptable neurotoxicity, including cortical blindness and death. (Spriggs *et al*., 1986; Warrell and Berman, 1986). Currently, fludarabine is used in the treatment of acute myeloid leukemia (AML) to potentiate the metabolism of cytarabine. To act as a cytotoxic agent, cytarabine must be phosphorylated to its 5′-triphosphate form (Ara-CTP). Furthermore, it is the cellular accumulation and retention of Ara-CTP that is associated with cytotoxicity (Kornblau *et al*., 1996). Work performed by Gandhi *et al*. (1993) has demonstrated that infusion of fludarabine prior to intermittent infusion of intermediate-dose cytarabine increases Ara-CTP accumulation in circulating blasts from patients with AML. It has also been shown that the inhibition of ribonucleotide reductase and subsequent lowering of endogenous deoxynucleosides are major factors for this interaction (Kufe *et al*., 1984). This combination has been investigated mainly in refractory or relapsed adult AML. At the M. D. Anderson Cancer Center, 30 mg/m^2 fludarabine once daily for five doses, and Ara-C 0.5 g/m^2/h for 2–6 h daily for six doses was examined in this setting (Estey *et al*., 1993). Fludarabine doses preceded those of Ara-C by 4 h. CR was achieved in 21 of 59 (36%) patients, with a median response duration of 39 weeks. In patients with new or relapsed AML, patients tolerated the protocol well, with myelosuppression as the principal toxicity (Kornblau *et al*., 1993). However, neurologic toxicity was also observed in 8 of 219 patients, with 2 patients developing cerebral dysfunction that was ultimately lethal. In another study, this combination was examined in newly diagnosed poor-prognosis patients with AML with CR achieved in 41% (Gandhi, 1993). As a randomized comparison to high-dose cytarabine has not been performed; this combination cannot be routinely recommended for clinical use. Despite initial enthusiasm for fludarabine and cytarabine combinations, recent (nonrandomized) comparisons have failed to show a benefit for fludarabine-containing combinations, and the group at the M. D. Anderson Cancer Center no longer recommends this regimen outside the setting of a clinical trial (Estey *et al*., 2001).

Cladribine has also been investigated in both pediatric and adult patients with AML (Gordon *et al*., 2000; Santana *et al*., 1991, 1992, 1994; Van Den Neste, 1998). Unfortunately, cladribine as monotherapy has limited activity and significant neurotoxicity (at the doses used to treat AML) in the adult population (Bryson and Sorkin, 1993; Vahdat *et al*., 1994). Similar to fludarabine, cladribine also increases Ara-CTP accumulation if given as pretreatment with cytarabine (Gandhi *et al*., 1996). Based on these observations, Kornblau *et al*. (1996) studied sequential therapy in relapsed patients with AML or MDS with cladribine at a dose of 12 mg/m^2/day and cytarabine at a dose of 1 g/m^2 over 2 h/day for 5 consecutive days. Such combinations (with or without other agents) have failed to offer an obvious advantage over regimens lacking the purine analog. A large randomized multicenter study in Poland comparing cladribine, cytarabine

(Ara-C), and daunorubicin (DAC-7) with standard 3 + 7 (DA-7) daunorubicin and cytarabine in 280 newly diagnosed patients failed to demonstrate a substantial benefit to the addition of cladribine, with CR seen in 70% of DAC-7 treated patients and 66% of DA-7 treated patients (Holowiecki *et al.*, 2001, 2002). Patients receiving the cladribine combination, however, were more likely to achieve CR with one course of induction.

One of the newer purine nucleoside analogs, clofarabine (2-chloro-2′fluoro-deoxy-9-β-D-arabinofuranoyladenine), is currently being studied in acute leukemia. It is similar to fludarabine and cladribine in that it is resistant to deamination by adenosine deaminase and requires intracellular phosphorylation by deoxycytidine kinase to the cytotoxic triphosphate form. However, clofarabine has favorable properties of both congeners by affecting DNA elongation (like fludarabine) and ribonucleotide reductase (like cladribine) (Parker *et al.*, 2001; Plunkett and Gandhi, 2001; Xie and Plunkett, 1996). In a Phase I study of clofarabine in acute leukemia, severe reversible hepatotoxicity defined the nonmyelosuppressive dose-limiting toxicity, suggesting a clofarabine dose of 40 mg/m^2 daily for 5 days (Kantarjian *et al.*, 2003b). Of the 32 evaluable patients, 2 complete responses and 3 marrow CRs were observed. Based on these results, the Phase II study proceeded at the set dose of 40 mg/m^2 intravenously over 1 h daily for 5 days repeated every 3–6 weeks (Kantarjian *et al.*, 2003a). Sixty-two patients with relapsed or refractory AML ($n = 31$), MDS ($n = 8$), CML in blastic phase ($n = 11$), and acute lymphocytic leukemia ($n = 12$) were included on the study. A complete response was noted in 20 patients (32%), and lesser responses were achieved in 10 additional patients. Responses were observed in all disease types studied. Severe reversible liver dysfunction was noted in 15–24%. As expected, the main toxicity was associated with myelosuppression with fever in 14 of 62 (23%) patients and documented infections in 31 of 62 (50%) patients. Five patients died during induction therapy. This study indicates that clofarabine has activity in acute leukemia and warrants further study.

VII. Purine Analogs in Chronic Myeloid Leukemia

In vivo studies have shown that purine analogs can inhibit proliferation and induce apoptosis of CML cells (Konwalinka *et al.*, 1992; Zinzani *et al.*, 1994). Thus, several Phase II studies have examined the role of these agents in CML. These studies indicate that purine analogs can suppress the leukocyte count in patients with CML, but no study has demonstrated a selective advantage, and cytogenetic responses have not been seen (Cortes *et al.*, 1997; Marks *et al.*, 1994; Saven *et al.*, 1994). Cladribine and fludarabine alone or in combination regimens have also been examined in patients with CML in blast crisis (Dann *et al.*, 1998; Martinelli *et al.*, 1996; Sacchi *et al.*,

1999; Tedeschi *et al*., 2000). These studies demonstrate some cytoreduction of leukemic cells in the blood, with responses seen in 28–47% of patients, but such treatments rarely produce cytogenetic remissions. In the era of imatinib, which is both extremely well tolerated and highly active, it is unlikely that purine analogs will play an important role in the treatment of CML. The one exception to this is their inclusion as potent immunosuppressants in reduced intensity (mini) transplants.

VIII. Purine Analogs in Allogeneic Stem Cell Transplantation

Nonmyeloablative conditioning regimens have been introduced for allogeneic transplantation in recent years (Bornhauser *et al*., 2001; Giralt *et al*., 1997, 2001; Khouri *et al*., 1998; Michallet *et al*., 2001; Saito *et al*., 2002; Schlenk *et al*., 2002; Slavin *et al*., 1998; Wasch *et al*., 2002). These low-intensity regimens, which are immunosuppressive but not myeloablative, can be safer for high-risk patients who are not candidates for fully myeloablative protocols. In addition, these reduced conditioning regimens allow allogeneic transplantation to be performed in older patients or in patients with comorbidities or advanced diseases. Initial studies by Giralt *et al*. (1997) with fludarabine (30 mg/m^2/day) and either cytarabine (2 g/m^2) or idarubicin (12 mg/m^2) demonstrated that purine-analog-containing nonmyeloablative chemotherapy followed by allogeneic transplant is feasible in older or debilitated patients with advanced AML or MDS who are not eligible for myeloablative therapy. Additional studies by this group (Giralt *et al*., 2001) with a nonmyeloablative regimen consisting of melphalan and either fludarabine or cladribine in 86 patients (43 with AML/MDS, 27 with CML) revealed excessive toxicity with the cladribine regimen. Disease-free survival at 1 year in patients in first remission or chronic phase was 57% and 49% in patients with untreated first relapse or in a second or later remission. Other investigators have evaluated cladribine, but at reduced doses than those in the study by Giralt *et al*. (1997). In one study by Saito *et al*. (2002), 16 patients (6 with AML, 2 with CML, 6 with MDS) received a regimen of cladribine, busulfan, and antithymocyte globulin. The incidence of grade II–IV graft-versus-host disease was 43%, and 1-year overall survival and disease-free survival rates were 69% and 50%, respectively. Because of the heterogenous populations studied, small cohort of patients, and short-term follow-up in these studies, it clear that additional studies and long-term follow-up is necessary to evaluate regimens containing purine analogs as a component of nonmyeloablative chemotherapy prior to allogeneic transplantation.

Several investigators have also examined the use of pentostain in nonmyeloablative stem cell transplantation conditioning regimens (Chan *et al*.,

2001; Liu *et al*., 2002; Pavletic *et al*., 2002). Liu *et al*. looked at a conditioning regimen of mitoxantrone (40 mg/m^2 for one cycle), cytarabine (2 g/m^2 for three cycles), and pentostatin (Phase I dose escalating) in 25 patients with either relapsed or poor prognostic hematologic diseases (10 NHL, 4 HD, 6 AML, 2 MDS-RAEBt, 1 CLL, 2 multiple myeloma). Thirteen had failed autologous transplantation. The maximum tolerated dose of pentostatin at 14 mg/m^2 given over 3 days was not reached. The regimen was well tolerated, median time to neutrophil recovery >500/μl was 2 days, and platelet recovery >20,000/μl was 9 days. Median donor chimerism at 3 months was 98% for the related stem cell transplantations. At a median follow-up of 9 months (0–22), the median survival was 9 months, disease-free survival was 32%, and overall survival for these patients with extremely poor prognosis was 44%. In another study by Chan *et al*. (2001), 42 patients (14 AML, 7 MDS, 7 CML, 6 NHL, 3 HD, 2 MF, 1 CLL 1 MM, 1 other) were treated with extracorporeal phototherapy, pentostatin, and total body irradiation (TBI). The overall survival was 63% at a median follow-up of 185 days. These results are particularly encouraging given the high-risk nature of the population treated, which included eight match unrelated donor transplants and seven mismatched donor transplants.

IX. Conclusion and Future Directions

Purine analogs have clearly demonstrated their role in low-grade lymphoid leukemias, specifically in the treatment of CLL and hairy cell leukemia, in which these agents play a dominant role. Their utility in the acute leukemias are more limited, but new agents such as clofarabine hold promise in treating acute leukemias as well. Purine analogs have also demonstrated a role in nonmyeloablative allogeneic transplantation. In conclusion, as the pharmacology of the established and the newer purine analogs is further elucidated, refinements in dosing and schedule can improve both their single-agent activity as well as their role in combination therapy in both acute and chronic leukemias.

References

Adkins, J. C., Peters, D. H., and Markhan, A. (1997). Fludarabine: An update of its pharmacology and use in the treatment of hematological malignancies. *Drugs* **53**, 1005–1037.

Anaissie, E., Kontoyiannis, D. P., Kantarjian, H., Elting, L., Robertson, L. E., and Keating, M. (1992). Listeriosis in patients with chronic lymphocytic leukemia who were treated with fludarabine and prednisone. *Ann. Intern. Med.* **117**, 466–469.

Bornhauser, M., Thiede, C., Schuler, U., Platzbecker, U., Freiberg-Richter, J., Helwig, A., Plettig, R., Rollig, C., Naumann, R., Kroschinsky, F., Neubauer, A., and Ehninger, G.

(2001). Dose reduced conditioning for allogenic blood stem cell transplantation: Durable engraftment without antithymocyte globulin. *Bone Marrow Transplant.* **26,** 119–125.

Bryson, H. M., and Sorkin, E. M. (1993). Cladribine. A review of its phmacodynamic and pharmacokinetic properties and therapeutic potential in haematological malignancies. *Drugs* **46,** 872–894.

Byrd, J. C., Peterson, B. L., Morrison, V. A., Park, K., Jacobson, R., Hobe, E., Vardiman, J. W., Rai, K., Schiffer, C. A., and Larson, R. A. (2003). Randomized phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic, untreated patients with B-cell chronic lymphocytic leukemia: Results from Cancer and Leukemia Group B 9712 (CALGB 9712). *Blood* **101,** 6–14.

Carson, D. A., Wassun, B., Taetle, R., and Yu, A. (1983). Specific toxicity of 2-chlorodeoxyadenosine toward resting and proliferating human lymphocytes. *Blood* **62,** 737–743.

Chan, G. W., Foss, F. M., Roberts, T., Spraque, K., Schenkein, D., and Miller, K. B. (2001). Age is not associated with adverse outcomes following pentostatin-based preparative regimen for allogeneic bone marrow transplant [Abstract]. *Blood* **98,** 313b.

Cohen, A., Lee, J. W., and Gelfand, E. W. (1983). Selective toxicity of deoxyguanosine and arabinosyl guanine for T-leukemic cells. *Blood* **61,** 660–666.

Cortes, J., Kantarjian, H., Talpaz, M., O'Brien, S., Beran, M., Koller, C., and Keating, M. (1997). Treatment of chronic myelogenous leukemia with nucleoside analogs deoxycoformycin and fludarabine. *Leukemia* **11,** 788–791.

Czuczman, M. S., Fallon, A., Mohr, A., Stewart, C., Bernstein, Z. P., McCarthy, I., Skipper, M., Brown, K., Miller, K., Wentling, D., Klippenstein, D., Loud, P., Rock, M. K., Benyunes, M., Grillo-Lopez, A. J., and Bernstein, S. H. (2002). Rituximab in combination with CHOP or fludarabine in low-grade lymphoma. *Semin. Oncol.* **29,** 36–40.

Dann, F. J., Anastasi, J., and Larson, A. (1998). High dose cladribine therapy for chronic myelogenous leukemia in the accelerated or blast phase. *J. Clin. Oncol.* **16,** 1498–1504.

Elias, L., Stock-Novack, D., Head, D. R., Grever, M. R., Weick, J. K., Chapman, R. A., Godwin, J. E., Metz, E. N., and Appelbaum, E. R. (1993). A phase I trial of combination fludarabine monophosphate and chlorambucil in chronic lymphocytic leukemia: A Southwest Oncology Group study. *Leukemia* **3,** 361–365.

Estey, E., Plunkett, W., Gandhi, V., Rios, M. B., Kantarjian, H., and Keating, M. J. (1993). Fludarabine and arabinosylcytosine therapy of refractory and relapsed acute myelogenous leukemia. *Leuk. Lymph.* **9,** 343–350.

Estey, E. H., Thall, P. F., Cortes, J. E., Giles, F. J., O'Brien, S., Pierce, S. A., Wang, X., Kantarjian, H. M., and Beran, M. (2001). Comparison of idarubicin + ara-C-, fludarabine + ara-C-, and topotecan + ara-C-based regimens in treatment of newly diagnosed acute myeloid leukemia, refractory anemia with excess blasts in transformation, or refractory anemia with excess blasts. *Blood* **98,** 3575–3583.

Flinn, I. W., Byrd, J., Morrison, C., Jamison, J., Diehl, L. F., Murphy, T., Piantadosi, S., Seifter, E., Ambinder, R. F., Vogelsang, G., and Grever, M. R. (2000). Fludarabine and cyclophosphamide with filgrastim support in patients with previously untreated indolent lymphoid malignancies. *Blood* **96,** 71–75.

Flinn, I. W., Kopecky, K. J., Foucar, M. K., Head, D., Bennett, J. M., Hutchison, R., Corbett, W., Cassileth, P., Habermann, T., Golomb, H., Rai, K., Eisenhauer, E., Appelbaum, F., Cheson, B., and Grever, M. R. (2000). Long-term follow-up of remission duration, mortality, and second malignancies in hairy cell leukemia patients treated with pentostatin. *Blood* **96,** 2981–2986.

Gandhi, V. (1993). Fludarabine for treatment of adult acute myelogenous leukemia. *Leuk. Lymph.* **11**(Suppl. 2), 13.

Gandhi, V., Estey, E., Keating, M. J., Chucrallah, A., and Plunkett, W. (1996). Chlorodeoxyadenosine and arabinosylcytosine in patients with acute myelogenous

leukemia: Pharmacokinetic, pharmacodynamic, and molecular interactions. *Blood* **87,** 256–264.

Gandhi, V., Estey, E., Keating, M. J., and Plunkett, W. (1993). Fludarabine potentiates metabolism of cytarabine in patients with acute myelogenous leukemia during therapy. *J. Clin. Oncol.* **11,** 116–124.

Gandhi, V., and Plunkett, W. (2002). Cellular and clinical pharmacology of fludarabine. *Clin. Pharm.* **41,** 93–103.

Gandhi, V., Plunkett, W., Weller, S., Du, M., Ayres, M., Rodriguez, C. O., Jr., Ramakrishna, P., Rosner, G. L., Hodge, J. P., O'Brien, S., and Keating, M. J. (2001). Evaluation of the combination of nelzarabine and fludarabine in leukemias: Clinical response, pharmacokinetics, and pharmacodynamics in leukemia cells. *J. Clin. Oncol.* **19,** 2142–2152.

Genini, D., Adachi, S., Chao, O., Rose, D. W., Carrera, C. J., Cottam, H. B., Carson, D. A., and Leoni, L. M. (2000). Deoxyadenosine analogs induce programmed cell death in chronic lymphocytic leukemia cells by damaging the DNA and by directly effecting the mitochondria. *Blood* **96,** 3537–3543.

Giralt, S., Estey, E., Albitar, M., van Besien, K., Rondon, G., Anderlini, P., O'Brien, S., Khouri, I., Gajewski, J., Mehra, R., Claxton, D., Andersson, B., Beran, M., Przepiorka, D., Koller, C., Kornblau, S., Korbling, M., Keating, M., Kantarjian, H., and Camplin, R. (1997). Engraftment of allogenic hematopoietic progenitor cells with purine analog-containing chemotherapy: Harnessing graft-versus-leukemia without myeloablative therapy. *Blood* **89,** 4531–4536.

Giralt, S., Thall, P., Khouri, S., Wang, X., Braunschweig, I., Ippolitti, C., Claxton, D., Donato, M., Bruton, J., Cohen, A., Davis, M., Andersson, B. S., Anderlini, P., Gajewski, J., Kornblau, S., Andreeff, M., Przepiorka, D., Ueno, N. T., Molldrem, J., and Champlin, R. (2001). Melphalan and purine analog-containing preparative regimens: Reduced-intensity conditioning for patients with hematologic malignancies undergoing allogenic progenitor cell transplantation. *Blood* **97,** 631–637.

Gordon, M. S., Young, M. L., Tallman, M. D., Cripe, L. D., Bennett, J. M., Paietta, E., Longo, W., Gerad, H., Mazza, J., and Rowe, J. M. (2000). Phase II trial of 2-chlorodeoxyadenosine in patients with relapsed/refractory acute myeloid leukemia: A study of the Eastern Cooperative Oncology Group (ECOG), E5995. *Leuk. Res.* **24,** 871–875.

Grever, M. R., Kopecky, K. J., Coltman, C. A., Files, J. C., Greenberg, B. R., Hutton, J. J., Talley, R., Von Hoff, D. D., and Balcerzak, S. P. (1988). Fludarabine monophosphate: A potentially useful agent in chronic lymphocytic leukemia. *Nouv. Rev. Fr. Hematol.* **30,** 457–459.

Grever, M., Kopecky, K., Foucar, M. K., Head, D., Bennett, J. M., Hutchinson, R. E., Corbett, W. E., Cassileth, P. A., Habermann, T., and Golomb, H. (1995). Randomized comparison of pentostatin versus interferon alfa-2a in previously untreated patients with hairy cell leukemia: An intergroup study. *J. Clin. Oncol.* **13,** 974–982.

Grever, M. R., Leiby, J. M., Kraut, E. H., Wilson, H. E., Neidhart, J. A., Wall, R. L., and Balcerzak, S. P. (1985). Low-dose deoxycoformycin in lymphoid malignancy. *J. Clin. Oncol.* **3,** 1196–1201.

Grever, M. R., Siaw, M. F. E., Jacob, W. F., Neidhart, J. A., Miser, J. S., Coleman, M. S., Hutton, J. J., and Balcerzak, S. P. (1981). The biochemical and clinical consequences of 2′-deoxycoformycin in refractory lymphoid malignancy. *Blood* **57,** 406–417.

Griffig, J., Koob, R., and Blakley, R. L. (1989). Mechanisms of inhibition of inhibition of DNA synthesis by 2-chlorodeoxyadenosine in human lymphoblastic cells. *Cancer Res.* **42,** 6923–6928.

Gutheil, J., and Kearns, C. (1997). Antimetabolites. *In* "The Chemotherapy Source Book" (M. Perry, Ed.), chap 20, pp. 330–336. Williams & Wilkins, Baltimore, MD.

Hallek, M., Schmitt, B., Wilhelm, M., Busch, R., Krober, A., Fostitsch, H. P., Sezer, O., Herold, M., Knauf, W., Wendtner, C. M., Kuse, R., Freund, M., Franke, A., Schriever, F., Nerl, C.,

Dohner, H., Thiel, E., Hiddemann, W., Brittinger, G., and Emmerich, B. (2001). Fludarabine plus cyclophosphamide is an efficient treatment for advanced chronic lymphocytic leukaemia (CLL): Results of a phase II study of the German CLL Study Group. *Br. J. Haematol.* **114**(2), 342–348.

Hirota, Y., Yoshioka, A., Tanaka, S., Watanabe, K., Otani, T., Minowada, J., Matsuda, A., Ueda, T., and Wataya, Y. (1989). Imbalance of deoxyribonucleoside triphosphates, DNA double-strand breaks, and cell death caused by 2-chlorodeoxyadenosine in mouse FM3A cells. *Cancer Res.* **49,** 915–919.

Hoffman, M. A., Janson, D., Rose, E., and Rai, K. R. (1997). Treatment of hairy-cell leukemia with cladribine: Response, toxicity, and long-term follow-up. *J. Clin. Oncol.* **15,** 1138–1142.

Holowiecki, J., Grosicki, S., Robak, T., Krzemien, S., Hellman, A., Skotnicki, A., Jedrzejczak, W., Kluczkowski, K., Konopka, L., Zdziarska, B., Pluta, A., Dmoszynska, A., Maj, S., Giebel, S., Zawilska, K., Komarnicki, M., and Kloczko, J. (2001). An original, multicenter randomized phase III trial comparing 2-CdA combined with cytarabine and daunorubicin (DAC-7) with standard cytarabine and daunorubicin (DA-7) for remission induction therapy in newly diagnosed, adult acute myeloid leukemia (Abstract). *Blood* **98,** 592a.

Holowiecki, J., Robak, T., Kyrcz-Krzemien, S., Grosicki, S., Hellmann, A., and Skotnicki, A. (2002). Daunorubicin, cytarabine and 2-CdA (DAC-7) for remission induction in "*de novo*" adult acute myeloid leukemia patients *Acta. Haematol. Polon.* **33,** 839–847.

Huang, P., Chubb, S., and Plunkett, W. (1990). Termination of DNA synthesis by 9-β-D-arabinosyl-2-fluoroadenine: A mechanism for cytotoxicity. *J. Biol. Chem.* **265,** 16617–16625.

Kantarjian, H., Gandhi, V., Cortes, J., Verstovsek, S., Du, M., Garcia-Manero, G., Giles, F., Faderl, S., O'Brien, S., Jeha, S., Davis, J., Shaked, Z., Craig, A., Keating, M., Plunkett, W., and Freireich, E. J. (2003). Phase II clinical and pharmacology study of clofarabine in patients with refractory or relapsed acute leukemia. *Blood* First Edition Paper, prepublished online June 5, 2003; DOI 10.1182/blood-2003-03-0925.

Kantarjian, H., Gandhi, V., Kozuch, P., Faderl, S., Giles, F., Cortes, J., O'Brien, S., Ibrahim, N., Khuri, F., Du, M., Rios, M. B., Jeha, S., McLaughlin, P., Plunkett, W., and Keating, M. (2003). Phase I clinical and pharmacology study of clofarabine (CL-F-ara-A; 2-chloro-2′-fluoro-deoxy-9-β-D-arabinofuranosyladenine) in patients with solid and hematologic cancers. *J. Clin. Oncol.* **21,** 1167–1173.

Keating, M. J., Kantarjian, H., O'Brien, S., Koller, C., Talpaz, M., Schachner, J., Childs, C. C., Freirich, E. J., and McCredie, K. B. (1991). Fludarabine: A new agent with marked cytoreductive activity in untreated chronic lymphocytic leukemia. *J. Clin. Oncol.* **9**(1), 44–49.

Keating, M. J., Kantarjian, H., Talpaz, M., Redman, J., Koller, C., Barlogie, B., Velasquez, W., Plunkett, W., Freirich, E. J., and McCredie, K. B. (1989). Fludarabine: A new agent with major activity against chronic lymphocytic leukemia. *Blood* **74,** 19–25.

Keating, M. J., O'Brien, S., Lerner, S., Koller, C., Beran, M., Robertson, L. E., Freirich, E. J., Estey, E., and Kantarjian, H. (1988). Long-term follow-up of patients with chronic lymphocytic leukemia (CLL) receiving fludarabine regimens as initial therapy. *Blood* **92,** 1165–1171.

Khouri, I. F., Keating, M., Korbling, M., Przepiorka, D., Anderlini, P., O'Brien, S., Giralt, S., Ippoliti, C., von Wolff, B., Gajewski, J., Donato, M., Claxton, D., Ueno, N., Andersson, B., Gee, A., and Champlin, R. (1998). Transplant-like: Induction of graft-versus malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. *J. Clin. Oncol.* **16,** 1958–1960.

Konwalinka, G., Helson, C., Braverman, S., Petzer, G., Bilgeri, R., and Zihan, U. (1992). Growth inhibitory properties of 2-chlorodeoxyadenosine (2-CdA): Growth inhibitory

system tumors. *In* Molecular biology of hematopoiesis (N. G. Abraham, G. Konwalinka, and P. Marks, Eds.), pp. 603–613. Intercept Ltd., Hanover, MA.

Kornblau, S. M., Cortes-Franco, J., and Estey, E. (1993). Neurotoxicity associated with fludarabine and cytosine arabinoside chemotherapy for acute leukemia and myelodysplasia. *Leukemia* **7,** 378–383.

Kornblau, S. M., Gandhi, V., Andreeff, H. M., Beran, M., Kantarjian, H. M., Koller, C. A., O'Brien, S., Plunkett, W., and Estey, E. (1996). Clinical and laboratory studies of 2-chlorodeoxyadenosine ± cytosine arabinoside for relapsed or refractory acute myelogenous leukemia in adults. *Leukemia* **10,** 1563–1569.

Krenitsky, T. A., Koszalka, G. W., Tuttle, J. V., Rideout, J. L., and Elion, G. B. (1981). An enzymatic synthesis of purine-D-arabinonucleosides. *Carbohydr. Res.* **97,** 129–146.

Kufe, D., Spriggs, D., Egan, E. M., and Munroe, D. (1984). Relationships among Ara-CTP pools, formation of (Ara-C) DNA, and cytotoxicity of human leukemic cells. *Blood* **64,** 54–58.

Kurtzberg, J., Ernst, T. J., Keating, M. J., Gandhi, V., Hodge, J. P., Kisor, D. F., Therriault, R., Stephens, C., Levin, J., Krenitsky, T., Elion, G. B., and Mitchell, B. S. (1999). A phase I study of 2-amino-9-β-D-arabinosyl-6-methoxy-9H-purine (506U78) administered on a consecutive five day schedule in children and adults with refractory hematologic malignancies. *Blood* **94**(Suppl.), 2794a.

Lamanna, N., Weiss, M. A., Maslak, P. G., Gencarelli, A. N., Scheinberg, D. A., and Horgan, D. (2003). Sequential therapy with fludarabine, high dose cyclophosphamide, and rituximab induces a high incidence of complete response in patients with chronic lymphocytic leukemia. *Proc. ASCO* **22,** 580. (abstract 2333).

Lambe, C. U., Averett, D. R., Paff, M. T., Reardon, J. E., Wilson, J. G., and Krenitsky, T. A. (1995). 2-amino-6-methoxypurine arabinoside: An agent for T-cell malignancies. *Cancer Res.* **55,** 3352–3356.

Liu, D., Seiter, K., Chiao, J. W., Nelson, J., Kancherla, R., Siddiqui, D., Cereb, N., Yang, S., and Ahmed, T. (2002). MAP regimen: A novel non-myeloablative conditioning regimen of mitoxantrone, Ara-C, and pentostatin (MAP regimen) for hematological malignancies [abstract]. *Blood* **100,** 622a.

Marks, R. S., Letendre, L., Reid, J. M., Bagniewski, P. G., and Ames, M. M. (1994). A Phase I and pharmacologic study of chlorodeoxyadenosine in patients with chronic myelomonocytic or chronic granulocytic leukemia. *Proc. ASCO* **13,** 155. (abstract 415).

Martinelli, G., Testoni, N., Zuffa, E., Visani, G., Zinzani, P. L., Zaccaria, A., Farabegoli, P., Arpinati, M., Amabile, M., and Tura, S. (1996). FLANG (fludarabine + cytosine arabinoside + novantrone + G-CSF) induces partial remission in lymphoid blast transformation of Ph + chronic myelogenous leukemia. *Leuk. Lymph.* **22,** 173–176.

Michallet, M., Bilger, K., Garban, F., Attal, M., Huyn, A., Blaise, D., Milpied, N., Moreau, P., Bordigoni, P., Kuentz, M., Sadoun, A., Cahn, J. Y., Socie, G., Thomas, X., Arnaud, P., Raus, N., Lheritier, V., Pigneux, A., and Boiron, J. M. (2001). Allogeneic hematopoietic stem-cell transplantation after nonmyeloablative preparative regimens: Impact of pretransplantation and posttransplantation factors on outcome. *J. Clin. Oncol.* **19,** 3340–3349.

Montgomery, J. A., and Hewson, K. (1957). Synthesis of potential anticancer agents. X. Fluroadenosine. *J. Am. Chem. Soc.* **79,** 4559.

Montillo, M., Tedeschi, A., O'Brien, S., Di Raimondo, F., Lerner, S., Ferrajoli, A., Morra, E., and Keating, M. J. (2003). Phase II study of cladribine and cyclophosphamide in patients with chronic lymphocytic leukemia and prolymphocytic leukemia. *Cancer* **97,** 114–120.

O'Brien, S., Kantarjian, H. M., Cortes, J., Beran, M., Koller, C. A., Giles, F. J., Lerner, S., and Keating, M. (2001). Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. *J. Clin. Oncol.* **19**(5), 1414–1420.

Parker, W. B., Shaddix, S. C., Chang, C. H., White, E. L., Rose, L. M., Brockman, R. W., Shortnacy, A. T., Montgomery, J. A., Secrist, J. A. 3rd, and Bennett, L. L., Jr. (2001). Effects of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine on K562 cellular metabolism and the inhibition of human ribonucleotide reductase and DNA polymerases by its 5′-triphosphate. *Cancer Res.* **51,** 2386–2394.

Pavletic, S. Z., Bociek, G., Foran, J., Hatcher, L., Rubocki, R., Kuszynsky, L., Lucas, D., Grever, M., Hock, L., Joshi, S., Hardiman, P., Bierman, P., Vose, J., Armitage, J., and Talmadge, J. (2002). Immunological effects of pentostatin in a minimally myelosuppressive regimen for allogeneic stem cell transplantation [Abstract]. *Blood* **100,** 626a.

Piro, L. D., Carrera, C. J., Carson, D. A., and Beutler, E. (1990). Lasting remissions in hairy-cell leukemia induced by a single infusion of 2-chlorodeoxyadenosine. *N. Engl. J. Med.* **322,** 1117–1121.

Plunkett, W., and Gandhi, V. (2001). Purine and pyrimidine nucleoside analogs. *In* "Cancer Chemotherapy and Biological Response Modifiers, Annual 19" (G. Giaccone, R. Schilsky, and P. Sondel, Eds.), pp. 21–45. Elsevier, Amsterdam.

Plunkett, W., Huang, P., and Gandhi, V. (1990). Metabolism and action of fludarabine phosphate. *Sem. Oncol.* **17**(Suppl. 8), 3–17.

Rai, K. R., Peterson, B. L., Appelbaum, F. R., Kolitz, J., Elias, L., Shepherd, L., Hines, J., Threatte, G. A., Larson, R. A., Cheson, B. D., and Schiffer, C. A. (2000). Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. *N. Engl. J. Med.* **343**(24), 1750–1757.

Rai, K. R., Peterson, B., Elias, L., Shepherd, L., Hines, J., Nelson, D., Cheson, B., Kolitz, J., and Schiffer, C. A. (1996). A randomized comparison of fludarabine and chlorambucil for patients with previously untreated chronic lymphocytic leukemia. A CALGB, SWOG, CTG/NCI-I and ECOG Intergroup study [abstract]. *Blood* **88,** 552.

Reist, E. J., and Goodman, L. (1964). Synthesis of 9-β-D-arabinofuranosylguanine. *Biochemistry* **3,** 15–18.

Robak, T., Blonski, J. Z, Kasznicki, M., Blasinska-Morawiec, M., Krykowski, E., Dmoszynska, A., Mrugala-Spiewak, H., Skotnicki, A. B., Nowak, W., Konopka, L., Ceglarek, B., Maj, S., Dwilewica-Trojaczek, J., Hellmann, A., Urasinski, I., Zdziarska, B., Kotlarek-Haus, S., Potoczek, S., and Grieb, P. (2000). Cladribine with prednisone versus chlorambucil with prednisone as first-line therapy in chronic lymphocytic leukemia: Report of a prospective, randomized, multicenter trial. *Blood* **96,** 2723–2729.

Robertson, I. E., Chubb, S., Meyn, R. E., Story, M., Ford, R., Hittelman, W. N., and Plunkett, W. (1993). Induction of apoptotic cell death in chronic lymphocytic leukemia by 2-chloro-2′-deoxyadenosine and 9-β-D-arabinosyl-2-fluoroadenine. *Blood* **81,** 143–150.

Robertson, L. E., Huh, Y. O., Butler, J. J., Pugh, N. C., Hirsch-Ginsberg, C., Stass, S., Kantarjian, H., and Keating, M. J. (1992). Response assessment in chronic lymphocytic leukemia after fludarabine plus prednisone: Clinical, pathologic, immunophenotypic and molecular analysis. *Blood* **80,** 29–36.

Sacchi, S., Kantarjian, H. M., O'Brien, S., Cortes, J., Rios, M. B., Giles, F. J., Beran, M., Koller, C. A., Keating, M. J., and Talpaz, M. (1999). Chronic myelogenous leukemia in nonlymphoid blastic phase: Analysis of the results of first salvage therapy with three different treatment approaches for 162 patients. *Cancer* **86,** 2632–2641.

Saito, T., Kanda, Y., Kami, M., Kato, K., Shoji, N., Kanai, S., Ohnishi, T., Kawano, Y., Nakai, K., Ogasawara, T., Matsubara, H., Makimoto, A., Tanosaki, R., Tobinai, K., Wakasugi, H., Takaue, Y., and Mineishi, S. (2002). Therapeutic potential of a reduced-intensity preparative regimen for allogeneic transplantation with cladribine, busulfan and antithymocyte globulin against advanced/refractory acute leukemia/lymphoma. *Clin. Cancer Res.* **8,** 1014–1020.

Santana, V. M., Hurwitz, C. A., Blakley, R. L., Crom, W. R., Luo, X., Roberts, W. M., Ribeiro, R., Mahmoud, H., and Krance, R. A. (1994). Complete hematologic remissions induced

by 2-chlorodeoxyadenosine in children with newly diagnosed acute myeloid leukemia. *Blood* **84,** 1237–1242.

Santana, V. M., Mirro, J., Jr., Harwood, F. C., Cherrie, J., Schell, M., Kalwinsky, D., and Blakley, R. L. (1991). A phase I clinical trial of 2-chlorodeoxyadenosine in pediatric patients with acute leukemia. *J. Clin. Oncol.* **9,** 416–422.

Santana, V. M., Mirro, J., Jr., Kearns, C., Schell, M. J., Crom, W., and Blakley, R. L. (1992). 2-Chlorodeoxyadenosine produces a high rate of complete hematologic remission in relapsed acute myeloid leukemia. *J. Clin. Oncol.* **10,** 364–370.

Saven, A., Burian, C., Koziol, J. A., and Piro, L. D. (1998). Long-term follow-up of patients with hairy cell leukemia after cladribine treatment. *Blood* **92,** 1918–1926.

Saven, A., Piro, L. D., Lemon, R. H., Figueroa, M. L., Kosty, M., Ellison, D. J., and Beutler, E. (1994). Complete hematologic remissions in chronic-phase, Philadelphia-chromosome-positive, chronic myelogenous leukemia after 2-chlorodeoxyadenosine. *Cancer* **73,** 2953–2963.

Schlenk, R. F., Hartmann, F., Hensel, M., Jung, W., Weber-Nordt, R., Gabler, A., Haas, R., Ho, A. D., Trumper, L., and Dohner, H. (2002). Less intense conditioning with fludarabine, cyclophosphamide, idarubicin, and etoposide (FICE) followed by allogenic unselected peripheral blood stem cell transplantation in elderly patients with leukemia. *Leukemia* **16,** 581–586.

Seto, S., Carrera, C. I., Kubota, M., Wasson, D. B., and Carson, D. A. (1985). Mechanism of deoxyadenosine and 2′-chlorodeoxyadenosine toxicity to non-dividing human lymphocytes. *J. Clin. Invest.* **75,** 377–383.

Seymour, J. F., Huang, P., Plunkett, W., and Gandhi, V. (1996). Influence of fludarabine on pharmacokinetics and pharmacodynamics of cytarabine: Implications of a continuous infusion schedule. *Clin. Cancer Res.* **2,** 653–658.

Seymour, J. F., Kurzrock, R., Freirich, E. L., and Estey, E. H. (1994). 2-chlorodeoxyadenosine induces durable remissions and prolonged suppression of CD4+ lymphocyte counts in patients with hairy cell leukemia. *Blood* **83,** 2906–2911.

Siaw, M. F., and Coleman, M. S. (1984). *In vitro* metabolism of deoxycoformycin in human T lymphoblastoid cells. Phosphorylation of deoxycoformycin and incorporation into cellular DNA. *J. Biol. Chem.* **259,** 9426–9433.

Slavin, S., Nagler, A., Naparstek, E., Kapelushnik, Y., Aker, M., Cividalli, G., Varadi, G., Kirschbaum, M., Ackerstein, A., Samuel, S., Amar, A., Brautbar, C., Ben-Tal, O., Eldor, A., and Or, R. (1998). Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic disease. *Blood* **91,** 756–763.

Spriggs, D. R., Stopa, E., Mayer, R. J., Schoene, W., and Kufe, D. W. (1986). Fludarabine phosphate (NSC 312878) infusions for the treatment of acute leukemia: Phase I and neuropathological study. *Cancer Res.* **46,** 5953–5958.

Tallman, M. S., Hakimian, D., Rademaker, A. W., Zanzig, C., Wollins, E., Rose, E., and Peterson, L. C. (1996). Relapse of hairy cell leukemia after 2-chlorodeoxyadenosine: Long-term follow-up of the Northwestern University experience. *Blood* **88,** 1954–1959.

Tedeschi, A., Montillo, M., Ferrara, F., Nosari, A., Mele, G., Copia, C., Leoni, P., and Morra, E. (2000). Treatment of chronic myeloid leukemia in the blastic phase with fludarabine, cytosine arabinoside and G-CSF (FLAG). *Eur. J. Haematol.* **64,** 182–187.

Vahdat, L., Wong, E., Wile, M., Rosenblum, M., Foley, K. M., and Warell, R. P., Jr. (1994). Therapeutic and neurotoxic effects of 2-chlorodeoxyadenosine in adults with acute myeloid leukemia. *Blood* **84,** 3429–3434.

Van Den Neste, E., Martiat, P., Mineur, P., Delannoy, A., Doyen, C., Zenebergh, A., Michaux, J. L., and Ferrant, A. (1998). A. 2-Chlorodeoxyadenosine with or without daunorubicin in relapsed or refractory acute myeloid leukemia. *Ann. Hematol.* **76,** 19–23.

Warrell, R. P., Jr., and Berman, E. (1986). Phase I and II study of fludarabine phosphate in leukemia: Therapeutic efficacy with delayed central nervous system toxicity. *J. Clin. Oncol.* **4,** 74–79.

Wasch, R., Reisser, I., Hahn, J., Bertz, H., Engelhardt, M., Kunzmann, R., Veelken, H., Holler, E., and Finke, J. (2002). Rapid achievement of complete donor chimerism and low regimen-related toxicity after reduced conditioning with fludarabine, carmustine, melphalan and allogeneic transplantation. *Bone Marrow Transplant.* **26,** 243–250.

Weiss, M. A., Glenn, M., Maslak, P., Rahman, Z., Noy, A., Zelenetz, A., Scheinberg, D. A., and Golde, D. W. (2000). Consolidation therapy with high-dose cyclophosphamide improves quality of response in patients with chronic lymphocytic leukemia treated with fludarabine as induction therapy. *Leukemia* **14,** 1577–1582.

Weiss, M. A., Lamanna, N., Maslak, P. G., Gencarelli, A. N., Scheinberg, D. A., Jurcic, J., and Horgan, D. (2003a). Pentostatin, cyclophosphamide and rituximab (PCR therapy): A new active regimen for previously treated patients with chronic lymphocytic leukemia (CLL). *Proc. ASCO* **22,** 580. (abstract 2334).

Weiss, M. A., Maslak, P. G., Jurcic, J. G., Scheinberg, D. A., Aliff, T. B., Lamanna, N., Frankel, S. R., Kossman, S. E., and Horgan, D. (2003b). Pentostatin and cyclophosphamide: An effective new regimen in previously treated patients with chronic lymphocytic leukemia. *J. Clin. Oncol.* **21**(7), 1278–1284.

Weiss, M., Spiess, T., Berman, E., and Kempin, S. (1994). Concomitant administration of chlorambucil limits dose intensity of fludarabine in previously treated patients with chronic lymphocytic leukemia. *Leukemia* **8,** 1290–1293.

Xie, K. C., and Plunkett, W. (1996). Deoxynucleotide pool deletion and sustained inhibition of ribonucleotide reductase and DNA synthesis after treatment of human lymphoblastoid cells with 2-chloro-(2-deoxy-fluoro-B-D-arabinofuranosyl) adenine. *Cancer Res.* **56,** 3030–3037.

Zinzani, P. L., Buzzi, M., Farabegoli, P., Martinelli, G., Tosi, P., Zuffa, E., Visani, G., Testoni, N., Salvucci, M., and Bendandi, M. (1994). Apoptosis induction with fludarabine on freshly isolated chronic myeloid leukemia cells. *Haemtaolog.* **79,** 127–131.

Thomas S. Lin and John C. Byrd

Division of Hematology and Oncology
The Ohio State University
The Arthur James Comprehensive Cancer Center
Columbus, Ohio 43210

Monoclonal Antibody Therapy in Lymphoid Leukemias

I. Chapter Overview

Monoclonal antibodies have played an increasingly important role in the treatment of hematological malignancies over the past several years. Monoclonal antibodies offer the potential of targeted therapy with minimal toxicity to normal cells, and clinical studies over the past decade have demonstrated the feasibility, safety, and clinical efficacy of these agents in myriad solid and hematological cancers. Monoclonal antibodies are at present used to treat diseases as diverse as acute myeloid leukemia (AML), diffuse large B-cell non-Hodgkin's lymphoma (NHL), mycosis fungoides, and chronic lymphocytic leukemia (CLL) (Byrd *et al.*, 2001; Coiffier *et al.*, 2002; Huhn *et al.*, 2001; McLaughlin in *et al.*, 1998; Olsen *et al.*, 2001; Osterborg *et al.*, 1997; Sievers *et al.*, 2001). This chapter focuses on monoclonal antibody therapy in lymphoid leukemias.

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

II. Introduction

A. Introduction of Monoclonal Antibodies into Clinical Practice

Several problems had to be surmounted to successfully introduce monoclonal antibody therapies into clinical practice. These obstacles included (1) identification of tumor-specific antigens, (2) antigen surface density, (3) antibody production, (4) internalization of antigen or antigen–antibody complex, (5) host sensitization and formation of antibodies against the monoclonal antibody, (6) infusion toxicity from host humoral response, and (7) penetration of antibody into bulky tumors. The ideal antigen should be expressed at relatively high density on tumor cells, but not on most normal cells, and should not undergo shedding, internalization, or other modification. B-cell lymphoid malignancies each express a unique immunoglobulin (Ig) idiotype (Id), which is generated by recombination of the genetic sequences for variable Ig light and heavy chains. Each clonal B-cell lymphoid malignancy produces a unique Id protein; thus, Id would be the ideal antigen for monoclonal antibody therapy. Early studies of immunotherapy focused on monoclonal antibodies directed against tumor-specific idiotype (anti-Id MoAb). These studies yielded promising results, and several patients with NHL achieved long-lasting remissions (Brown *et al.*, 1989a,b; Maloney *et al.*, 1992; Meeker *et al.*, 1985b; Miller *et al.*, 1982). However, relapse was common and was usually due to genetic mutation of the Id protein (Cleary *et al.*, 1986; Levy *et al.*, 1988; Meeker *et al.*, 1985a). Anti-Id MoAb therapy was tested primarily in patients with indolent B-cell NHL, although a small number of patients with CLL responded to anti-Id MoAb (Allebes *et al.*, 1988, 1991; Caulfield *et al.*, 1989).

Although patient-specific anti-Id MoAb is attractive theoretically, identification of an individual patient's Id protein sequence and generation of an individualized anti-Id MoAb for each patient are not practical on a large scale with current available technology. Id vaccines might be one alternative approach; such vaccines have shown promise in B-cell NHL and are now entering clinical trials in CLL. Thus, the focus has turned toward development of monoclonal antibodies targeted against tumor-specific, rather than patient-specific, antigens. This change has allowed the development of monoclonal antibodies with much broader therapeutic applicability than that by the initial anti-Id MoAb approach.

B. Humanization of Monoclonal Antibodies

The clinical utility of initial monoclonal antibodies was limited by significant infusion-related toxicity due to host recognition of xenotropic sequences in the murine monoclonal antibodies. Patients developed human

antimouse antibodies (HAMAs) that limited the use of these antibodies, and patients experienced significant serum sickness (Goodman *et al.*, 1990; Scheinberg *et al.*, 1990). However, recombinant DNA technology has allowed the generation of chimeric and humanized murine monoclonal IgG antibodies. Murine sequences are replaced with the human Fc fragment, resulting in humanized IgG molecules whose Fab portions contain only the murine sequences required to recognize the target tumor-specific antigen. These chimaeric and humanized antibodies are significantly less immunogenic and produce less infusion toxicity (Baselga *et al.*, 1999; Maloney *et al.*, 1997; Pegram *et al.*, 1998). In addition, the human Fc fragment allows chimeric antibodies to activate patients' host immune systems through induction of antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Thus, patients and more effective better tolerate humanized monoclonal antibodies. Despite these technological advances, technical problems must be addressed with each new individual monoclonal antibody.

C. Limitations of Radioimmunotherapy in Lymphoid Leukemias

A growing field of research is the use of radioisotope-conjugated antibodies to deliver targeted radiotherapy to tumor cells. Most work to data in this field has focused on indolent and aggressive NHL, although a few groups have examined radioimmunotherapy in lymphoid leukemias. A preliminary report indicated that the ^{131}I-labeled anti-CD20 antibody tositumomab (Bexxar®) is effective in previously treated patients with advanced CLL (Gupta *et al.*, 2001). To date, no clinical data are available regarding the use of the ^{90}Y-labeled anti-CD20 antibody ibritumomab (Zevalin®) in the treatment of CLL. Several technical limitations make it doubtful that radioisotope-conjugated antibodies will play a significant role in the nontransplant therapy of lymphoid leukemias. The primary limitation is the high degree to which leukemia cells infiltrate the blood, bone marrow, and spleen. The effectiveness of radioimmunotherapy is predicated on the ability to deliver targeted radiation therapy to a single or several sites of concentrated tumor cells. The radioisotope is targeted directly to an individual cell, and each cell is also subjected to emitted radiation from delivery of radioisotope-conjugated antibodies to neighboring cells. Marrow toxicity or myelodysplasia, because of exposure of "bystander" normal hematopoietic stem cells to radiation, will likely limit the use of radioimmunotherapy in lymphoid leukemias. Myelosuppression was the dose-limiting toxicity in CLL patients given tositumomab and was related to the total body dose of radiation (Gupta *et al.*, 2001).

D. Summary

Monoclonal antibody therapy in lymphoid leukemias, especially in CLL, is an active area of laboratory and clinical research. Advances in

recombinant DNA technology now allow production of monoclonal antibodies against many potential tumor-specific antigens, and several antibodies are in clinical use. However, the identification of new monoclonal antibodies and the incorporation of these antibodies into clinical therapy remain active areas of research. The development of new antibodies against different cell surface antigens and the optimal use of current monoclonal antibodies in combination chemotherapy regimens are primary areas of ongoing investigation.

III. Chronic Lymphocytic Leukemia

A. The Disease

Indolent B-cell lymphoproliferative disorders such as CLL are ideal targets for monoclonal antibody therapies. Although CLL responds to cytotoxic chemotherapy, most patients do not achieve complete remission (CR) and treatment remains palliative. For many years therapy for CLL consisted of oral alkylating agents, such as chlorambucil, and combination chemotherapeutic regimens, such as cyclophosphamide, vincristine, and prednisone (CVP) (Montserrat *et al*., 1985; Raphael *et al*., 1991; Sawitsky *et al*., 1977). In recent years the purine analog fludarabine and fludarabine-containing combination regimens have shown significant clinical efficacy in relapsed and previously untreated CLL (Boogaerts *et al*., 2001; Flinn *et al*., 2000a; Grever *et al*., 1990; Keating *et al*., 1989, 1991, 1993, 1998; O'Brien *et al*., 2001a). Despite improved response rates and durations of response, these new regimens are not curative. The failure of traditional cytotoxic agents to cure CLL, as well as other indolent B-cell lymphoproliferative disorders, might result from these diseases' indolent nature as well as intrinsic resistance mechanisms to chemotherapy. Only a small fraction of CLL cells undergo growth and division at a time. Cytotoxic chemotherapy often acts only against actively dividing cells undergoing transcription and DNA replication and is ineffective against resting cells. Fludarabine, which acts against both dividing and nondividing cells, is an exception to this rule.

The inherent resistance of CLL and other indolent B-cell lymphoproliferative disorders to chemotherapy is due to defective apoptosis. Unlike acute leukemias or aggressive lymphomas, which are characterized by uncontrolled growth, CLL arises from cellular defects in programmed cell death. Antiapoptotic proteins such as Bcl-2, Mcl-1, and X-linked inactivator of apoptosis (XIAP) are overexpressed in CLL, and high levels of Mcl-1 might be associated with failure to achieve CR to fludarabine (Kitada *et al*., 1998). ADCC and CDC are observed after antibody therapy (Golay *et al*.,

2000; Treon *et al.*, 2001), but monoclonal antibodies might exert their anticancer effects in CLL primarily by inducing apoptosis (Byrd *et al.*, 2002; Pedersen *et al.*, 2002). Although multiple mechanisms of action might play a role, monoclonal antibodies act directly against the cellular defects in apoptosis that give rise to CLL.

B. Rituximab

1. Preclinical Studies

Rituximab (Rituxan®, IDEC-C2B8), a chimeric murine monoclonal antibody that recognizes the CD20 antigen on the surface of normal and malignant B cells, is the best studied and most widely used monoclonal antibody in lymphoid malignancies. CD20 is a calcium channel that interacts with the B-cell immunoglobulin receptor complex (Bubien *et al.*, 1993; Leveille *et al.*, 1999). CD20 is an excellent target; it is expressed in 90–100% of CLL and B-cell NHL, and the antigen is not internalized or shed. However, significant levels of soluble CD20 have been detected in sera of patients with CLL and B-cell NHL, and increased levels of soluble CD20 correlated with poorer survival (Keating *et al.*, 2002; Vose *et al.*, 2001a). Circulating CD20 levels correlated with beta-2-microglobulin levels and advanced stage disease. Circulating CD20 levels correlated with poor survival, and the prognostic value was independent of Rai stage (Manshouri *et al.*, 2003).

Rituximab exerts its anticancer effects on CLL cells by several mechanisms. Rituximab induces both ADCC and CDC, and the antibody activates caspase 3 and induces apoptosis (Byrd *et al.*, 2002; Golay *et al.*, 2000; Pederson *et al.*, 2002; Treon *et al.*, 2001). Rituximab also induces calcium influx, contributing to apoptosis. Rituximab induces apoptosis *in vitro* within 4 h; this induction is independent of complement but requires cross-linking with anti-Fcγ antibody. The ratio of the antiapoptotic protein Mcl-1 to the proapoptotic protein Bax was significantly elevated in CLL patients who did not respond to rituximab compared with that in responders (Bannerji *et al.*, 2003). Complement activation might be important, as increased expression of complement inhibitors CD55 and CD59 resulted in resistance to rituximab in NHL cell lines and CLL cells (Golay *et al.*, 2001; Treon *et al.*, 2001). Blocking CD55 and CD59 resulted in a fivefold to sixfold increase in rituximab-induced cell lysis of poorly responding CLL samples, although CD55 and CD59 levels did not predict complement susceptibility (Golay *et al.*, 2001). However, baseline expression of CD55 and CD59 were not associated with clinical response to rituximab in 21 treated patients (Bannerji *et al.*, 2003). Thus, rituximab might exert its anticancer effects through more than one mechanism of action, but induction of apoptosis appears to be critical.

2. *Clinical Studies: Single-Agent Weekly Rituximab*

Phase I clinical studies in indolent B-cell NHL established a dose of 375 mg/m^2 given by intravenous (IV) infusion weekly for four doses, although the length of treatment was empirically established. In the pivotal Phase II trial in 166 patients with relapsed or refractory indolent B-cell NHL or CLL, an overall response rate (ORR) of 48% was seen (CR 6%), with a median response duration of 12 months (McLaughlin *et al.*, 1998). Subsequent analysis of this study showed that patients with indolent follicle center B-cell NHL had an ORR of 60%, whereas only 4 of 30 patients with SLL/CLL (13%) responded. A British study of 48 patients employing the same dosing schedule achieved only one PR in 10 patients (10%) with relapsed or refractory SLL/CLL, although the ORR was only 27% in patients with follicular lymphoma (Nguyen *et al.*, 1999). A similar study observed only one PR in nine evaluable patients (11%) with fludarabine-refractory CLL, although seven patients had stable disease (Winkler *et al.*, 1999). A study of seven patients with refractory or relapsed CLL observed a striking, but transient, reduction (median 93%) in peripheral lymphocyte count, but nodal disease was not affected (Ladetto *et al.*, 2000). The German CLL Study Group administered weekly rituximab to 28 patients with previously treated CLL; seven patients (25%) achieved PR with a median duration of 20 weeks. Forty-five percent of patients experienced at least 50% reduction of peripheral lymphocyte count lasting 4 weeks or longer (Huhn *et al.*, 2001). Finally, a Nordic multicenter study observed an ORR of 35% in 24 heavily pretreated CLL patients, with a median duration of response of only 12.5 weeks. Interestingly, 17 of 20 (85%) patients with adenopathy experienced >50% reduction in nodal disease, whereas only 2 of 18 (11%) patients (Itala *et al.*, 2002) had reduction of marrow infiltration.

Thus, weekly administration of rituximab has limited activity in CLL. Rituximab effectively reduces peripheral blood lymphocytosis, but is less effective at reducing bone marrow or nodal disease. The preferential response of peripheral lymphocyte count might be due to increased CD20 expression on circulating CLL cells compared with that in bone marrow cells. In quantitative flow cytometric studies, circulating CLL cells bound an average of 9050 anti-CD20 molecules, compared with only 4070 molecules for bone marrow CLL cells and 3950 molecules for lymph node CLL cells (Huh *et al.*, 2001). This increased binding to circulating peripheral blood CLL cells might explain the ability of rituximab to preferentially reduce peripheral lymphocyte count. Stromal cells in bone marrow and lymph nodes might also provide an additional survival advantage to CLL cells in these environments over circulating CLL cells.

3. *Upfront Therapy with Rituximab*

Data suggest that weekly rituximab might be more effective in previously untreated SLL/CLL. Forty-four previously untreated patients with SLL/CLL

TABLE I Selected Phase II trials of weekly Rituximab in CLL/SLL

Reference (Authors/year)	*Doses*	*Prior therapy*	*Evaluable patients*	*Response rate (ORR)*
McLaughlin *et al.*, 1998	4	Yes	30	13%
Nguyen *et al.*, 1999	4	Yes	10	10%
Winkler *et al.*, 1999	4	Yes	9	11%
Ladetto *et al.*, 2000	4	Yes	7	0%
Huhn *et al.*, 2001	4	Yes	28	25%
Hainsworth *et al.*, 2003	4	No	44	51%
Thomas *et al.*, 2001	8	No	21	90%

received four weekly doses of rituximab 375 mg/m^2; the ORR after the first course of rituximab was 51% (CR 4%). Twenty-eight patients with stable or responsive disease received additional maintenance therapy with 4-week courses of rituximab every 6 months for up to four cycles. Maintenance therapy increased the ORR to 58% (CR 9%). However, the median progression-free survival (PFS) of 19 months was shorter than the 36–40 median PFS obtained by the same group, using an identical regimen in previously untreated patients with follicle center NHL (Hainsworth *et al.*, 2003). A study of eight weekly doses of rituximab 375 mg/m^2 in 31 untreated, early-stage CLL patients (21 evaluable) with beta-2 microglobulin levels $\geq$ 2.0 mg/dl showed an ORR of 90% (CR 19%, nodular PR 19%) (Thomas *et al.*, 2001). Even in this group of previously untreated patients with limited disease, the majority of patients achieved PR only, with few patients attaining CR. In addition, initial results indicate that median PFS in response to upfront rituximab is inferior to that observed in response to frontline fludarabine therapy. Thus, rituximab as a single agent is unlikely to significantly alter long-term survival in CLL. The results of clinical trials using weekly dosing of rituximab in CLL are summarized in Table I.

4. Limitations of Weekly Rituximab in CLL

Several theories may explain the inferior efficacy of weekly rituximab in CLL compared to its activity in indolent follicle center NHL. First, CLL/SLL cells express lower CD20 density than follicle center NHL cells, decreasing the number of target antigen sites and the amount of antibody delivered to individual tumor cells. In an analysis of 70 patients with chronic B-cell leukemias and 17 normal donors, normal B lymphocytes expressed 94,000 CD20 molecules per cell. Other chronic B-cell leukemias such as mantle cell lymphoma and hairy cell leukemia expressed between 123,000 and 312,000 CD20 molecules per cell, but CLL cells expressed only 65,000 CD20 molecules per cell (Ginaldi *et al.*, 1998). However, an analysis of 10 patients with

CLL did not identify a correlation between CD20 expression and clinical response to rituximab (Perz *et al.*, 2002).

A more plausible explanation is that the large intravascular burden of circulating CLL cells might alter the pharmacokinetics of rituximab and result in accelerated clearance of antibody from plasma. Lower trough concentrations of rituximab were observed in CLL patients who did not respond to therapy, and the importance of serum rituximab levels was previously documented in follicle center NHL (Berinstein *et al.*, 1998; J. C. Byrd, personal communication, 2001). Detectable plasma levels of rituximab are seen for more than 6 months after therapy in follicle center NHL, but serum concentrations of rituximab decrease more rapidly after treatment in CLL. In addition, the presence of soluble CD20 in the serum of CLL patients suggests that free CD20, derived from cell membrane fragments or shed antigen, might contribute to rapid clearance of rituximab. However, a relationship between soluble CD20 levels and response to rituximab has not been demonstrated (Keating *et al.*, 2002d). Finally, intrinsic mechanisms of resistance, such as overexpression of the antiapoptotic proteins Bcl-2, Mcl-1, and XIAP or p53 mutations or deletions, might contribute to the common resistance of CLL to rituximab and cytotoxic therapy. As previously discussed, the ratio of Mcl-1 to Bax was significantly elevated in nonresponding compared with responding CLL patients treated with rituximab (Bannerji *et al.*, 2003).

5. Dose Escalation: Improved Clinical Response

Investigators have taken two different strategies to overcome these pharmacokinetic and pharmacodynamic obstacles. In a single-institution study, 50 patients with previously treated CLL ($n = 40$) or other B-cell leukemias ($n = 10$) received weekly rituximab dose escalated to 2250 mg/m^2 (Keating and O'Brien, 2000; O'Brien *et al.*, 2001b). Although no CLL patient achieved CR, the ORR was 40% and a statistically significant dose–response relationship was observed; 22% of patients treated with 500–850 mg/m^2 responded, compared with 75% of patients treated with 2250 mg/m^2. The ORR was 36% for CLL and 60% for other B-cell leukemias; median response duration was 8 months. Eight of 12 patients (67%) at 2250 mg/m^2 developed grade 2 toxicity, primarily fatigue, but no grade 3 or 4 toxicity was observed.

In an alternative approach, 33 patients with relapsed or refractory SLL/CLL received thrice-weekly rituximab for 4 weeks (Byrd *et al.*, 2001). Patients received 100 mg over 4 h on the first day of therapy and 375 mg/m^2 thereafter. This stepped-up dosing schedule was designed to minimize infusion-related toxicity. The ORR was 45% (CR 3%), and median response duration was 10 months. Thirteen patients developed transient infusion-related toxicity that appeared to be related to cytokine release (TNF-α, IFN-γ, IL-8, and IL-6) and resolved by the third infusion. Thus, both dose

escalation and thrice-weekly dosing improved the response rate in SLL/CLL and established a role for rituximab in the treatment of relapsed CLL. Although both approaches produced few complete responses, it is important to remember that no therapeutic agent achieves a significant CR rate in relapsed or refractory CLL.

6. *Toxicity*

Infusion-related side effects constitute the most common toxicity of rituximab; however, these toxicities are generally manageable, particularly with use of a stepped-up dosing schedule. Patients can develop transient hypoxemia, dyspnea, and hypotension, which are partly due to inflammatory cytokine release. Although initial studies suggested that patients with lymphocyte counts higher than 50,000/μl might be at greater risk of this cytokine release syndrome, subsequent larger studies failed to support this finding. Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) peak 90 min after start of infusion, and their rise is accompanied by fever, chills, hypotension, and nausea (Winkler *et al.*, 1999). These side effects are most severe with the first rituximab infusion and resolve by the third infusion in the thrice-weekly dosing schedule (Byrd *et al.*, 2001). An uncommon but potentially severe toxicity is tumor lysis syndrome, which is generally observed in patients with high numbers of circulating CLL cells (Byrd *et al.*, 1999; Jensen *et al.*, 1998). Patients at risk should receive prophylactic allopurinol, hydration, and careful observation, and it might be necessary to administer the first dose of rituximab in an in-patient setting. However, patients who develop tumor lysis syndrome to the first dose of rituximab can safely receive subsequent doses, especially after the number of circulating CLL cells is reduced (Byrd *et al.*, 1999). Other toxicities are minimal and should not affect administration of this antibody. Rare toxicities that can be serious include skin toxicity, pure red cell aplasia, and hepatitis B reactivation.

7. *Combination Therapy*

There is great interest in combining monoclonal antibody therapy with cytotoxic chemotherapy in the treatment of lymphoid malignancies, and several studies have specifically examined rituximab. The low CR rates to single-agent rituximab indicate that combination with traditional cytotoxic drugs or other monoclonal antibodies might be necessary for rituximab to significantly impact long-term survival in CLL. Several clinical trials examined the use of rituximab in combination regimens against B-cell lymphoid malignancies, including CLL (Coiffier *et al.*, 2002; Czuczman *et al.*, 1999; Keating *et al.*, 2000b; McLaughlin *et al.*, 2000; Vose *et al.*, 2001b). The results of these studies are summarized in Table II. Rituximab was successfully combined with fludarabine in both NHL and CLL (Byrd *et al.*, 2003; Czuczman *et al.*, 2000). Concurrent administration of these two agents to

TABLE II Selected trials of Fludarabine combination regimens in previously untreated CLL

Reference (Authors/year)	*Regimen*	*Evaluable patients*	*Response ORR (CR)*	*Median PFS (mo)*
Flinn *et al.*, 2000a	FC	17	100% (47%)	N/A
O'Brien *et al.*, 2001a	FC	34	88% (35%)	N/A
Byrd *et al.*, 2003	FR	51	90% (47%)	N/A
Keating *et al.*, 2002	FCR	135	95% (67%)	N/A

Abbreviations: Fludarabine (F), Cyclophosphamide (C), Rituximab (R).

104 previously untreated CLL patients in a randomized Phase II CALGB trial yielded a higher CR rate (47%) than did sequential administration (28%) (Byrd *et al.*, 2003). Patients received standard fludarabine 25 mg/m^2 Days 1–5 every 4 weeks for six cycles. Patients were randomized to receive concurrent rituximab 375 mg/m^2 on Day 1 of each cycle, with an additional Day 4 dose during Cycle 1, or sequential rituximab 375 mg/m^2 weekly for 4 doses beginning 2 months after completion of fludarabine. The median duration of response was not reached at 23 months.

A multicenter European Phase II study of concurrent fludarabine and rituximab in 31 evaluable patients with CLL achieved an ORR of 87% (CR 32%), with a median duration of response of 75 weeks. Patients received fludarabine 25 mg/m^2 Days 1–5 every 4 weeks for four cycles, and rituximab 375 mg/m^2 every 4 weeks for four doses, beginning Day 1 of Cycle 3 of fludarabine. ORR and CR were similar in previously treated (ORR 91%, CR 45%) and untreated patients (ORR 85%, CR 25%). Sixteen patients developed 32 infections, and one patient died of cerebral hemorrhage due to prolonged thrombocytopenia (Schulz *et al.*, 2002). The highest CR rate was achieved by a single-institution study, using a combination regimen of fludarabine, cyclophosphamide, and rituximab (FCR). A total of 102 evaluable patients received fludarabine 25 mg/m^2 and cyclophosphamide 250 mg/m^2 on Days 2–4 of Cycle 1 and on Days 1–3 of Cycles 2–6, in addition to rituximab 375 mg/m^2 on Day 1 of Cycle 1 and 500 mg/m^2 on Day 1 of Cycles 2–6. The ORR was 73% (CR 23%), and 5 of 13 patients in CR achieved molecular remission (Garcia-Manero *et al.*, 2001). The same authors administered FCR to 135 previously untreated CLL patients with symptomatic disease requiring initiation of therapy by NCI criteria, achieving a CR rate of 67% (Keating *et al.*, 2002). Molecular remissions were observed in 35 of 61 (57%) tested patients who achieved CR; 11 of these 35 patients developed molecular evidence of relapse, usually within 6 months of completing treatment. The major toxicities of this regimen were grade 4 neutropenia and infection, which occurred in 20% and 17%, respectively, of treatment cycles (Wierda *et al.*, 2001).

Finally, an aggressive upfront regimen incorporating fludarabine and rituximab has been given to previously untreated patients with CLL, as cytoreductive therapy prior to autologous stem cell transplantation. Thirteen patients, with a median age of 47 years, received fludarabine 25 mg/m^2 Days 1–3, cyclophosphamide 200 mg/m^2 Days 1–3, and mitoxantrone 10 mg/m^2 Day 1 every 4 weeks for four to six cycles, followed by rituximab 375 mg/m^2 weekly for four doses. All patients responded (ORR 100%, CR 77%), and four patients (31%) achieved a molecular remission (Polliack *et al.*, 2001). The ability of this regimen to induce complete hematologic and molecular remissions is promising, although patients have not been followed long enough to determine whether these initial remissions will be durable.

8. Radioisotope Conjugates of Anti-CD20

Anti-CD20 monoclonal antibody has been conjugated to the radioisotopes yttrium-90 (^{90}Y-ibritumomab, Zevalin) and iodine-131 (^{131}I-tositumomab, Bexxar). Several published clinical trials have demonstrated the efficacy of Zevalin and Bexxar in indolent B-cell NHL, particularly follicle center grades I and II NHL (Kaminski *et al.*, 1993, 1996, 2001; Press *et al.*, 1993; Vose *et al.*, 2000b; Wiseman *et al.*, 1999; Witzig *et al.*, 1999). The results of these trials have been extensively reviewed and are not discussed here. There has been reluctance to use Zevalin and Bexxar in patients with SLL/CLL, because of the concern about myelotoxicity resulting from bystander radiation to normal hematopoietic cells in patients with significant marrow disease. However, results of a Phase I dose escalation study indicated that Bexxar is effective in previously treated patients with advanced CLL. Eleven patients with heavily pretreated CLL received a total body dose of 35–55 cGy; three patients (27%) achieved PR and six patients (55%) had stable disease (Gupta *et al.*, 2001). As expected, myelosuppression was the dose-limiting toxicity and was related to the total radiation dose. Thus, though CLL cells are sensitive to radiation, myelotoxicity limits the clinical use of Zevalin and Bexxar in patients with significant marrow involvement. However, radioisotope conjugates might be more effective than rituximab in SLL/CLL patients with bulky nodal disease, because of delivery of radiation to surrounding tumor cells. Future studies of Zevalin and Bexxar in SLL/CLL should focus on patients with primarily bulky lymphadenopathy and limited marrow involvement. In addition, sequential combination regimens with agents such as Campath-1H, which effectively reduces blood and marrow disease but has limited activity against nodal disease, should be investigated.

9. Summary

Rituximab, the best characterized and most widely used monoclonal antibody in hematological malignancies, is active in CLL, although dose

intensification is necessary to obtain maximal clinical benefit. However, single-agent rituximab produces few complete responses and does not, by itself, substantially improve long-term survival in CLL. The combination of rituximab with fludarabine has yielded improved CR rates, and further studies of such combination regimens are needed to determine whether these higher CR rates result in improved long-term survival. Although most studies have combined rituximab with traditional cytotoxic agents, several trials are now examining the use of rituximab with other monoclonal antibodies such as Campath-1H. Thus, the optimal role of rituximab in treatment of CLL remains to be established.

C. Campath-1H

1. Preclinical Studies

Campath-1H® (alemtuzumab) is a humanized anti-CD52 monoclonal antibody that effectively fixes complement and depletes normal lymphocytes and lymphoma cells (Flynn and Byrd, 2000; Hale *et al*., 1983, 1988). CD52 is a 21- to 28-kDa glycopeptide expressed on the surface of nearly all human lymphocytes, monocytes, and macrophages (Domagala and Kurpisz, 2001; Rowan *et al*., 1998; Treumann *et al*., 1995). CD52 is expressed on a small subset of granulocytes, but CD52 is not expressed on erythrocytes, platelets, or bone marrow stem cells. CD52 is expressed on all CLL cells and indolent B-cell NHL cells (Hale *et al*., 1985; Salisbury *et al*., 1994). Its physiological function remains unknown, but cross-linking of CD52 on B-cell and T-cell lymphoma cell lines resulted in growth inhibition (Rowan *et al*., 1998). Despite its small size, antibody binding of the CD52 antigen results in profound complement activation and ADCC. In addition, CD52 is not shed, internalized, or modulated. Thus, CD52 is an ideal antigen for targeted immunotherapy. However, the ubiquitous expression of CD52 on normal lymphocytes and monocytes has resulted in increased hematological and immune toxicity with Campath-1H, manifested by neutropenia, prolonged lymphopenia, and infectious complications.

Campath-1H acts *in vivo* by inducing programmed cell death. *In vivo* blood samples showed 19–92% reduction in expression of the antiapoptotic protein Bcl-2 in 6 of 8 patients undergoing Campath-1H therapy (J. C. Byrd, personal communication, 2001). In addition, expression of the antiapoptotic proteins Mcl-1 and XIAP was downregulated by treatment with Campath-1H. Campath-1H induced activation of caspase 3 and cleavage of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP), indicating that apoptosis is an important mechanism of action of this antibody (J. C. Byrd, personal communication, 2001).

2. *Clinical Trials*

Phase I studies established a dose of 30 mg IV thrice weekly for 4–12 weeks. Campath-1H induced significantly more infusion toxicity than did rituximab, and a stepped-up dosing schedule was necessary to diminish initial infusion toxicity and make the antibody tolerable. An initial dose of 3 mg was given on Day 1, 10 mg on Day 2, and 30 mg on Day 3; once the full dose of 30 mg was achieved, patients were given 30 mg thrice weekly. Several clinical studies established the efficacy of Campath-1H in CLL (Bowen *et al.*, 1997; Keating *et al.*, 2000a, 2002; Osterborg *et al.*, 1996, 1997). The results of these studies are summarized in Table III. A multicenter European Phase II study administered Campath-1H 30 mg thrice weekly for up to 12 weeks to 29 recurrent and refractory CLL patients. The ORR was 42%, but only one patient (4%) achieved CR (Osterborg *et al.*, 1997). The antibody cleared CLL cells from the peripheral blood in 97% of patients, but was substantially less effective at eliminating marrow (36%) or nodal disease (7%).

The pivotal trial administered the same Campath-1H regimen to 93 heavily pretreated, fludarabine-refractory CLL patients; an intent-to-treat ORR of 33% was observed, although only 2% of patients achieved CR (Keating *et al.*, 2002c). Median time to progression for responders was 9.5 months, with a median overall survival of 16 months for all patients and 32 months for responders. The median peripheral blood CLL count decreased by more than 99.9%, but the antibody was less effective against nodal disease. Although 74% of all patients with nodal disease responded, with 27% experiencing resolution of their adenopathy, patients with bulky lymph nodes did significantly poorer. Whereas 90% of patients with lymph nodes measuring $\leq$2 cm responded, with 64% achieving resolution of their adenopathy, only 59% of patients with lymph nodes $>$5 cm responded, with no patients enjoying resolution of their adenopathy. All patients were placed on prophylactic antibacterial and antiviral agents, and toxicity was

TABLE III Selected phase II trials of thrice weekly Campath-1H in CLL

Reference (Authors/year)	*Weeks*	*Route*	*Prior therapy*	*Evaluable patients*	*ORR (CR)*
Osterborg *et al.*, 1997	12	IV	Yes	29	42% (4%)
Ferrajoli *et al.*, 2003	4	IV	Yes	78	35% (12%)
Rai *et al.*, 2001	12	IV	Yes	136	40% (7%)
Keating *et al.*, 2002c	12	IV	Yes	92	33% (2%)
Lundin *et al.*, 2002	18	SC	No	38	87%

Abbreviations: Intravenous (IV), Subcutaneous (SC).

manageable, in contrast to previous trials of Campath-1H. However, patients with poor performance status did markedly worse than patients with no or minimal symptoms from their disease. As a result of this pivotal CAM211 study, Campath-1H was recently approved for the treatment of fludarabine-refractory CLL in the United States.

The activity of Campath-1H in CLL was confirmed by a multi-institutional study in 136 patients with fludarabine-refractory B-CLL who received Campath-1H 30 mg thrice weekly for up to 12 weeks on a compassionate basis (Rai *et al.*, 2001). The ORR was 40% (CR 7%), and the median progression-free and overall survivals of responders were 7.3 and 13.4 months, respectively. Similarly, 41 patients with relapsed B-CLL and one patient with T-CLL were treated with Campath-1H 30 mg IVB thrice weekly for 4 weeks in a single-institution study (Ferrajoli *et al.*, 2001). Two patients with B-CLL achieved CR (5%), and nine patients achieved PR (21%) for an ORR of 26%. Interestingly, 7 of 12 patients with B-cell or T-cell prolymphocytic leukemia (B- or T-PLL) responded (3 CR, 4 PR, ORR 58%). Although Campath-1H was more effective at eliminating disease in peripheral blood (CR 36%, PR 36%) and bone marrow (CR 41%, PR 28%) than in lymph nodes (CR 23%, PR 13%), a greater response in nodal disease was seen in this study than in previous trials that used Campath-1H. A recent update of this study showed an ORR of 35% (CR 12%) in 78 patients with indolent lymphoproliferative disorders (42 CLL), with a median duration of response of 18 months (Ferrajoli *et al.*, 2003). Although Campath-1H is effective therapy in previously treated patients with CLL, the antibody is less effective against bulky lymphadenopathy than it is against peripheral blood or bone marrow disease.

3. Upfront Therapy in Previously Untreated Patients

A Phase II clinical trial administered subcutaneous (SC) Campath-1H to 41 previously untreated patients with CLL. Patients received a prolonged course of Campath-1H 30 mg SC three times per week for up to 18 weeks. Except for transient grade I or II fever, first-dose reactions were minimal. The ORR was 87% in the 38 patients who received at least 2 weeks of treatment, and the intent-to-treat ORR was 81% (Lundin, 2002). Campath-1H was most effective at clearing disease from peripheral blood (CR 95%), but bone marrow (CR + nodular PR 66%) and nodal disease (ORR 87%, CR 29%) also responded to therapy. Interestingly, some patients who achieved CR in the bone marrow required the full 18 weeks of therapy to do so, suggesting that prolonged administration of Campath-1H might be necessary to clear CLL from the bone marrow. Median time to treatment failure had not been reached at time of study report (18+ months). These results confirmed that subcutaneous administration of Campath-1H is feasible, as had been initially shown in trials in rheumatoid arthritis, and indicate that longer courses of Campath-1H might produce ORR and CR

rates similar to those observed with fludarabine. However, subcutaneous administration of Campath-1H is currently not FDA-approved for CLL.

4. Immunosuppression and Infectious Complications

Infections constitute the major complication of Campath-1H therapy (Flynn and Byrd, 2000; Khorana *et al*., 2001; Tang *et al*., 1996). All 50 previously treated indolent NHL patients in a multicenter European study developed profound lymphopenia. Opportunistic infections and bacterial septicemia occurred in 14% and 18% of patients, respectively, and 6% of patients died of infectious complications (Lundin *et al*., 1998). Infections occurred in 55% of patients (27% grade 3 or 4) in the CAM211 study, and 13% developed septicemia (Keating *et al*., 2002c). Campath-1H also inhibits B-cells, CD8+ T-cells, natural killer (NK) cells, and monocytes, but the antibody's most profound effects are on CD4+ T-lymphocytes (Bretl *et al*., 1996; Condiotli and Nagler, 1996; Fabian *et al*., 1993). Treatment with 5–10 daily IV infusions of Campath-1H almost completely depleted lymphocytes, and lymphocyte subsets recovered with varying kinetics. NK cells and monocytes recovered to normal levels within 1–2 months, whereas B-cell numbers returned to normal within 5 months. However, CD8+ T-cells returned to 50% of pretreatment levels by 2 months but did not increase further, and CD4+ T-cells never reached 20% of pretreatment levels despite a 18-month follow-up (Brett *et al*., 1996). In a Swedish study of 41 CLL patients given SC Campath-1H for up to 18 weeks, NK and NK-T cells remained severely suppressed more than 12 months afterwards; however, no late infectious complications were observed (Rezvany *et al*., 2002).

Paroxysmal nocturnal hemoglobinuria (PNH)-like T-cells emerge during or immediately after Campath-1H treatment in many patients. These PNH-like cells cannot synthesize glycosylphosphatidylinositol (GPI) anchor glycans and therefore lack GPI-linked surface proteins, including CD52. As a result, these cells are resistant to CD52-mediated killing. Preliminary studies by one group demonstrated that, despite lacking GPI-linked proteins, these PNH-like T-cells are functional immune effector cells (Kennedy *et al*., 2000). This finding might explain why the great majority of severe opportunistic infections that occur with Campath-1H are observed during active Campath-1H therapy rather than after treatment. Such escape has not been observed with normal B-cells or malignant CLL cells.

Lymphocyte recovery might depend on the dosing schedule, as the absolute CD4+ T-cell count reached a nadir of 2/μl by Week 4 but increased to 84/μl by Week 12 in the CAM211 trial (Keating *et al*., 2002c). In 42 refractory CLL patients (median CD3+ T-cell count 1900/μl) treated with Campath-1H, extreme lymphopenia of less than 30/μl was seen in all patients after a median of 2 weeks of therapy. At a median follow-up of 14 months, the median CD3+ T-cell count recovered to 930/μl and the median CD4+ T-cell count to 320/μl (Kennedy *et al*., 2000). Campath-1H depleted

CD52+ myeloid peripheral blood dendritic cells, resulting in inhibition of the stimulatory activity of peripheral blood mononuclear cells (PBMCs) in allogeneic mixed lymphocyte reactions. Depletion of CD52+ dendritic cells also inhibited the ability of PBMCs to present antigen to purified CD4+ T lymphocytes (Buggins *et al.*, 2001). This effect might explain the low rate of graft-versus-host disease (GVHD) in allogeneic stem cell transplants using Campath-1H (Hale *et al.*, 1998, 2000).

This prolonged inhibition of T lymphocyte and dendritic cell function will likely limit the clinical use of Campath-1H, particularly in combination regimens with other immunosuppressive agents such as fludarabine. Patients receiving Campath-1H must be placed on appropriate prophylaxis for *Pneumocystis pneumoniae* (PCP) and varicella zoster virus (VZV). In addition, patients should also be monitored for cytomegalovirus (CMV) reactivation during and immediately after therapy (minimum 2 months). With these prophylactic measures, Campath-1H can be administered safely and with acceptable toxicity.

5. Infusion Toxicity

Infusion-related toxicity occurred in 93% of patients in the CAM211 study, although the majority of reactions were grade 1 or 2. Rigors (90% overall, 14% grade 3), fever (85% overall, 17% grade 3, 3% grade 4) and nausea (53%) were the most infusion-related toxicities (Keating *et al.*, 2002c). Similar rates of rigors (71%), fevers (65%), and nausea (45%) were reported in the multicenter study of 136 B-CLL patients, and almost all infusion toxicities were grade 1 or 2 (Rai *et al.*, 2001). Most toxicity was observed with the first infusion (Keating *et al.*, 2002c). This first-dose cytokine release syndrome involves TNF-α, interferon (IFN)-γ, and IL-6 (Wing *et al.*, 1996). TNF-α levels increase by more than 1000-fold after Campath-1H infusion, and TNF-α is most important cytokine in this syndrome (Flynn *et al.*, 2000; Pruzanski *et al.*, 1995). Ligation of the low-affinity Fc receptor for IgG, FcγR, on NK cells results in release of TNF-α and might play a central role in inducing infusion toxicity to Campath-1H (Wing *et al.*, 1996).

6. Hematological Toxicity

Campath-1H has significant hematological toxicity, given the presence of CD52 on many hematopoietic cells. The multicenter study of 136 CLL patients noted 26% neutropenia (22% grade 3 or 4), 35% thrombocytopenia (23% grade 3 or 4), and 21% anemia (11% grade 3) (Rai *et al.*, 2001). In contrast to infusion toxicity, which is predominantly grade 1 or 2, many patients who develop cytopenias develop grade 3 or 4 toxicity, resulting in severe infectious complications. Fever, rigors, and nausea might be bothersome and uncomfortable to patients, but cytopenias and infectious complications constitute the medically serious toxicities of Campath-1H. However, these toxicities are clinically manageable with proper monitoring

of peripheral blood counts and appropriate antibiotic prophylaxis. GMCSF should be avoided, as GMCSF exacerbates infusion-related toxicity by inducing TNF-α, without significantly improving granulocyte recovery (Flinn *et al.*, 2000b).

7. Combination Therapy

Laboratory evidence from our institution indicates that Campath-1H might synergize with fludarabine *in vivo* (J. C. Byrd, personal communication, 2001). A small study of six CLL patients, refractory to fludarabine alone and Campath-1H alone, suggests that such synergy exists. Fludarabine was given at a dose of 25 mg/m^2 IV for 3–5 days, and Campath-1H was given at 30 mg IV thrice weekly for 8–16 weeks. One patient achieved a CR (17%), and five patients achieved a PR for an ORR of 83%; flow cytometric analysis could not detect residual CLL cells in the two patients. Patients received prophylactic cotrimoxazole and acyclovir, and no serious adverse events were noted (Kennedy *et al.*, 2002).

Two studies indicate that the combination of Campath-1H and rituximab can be given safely and might have clinical activity in patients with relapsed CLL. Nine patients received rituximab 375 mg/m^2 Weeks 1 and 3–5, in combination with Campath-1H 3, 10, or 30 mg thrice weekly Weeks 2–5 in a single-institution Phase I dose escalation study (Nabhan *et al.*, 2001). Toxicity was acceptable, with no opportunistic infections or treatment-related deaths. Eight (89%) patients experienced significant reduction (median 95% decrease) in peripheral lymphocyte count, but no objective responses by NCI criteria were seen. A second study administered rituximab 375 mg/m^2 weekly for four doses, with Campath-1H 30 mg on Days 3 and 5 of each week, to 48 patients with relapsed or refractory lymphoproliferative disorders, including 32 patients with CLL and nine patients with CLL/PLL (Faderl *et al.*, 2003). The ORR was 52% (CR 8%), with a median time to progression of 6 months. Similar to results observed with single-agent Campath-1H, nearly all CLL and CLL/PLL patients had resolution of peripheral blood lymphocytosis, but only 11 of 33 (33%) patients had clearing of marrow disease and 24 of 41 (59%) patients had >50% reduction of nodal disease. Infections developed in 52% of patients, and CMV reactivation was seen in 27% of patients.

8. Summary

Because of the ubiquitous expression of CD52 on lymphocytes and monocytes, Campath-1H causes significantly more hematologic and immune toxicity than does rituximab. However, infectious complications are manageable with adequate antibiotic prophylaxis. The majority of patients receiving Campath-1H experience infusion toxicity, but toxicity is manageable with a stepped-up dosing schedule. In addition, infusion toxicity usually diminishes as therapy progresses. Campath-1H has clinical activity in CLL,

and the antibody demonstrates particular efficacy in T-PLL, a disorder for which few therapies exist at present. Campath-1H demonstrates greatest activity against CLL cells in blood, although prolonged therapy might be able to achieve CR in bone marrow. The antibody is less effective against nodal disease; responses, while common, are almost exclusively PR. Further studies of Campath-1H, especially in combination with cytotoxic agents or other monoclonal antibodies, are warranted.

D. Hu1D10

1. Preclinical Studies

Hu1D10 (apolizumab, Remitogen™) is a humanized murine IgG monoclonal antibody whose antigenic epitope is a polymorphic determinant on the MHC class II HLA-DR beta chain (Kostelny *et al.*, 2001). The 1D10 epitope is a variant of the HLA-DR beta chain and is not shed or downregulated by antibody binding (Gingrich *et al.*, 1990). The 1D10 antigen is present on normal and malignant B lymphocytes, dendritic cells, macrophages, and some activated T lymphocytes. The 1D10 antigen is expressed in 50% of acute lymphocytic leukemia, 50% of diffuse large-cell NHL, 50–70% of follicular center cell NHL, and 80–90% of CLL (J. C. Byrd, personal communication, 2002). Expression is uniformly strong in tumors that are ID10 positive. The secondary structure of the beta chain is important for recognition of the epitope, but N-linked glycosylation does not appear to be involved in antigen recognition. The 1D10 antigen is similar, but not identical, to the Lym-1 epitope on HLA-DR.

Hu1D10 induces both ADCC and CDC, and Hu1D10 is more effective at mediating ADCC than is murine 1D10 in standard chromium release assays (Kostelny *et al.*, 2001). Hu1D10 also induces apoptosis, induces changes in intracellular calcium concentrations, and increases tyrosine phosphorylation in 1D10-positive cells. Data indicate that apoptosis occurs by a caspase-independent pathway. Preliminary *in vitro* data from our laboratory demonstrated maximal induction of apoptosis after incubation with 10 μg/ml Hu1D10 and goat antihuman Fc antibody, and apoptosis occurred in the absence of complement or effector cells (J. C. Byrd, personal communication, 2002). Incubation with Hu1D10 alone did not induce apoptosis, and further experiments with anti-Fcγ and secondary F(ab′)2 fragments provided further evidence that Fc-specific binding is necessary for apoptosis. Pharmacokinetic data obtained in rhesus monkeys indicated a significantly shorter half-life in 1D10+ animals (2.6 days) than in ID10− animals (8.4 days), with a 2.6-fold lower area under the curve (AUC). A rapid decline in serum Hu1D10 concentration was seen in 1D10+ animals, likely because of a large antigen sink and development of anti-Hu1D10 antibodies. Preliminary pharmacokinetic data in humans indicate a median serum half-life of approximately 11 days, although profound interpatient variability was observed.

2. *Clinical Studies*

An initial Phase I study in 20 patients with NHL demonstrated that Hu1D10 can be given safely at doses that show potential clinical efficacy (Link *et al*., 2001b). Patients received weekly doses ranging from 0.15 to 5 mg/kg, and a regimen giving the drug on 5 consecutive days was also examined. As is the case with other monoclonal antibody therapies, infusion-related toxicity was common but manageable. Observed side effects included fever, chills, nausea, vomiting, rash, flushing, and hypotension, but most toxicities were grade 1 or 2. Hu1D10 showed exciting clinical promise in this Phase I trial; 4 of 8 patients with follicular lymphoma achieved clinical response (1 CR, 3 PR), with a median time to response of 106 days. A recent report summarized preliminary results of a Phase II multicenter study in patients with relapsed or refractory indolent B-cell lymphoproliferative disorders. Twenty-one patients have received Hu1D10 0.5 mg/kg or 1.5 mg/kg weekly for four doses, including five patients with SLL (Link *et al*., 2001a). Therapy has been well tolerated, although no response data have been reported. We are currently conducting a Phase I dose escalation study of thrice-weekly Hu1D10 in patients with relapsed CLL; initial results have been promising (Abhyankar *et al*., 2002).

3. *Summary*

Hu1D10 is a promising monoclonal antibody that is being evaluated in ongoing clinical trials. Antigen expression appears to be more uniform than CD20 expression, and 80–90% of CLL cells express 1D10. Hu1D10 administration appears to be safe, and preliminary data indicate that 3–6 months might be necessary to see maximal response to the antibody. Although there is little information on Hu1D10's clinical efficacy in CLL, an ongoing Phase I and subsequently planned Phase II study will address this question.

E. ^{131}I-Lym-1

Whereas monoclonal antibodies such as rituximab and Campath-1H rely on host immune mechanisms such as CDC and ADCC to kill tumor cells, radioimmunotherapy uses the antigen specificity of a monoclonal antibody to deliver targeted radiation therapy to tumor cells. The monoclonal antibody Lym-1 recognizes an antigenic determinant on HLA-DR, near the 1D10 epitope. However, the epitopes of the two antibodies are distinct. Initial studies in human tumor cell lines demonstrated that Lym-1 stained B-cell leukemia and lymphoma cell lines but did not react with cells of T-cell, myeloid, or erythroid lineage. Approximately 8% of normal circulating peripheral blood lymphocytes stained for Lym-1 by flow cytometry. Of the B-CLL samples, 40% were positive for Lym-1, whereas T lymphocytes and T-cell lymphomas were negative by both immunoperoxidase stains and flow

cytometry. Thus, Lym-1 specifically recognizes B-cell malignancies but reacts with fewer than 40% of B-CLL.

Lym-1 has been conjugated to ^{131}I in order to effect targeted delivery of this radioactive isotope to tumor cells of B-cell origin. ^{131}I-Lym-1 has been tested primarily in patients with advanced NHL, but the antibody has been given to several patients with B-CLL. Twenty-five patients with previously treated, advanced B-NHL and five patients with relapsed B-CLL were treated with fractionated, low-dose ^{131}I-Lym-1, with a goal of 300 mCi per patient (DeNardo *et al.*, 1998). Thirty percent of patients developed HAMA, but only 3 patients had therapy interrupted as a result. Four of the five CLL patients responded (80%). The same group also reported that patients who responded to ^{131}I-Lym-1 therapy enjoyed improved survival (84 vs. 22 weeks) (De Nardo *et al.*, 1997). Radiation dosimetry studies revealed a lower tumor radiation dose and a higher liver radiation exposure in CLL patients compared with NHL patients, resulting in a lower therapeutic index for patients with CLL (De Nardo *et al.*, 1999a). Toxicity was acceptable, and the dose-limiting toxicity was thrombocytopenia (DeNardo *et al.*, 1999b).

F. ^{90}Y-T101 and Other Anti-CD5 Antibodies

CD5 (T1, Leu-1), a mature T-cell marker that is also expressed in CLL, is the ligand for CD72, which is expressed on all B lymphocytes. Evidence suggests that CD5 stimulates splenic B-cell activation and proliferation through its interaction with CD72 on splenic B lymphocytes (Bikah *et al.*, 1998). Interestingly, *in vitro* studies showed that crosslinking of CD5 on resting B lymphocytes, but not on T lymphocytes, led to apoptosis (Cioca and Kitano, 2002; Pers *et al.*, 1998, 2002). The T101 monoclonal antibody, which recognizes CD5, has been conjugated to ^{90}Y in order to increase its activity against tumor cells. Preclinical studies in human leukemia CEM cells demonstrated that T101 is internalized slowly and undergoes little lysosomal degradation. Instead, T101 undergoes recycling to the cell surface, thus providing a possible explanation for the unmodified antibody's low anticancer efficacy (Ravel *et al.*, 1992). Thus, conjugation to a radioisotope was necessary to increase the clinical activity of T101.

In a Phase I study, two patients with CLL and eight patients with cutaneous T-cell lymphoma (CTCL) were treated with 5 or 10 mCi of ^{90}Y-T101 (Foss *et al.*, 1998). Therapy was complicated by development of HAMA after one cycle in 9 of 10 patients. Despite the fact that only one patient received a second cycle of therapy, both CLL patients and three CTCL patients achieved PR (50%), with a median response duration of 23 weeks. However, significant hematologic toxicity was observed, with T-cell and B-cell suppression lasting 2–3 weeks and more than 5 weeks, respectively.

Finally, the anti-CD5 monoclonal antibody OKT1 has been conjugated to saporin-6 (SAP), a plant ribosome inactivating protein. Fresh CLL cells from 31 patients were exposed *in vitro* to OKT1-SAP. OKT1-SAP inhibited CLL proliferation in 90% of patients; this inhibition was dose dependent, with a 50% inhibitory concentration (IC_{50}) of 4–7 nM (Siena *et al.*, 1989).

G. LMB-2

An alternative approach to radioimmunotherapy is conjugation of a monoclonal antibody to a toxin. The antibody delivers the toxin to the tumor cell, which is killed by action of the toxin. LMB-2 (anti-Tac(Fv)-PE38) is a recombinant immunotoxin derived by fusion of the variable Fv portion of the anti-CD25 monoclonal antibody anti-Tac to a truncated form of *Pseudomonas* exotoxin A (Kreitman *et al.*, 1999). CD25 (Tac) is the beta chain of the high-affinity IL-2 receptor and is expressed on the cell surface of T-cell malignancies, including T-CLL (Uchiyama *et al.*, 1981a,b). LMB-2 induced major responses, including 1 CR, in 4 of 4 patients with refractory hairy cell leukemia (HCL) in an initial Phase I clinical study, demonstrating that Fv-based agents can be effective clinically (Kreitman *et al.*, 1999). In a Phase I dose escalation trial, 35 patients with CD25+ lymphomas and leukemias received LMB-2 at dose levels ranging from 2 to 63 μg/kg IVB every other day for three doses; the maximum tolerated dose was 40 μg/kg (Kreitman *et al.*, 2000b). One patient with CLL achieved PR, and seven other patients with other diseases also responded (1 CR, 6 PR). Recently, *in vitro* studies of DSP30, an immunostimulatory phosphorothioate oligodeoxynucleotide, demonstrated that DSP30 increased CD25 expression in 14 of 15 CLL samples. More importantly, DSP30 increased the cytotoxicity of LMB-2 in 12 of 13 CLL samples (Decker *et al.*, 2002). These results indicate that immunomodulatory molecules can increase expression of target antigens on CLL cells and thereby increase activity of monoclonal antibodies against CLL cells. The use of such molecules to increase the antitumor activity of monoclonal antibody therapy is an area of active research.

H. hLL2 and BL22

CD22 (Leu-14), the ligand for CD45RO, is expressed on normal B lymphocytes and B-cell malignancies, including CLL; CD22 is recognized by the murine IgG2 monoclonal antibody LL2 (Stein *et al.*, 1993). Humanized anti-CD22 (hLL2, epratuzumab) is undergoing Phase I and Phase II clinical trials in indolent B-cell NHL, and studies in CLL are being planned (Leung *et al.*, 1994, 1995). Preclinical *in vitro* studies demonstrated that LL2 was rapidly internalized after binding to Raji lymphoma cells, eventually undergoing lysosomal degradation (Shih *et al.*, 1994). To take advantage of this rapid internalization and degradation, LL2 has been conjugated to radioisotopes and biological effectors. LL2 has been conjugated to both ^{131}I and ^{90}Y.

An initial Phase I clinical study with ^{131}I-LL2 revealed no acute toxicities, and 2 of 5 evaluable patients achieved PR. However, grade IV marrow toxicity was observed in 3 of 7 patients who received total doses of 50 mCi, and 3 of 8 patients who received at least two injections developed HAMA (Goldenberg *et al.*, 1991). Subsequent clinical trials of ^{131}I-LL2 in relapsed NHL have demonstrated promising activity, with 3 of seven patients achieving PR in one study and 7 of 21 patients (5 CR, 2 PR) responding in another trial (Linden *et al.*, 1999; Vose *et al.*, 2000a). Two recent reports described the preliminary results of clinical trials of ^{90}Y-hLL2. In one of the reports, 18 patients with indolent B-cell lymphoproliferative disorders received two to four weekly infusions of 2.5 or 5.0 mCi ^{90}Y-hLL2; seven responses were seen, although the single CLL patient did not respond (Linden *et al.*, 2001). In the second study, 20 evaluable patients with recurrent B-cell NHL received ^{131}I-hLL2 (13 patients) or ^{90}Y-hLL2 (7 patients). Myelosuppression was the primary toxicity, and ^{90}Y-hLL2 appeared to exhibit more favorable tumor dosimetry than ^{131}I-hLL2 did (Juweid *et al.*, 1999).

LL2 has also been conjugated to biological effectors. BL22 (RFB4(dsFv)-PE38) is a recombinant immunotoxin generated by fusion of the variable Fv portion of the anti-CD22 monoclonal antibody RFB4 to a truncated form of *Pseudomonas* exotoxin A. In *ex vivo* experiments with fresh tumor cells from 28 patients with B-cell malignancies, including CLL and follicle center NHL, BL22 was cytotoxic to cells of 25 patients (89%), indicating the potential clinical use of this immunotoxin (Kreitman *et al.*, 2000). A recent report documented the efficacy of BL22 in 16 patients with cladribine-resistant HCL (Kreitman *et al.*, 2001). Eleven patients achieved CR (69%), and two patients attained PR (13%). In addition, LL2 has been conjugated to onconase, an amphibian ribonuclease. In preclinical studies, exposure to LL2–onconase was lethal to human Daudi lymphoma cells. LL2–onconase was tolerable to mice and increased the lifespan of SCID mice inoculated with Daudi lymphoma cells (Newton *et al.*, 2001). The results of *in vitro* and animal studies have been intriguing, but there are no data on the safety or efficacy of LL2–onconase in humans. Although there are no data on the use of LL2 or its radioisotope or immunotoxin conjugates in CLL, studies of hLL2 are warranted, given preclinical evidence of activity against CLL cells (Kreitman *et al.*, 2000a).

I. Anti-B4

CD19 (B4, Leu-12) is expressed on pre-B and B lymphocytes. In preclinical studies, the anti-CD19 monoclonal antibody HD37 was conjugated to the ribosome-inactivating protein SAP. HD37–SAP inhibited DNA synthesis in fresh CLL cells and was able to exert greater than a two log kill in B-NHL cells (Bregni *et al.*, 1989). HD37 has been conjugated to a deglycosylated ricin A chain and tested in patients with NHL although no

CLL patients were enrolled in the Phase I trial (Stone *et al.*, 1996). Anti-B4 was conjugated to blocked ricin to generate an immunotoxin (anti-B4-bR), which was administered by 7-day CIVI to 34 patients with relapsed or refractory B-cell neoplasms in a Phase I clinical trial, including 4 patients with CLL. Five clinical responses (2 CR, 3 PR) were observed, in addition to 11 transient responses (Grossbard *et al.*, 1993). The same authors also administered anti-B4-bR by five consecutive daily bolus infusions to 25 patients with refractory B-cell neoplasms; three responses (1 CR, 2 PR) were observed (Grossbard *et al.*, 1992).

J. Anti-CD23

CD23 is another potential target of monoclonal antibody therapy; like CD20 and CD5, CD23 is expressed on the overwhelming majority of CLL cells. A chimeric macaque-derived anti-CD23 antibody, p6G5G1, has been developed (Yabuuchi *et al.*, 2002). Although these antibodies have been developed as possible therapies for asthma and other allergic disorders, the ubiquitous expression of CD23 on CLL cells indicates that preclinical studies of these compounds in CLL should be pursued. Recently, *in vitro* studies of a humanized anti-CD23 monoclonal antibody, IDEC-152, demonstrated that cross-linked IDEC-152 induced apoptosis in fresh CLL cells from 5 patients. In addition, IDEC-152-induced apoptosis was enhanced in the presence of fludarabine or rituximab (Pathan *et al.*, 2001). These promising preclinical results formed the basis for a Phase I study of this agent in CLL, which is at present ongoing.

K. Anticytokine Monoclonal Antibodies

1. Cytokine Modulation

A growing area of interest in monoclonal antibody research is the use of cytokine modulation to increase the activity of antibody therapies. Cytokine modulation can induce apoptosis and increase activity of host immune effector cells, thereby enhancing the antitumor activity of monoclonal antibodies. There are many ongoing clinical trials of combined immunotherapy with cytokines and monoclonal antibodies in hematologic and solid malignancies. Rituximab has been combined with IL-2 and IL-12 in Phase I and Phase II clinical trials in B-cell NHL, and initial results have been promising (Ansell *et al.*, 2002; Friedberg *et al.*, 2000; Keilholz *et al.*, 1999). An alternative approach to cytokine-based immunotherapy is the development of anticytokine monoclonal antibodies. Such antibodies might be effective as single agents or be used as immunomodulators to enhance the efficacy of tumor-targeted antibodies such as rituximab and Campath-1H. Several such antibodies are in preclinical development, and the following section focuses on the scientific rationale for the use of each of these antibodies.

2. *Anti-TNF Alpha*

TNF-α stimulates the proliferation of CLL cells (Digel *et al.*, 1989; Moberts *et al.*, 1989). Serum levels of TNF-α are increased in patients with CLL, and CLL cells produce TNF-α as an autocrine growth regulator (Foa *et al.*, 1990; Mainou-Fowler *et al.*, 2001). Two TNF-α antagonists have been approved by the FDA for clinical use: (1) infliximab, a chimeric anti-TNF-α monoclonal antibody, and (2) etanercept, a recombinant soluble TNF-α receptor/Fc fusion protein. Etanercept (Enbrel®) has been approved for use in rheumatoid arthritis (Moreland *et al.*, 1999; Weinblatt *et al.*, 1999) and there are data supporting its use in the treatment of myelodysplastic syndrome (MDS), myelofibrosis with myeloid metaplasia, and chronic GVHD (Chiang *et al.*, 2002; Deeg *et al.*, 2002; Steensma *et al.*, 2002). In addition, etanercept is undergoing active clinical study in a number of other malignancies. Infliximab (cA2), which has been approved for use in rheumatoid arthritis and Crohn's disease (Elliott *et al.*, 1994; Targan *et al.*, 1997; van Dullemen *et al.*, 1995) has shown activity in the therapy of steroid-refractory GVHD (Kobbe *et al.*, 2001). In addition to neutralizing soluble TNF-α and depriving CLL cells of a vital growth signal, infliximab might also act by binding of transmembrane TNF-α, leading to lysis of TNF-α-expressing cells by ADCC and CDC (Scallon *et al.*, 1995). Infliximab is undergoing clinical investigation in several malignancies, and investigation of infliximab and etanercept as potential therapies in CLL, either as single agents or in combination with antibodies such as rituximab, is warranted.

3. *Anti-Interferon-Gamma*

Another cytokine important in maintaining the survival of CLL cells is IFN-γ. In preclinical laboratory studies, IFN-γ inhibited apoptosis of CLL cells in culture and resulted in prolonged survival. Purified CLL cells synthesized high levels of IFN-γ, indicating an autocrine pathway of tumor cell activation (Buschle *et al.*, 1993). In the same report, 7 of 10 CLL patients demonstrated increased serum levels of IFN-γ, compared to none of 10 healthy control individuals. A later report showed overexpression of IFN-γ receptors by CLL cells, as well as increased numbers of IFN-γ-producing T lymphocytes in patients with CLL (Zaki *et al.*, 2000). These studies provided *in vitro* and *in vivo* evidence of the antiapoptotic activity of IFN-γ in CLL. Anti-IFN-γ monoclonal antibodies have been administered in clinical trials of rheumatoid arthritis; initial results indicate that these antibodies are safe and have clinical activity (Sigidin *et al.*, 2001). Similar to the TNF-α antibodies infliximab and etanercept, the IFN-γ antibodies were developed for treatment of rheumatological disorders, but should be investigated in Phase I and Phase II clinical trials in CLL.

4. Anti-IL-4

Several interleukins inhibit apoptosis of CLL cells and are therefore potential targets of pharmacologic intervention by interleukin antagonists. IL-4 is one of the best studied of these interleukins. Initial *in vitro* studies demonstrated that IL-4 inhibits the TNF-α-induced proliferation of CLL cells, leading to interest in IL-4 as a cytokine therapy for CLL (van Kooten *et al.*, 1992, 1993). However, a Phase I dose escalation trial of IL-4 in 14 patients with CLL who were in PR after treatment with chemotherapy yielded no responses. In fact, 10 patients (71%) had progressive disease, with a twofold to fourfold increase in the blood lymphocyte count, providing *in vivo* evidence of the antiapoptotic effects of IL-4 (Lundin *et al.*, 2001). Interestingly, the blood lymphocyte count decreased after cessation of IL-4 therapy in 8 of 12 evaluable patients. These clinical results concurred with more recent *in vitro* studies, which showed that IL-4 inhibits apoptosis and maintains viability of CLL cells (Frankfurt *et al.*, 1997; Pu and Bezwoda, 1997). In addition, IL-4 conferred greater protection against apoptosis on CLL cells from previously treated patients than on tumor cells from untreated patients, suggesting that the antiapoptotic action of IL-4 might be one mechanism by which CLL becomes resistant to therapy (Frankfurt *et al.*, 1997). Recent studies revealed that T lymphocytes from patients with B-CLL secrete IL-4 and protect B-CLL cells from apoptosis (Kay *et al.*, 2001; Mainou-Fowler *et al.*, 2001). Further indication of the importance of IL-4 in maintaining CLL viability and growth was shown by the increased expression of mRNA for IL-4 receptor in fresh CLL cells (Mainou-Fowler *et al.*, 2001). Thus, preclinical and clinical data support clinical trials of IL-4 antagonists as potential therapeutic agents in CLL.

5. Anti-IL-8

Another potential cytokine target of monoclonal antibody therapy in CLL is IL-8. *In vitro* studies demonstrated constitutive secretion of IL-8 by CLL cells; in contrast, several B-cell lines and cells from HCL patients did not express IL-8 (di Celle *et al.*, 1994). The same authors later showed that although IL-8 did not induce proliferation of CLL cells, IL-8 protected CLL cells against steroid-induced death. IL-8 increased expression of bcl-2 mRNA and protein, and exogenous IL-8 induced overexpression of IL-8 mRNA, suggesting an autocrine role for IL-8 in maintaining CLL cell survival (di Celle *et al.*, 1996). Fully human anti-IL-8 monoclonal antibodies have been synthesized, as well as a polyethylene glycol (PEG)-conjugated form of a humanized anti-IL-8 F(ab′)2 antibody (Koumenis *et al.*, 2000; Yang *et al.*, 1999). Preclinical evidence suggests that further work should be undertaken to determine whether clinical studies with these antibodies should be pursued in CLL.

IV. T-Cell Prolymphocytic Leukemia

A. The Disease

Patients with T-PLL do extremely poorly, with a median survival of less than 12 months. Patients are typically treated with deoxycoformycin (pentostatin), but novel therapies are desperately needed in this disease.

B. Campath-1H

Several studies have demonstrated significant clinical activity of the anti-CD52 monoclonal antibody Campath-1H in T-PLL. Campath-1H was given to 15 patients with T-PLL, most of whom had failed deoxycoformycin (Pawson *et al*., 1997). The ORR was 73%, and nine patients achieved CR (60%); in addition, retreatment with Campath-1H induced second CR in three patients who relapsed after initial Campath-1H therapy. In comparison, only 3 of 25 (12%) similar T-PLL patients at the same institution achieved CR to deoxycoformycin. Two patients developed severe bone marrow aplasia, and one patient died of this complication. In a subsequent study by the same authors, Campath-1H 30 mg IV was administered thrice weekly until maximal response to 39 patients with T-PLL, including 30 who had failed deoxycoformycin (Dearden *et al*., 2001). The ORR was 76% (CR 60%), with a median disease-free interval of 7 months (range 4–45 months). Finally, a retrospective report of 76 T-PLL patients given Campath-1H thrice weekly for 4–12 weeks demonstrated an ORR of 51% (CR 40%), with a median duration of CR of 9 months (Keating *et al*., 2002a). Median overall survival was 7.5 months, although patients who achieved CR had a median survival of 15 months. Ten patients (13%) developed 15 infections, and severe cytopenias occurred in six patients (8%). Two patients (3%) died of treatment-related mortality. Thus, Campath-1H is the most active single agent in T-PLL, and, in contrast to its results in CLL, is able to produce CR in up to 60% of patients with relapsed T-PLL.

C. Correlation of CD52 Expression with Clinical Response

The higher activity of Campath-1H in T-PLL might be due to increased expression of CD52 on T-PLL cells. Quantitative flow cytometry was used to measure CD52 expression in 24 B-CLL patients, 21 T-PLL patients, and 12 normal volunteers (Ginaldi *et al*., 1998). Interestingly, CD52 expression was significantly higher on normal T lymphocytes than on normal B lymphocytes, and T-PLL cells expressed higher levels of CD52 than did B-CLL cells. In addition, CD52 expression was slightly higher in patients who responded to Campath-1H. These results suggest that the likelihood of

clinical response to Campath-1H might be related to the level of CD52 expression.

V. Hairy Cell Leukemia

A. The Disease

HCL is another disease within the family of indolent B-cell lymphoproliferative disorders. In contrast to CLL or follicle center lymphoma, in which standard chemotherapy is strictly palliative, many patients with HCL are cured with conventional chemotherapeutic agents such as deoxycoformycin (pentostatin) and 2-chlorodeoxyadenosine (2-CDA, cladribine). However, HCL that is resistant to therapy with purine analogs has a poor prognosis, and treatment options are limited for patients who fail pentostatin and cladribine. Thus, despite the generally favorable prognosis of this disease, monoclonal antibody therapy is a treatment modality of significant interest in HCL.

B. Rituximab

The monoclonal antibody that has been best studied in HCL is the anti-CD20 antibody rituximab. Several case reports and a few series have demonstrated rituximab's efficacy in this disease. Ten patients with relapsed or progressive HCL who had previously received treatment with cladribine or pentostatin/IFN-α received four weekly doses of rituximab 375 mg/m^2. The ORR was 50%, with one patient achieving CR and four patients attaining PR. Grade 1 or 2 infusion toxicity occurred during the first dose of rituximab but was extinguished with subsequent doses. Fifty percent of patients experienced >50% reduction of bone marrow involvement 1, 3, and 6 months after completion of rituximab therapy (Lauria *et al.*, 2001). A second study administered a similar schedule of rituximab to eight patients with relapsed HCL and three patients with previously untreated HCL. The ORR was 64%, with six patients achieving CR and one patient PR. The median duration of response was 14 months (range 0–34 months), and infusion-related toxicity was minimal (Hagberg and Lundholm, 2001). To examine whether an increased number of doses results in improved clinical response, a group administered eight weekly doses of rituximab 375 mg/m^2 to 15 patients with relapsed or primary refractory HCL. Patients who achieved PR but not CR received an additional four weekly doses of rituximab. The ORR was 80%; eight patients (53%) attained CR, two patients (13%) achieved hematological CR but had residual (1–5%) marrow involvement by HCL, and two patients (13%) experienced PR. Median duration of response was not reached after a median follow-up of 32 months; five

patients relapsed after 8, 12, 18, 23, and 39 months, and seven remained in remission. Toxicity was minimal, and no infections were noted (Thomas *et al.*, in press).

Thus, rituximab has significant activity in HCL. In contrast to CLL, weekly dosing achieves a significant response rate in HCL, although preliminary data suggest that a longer course of therapy might result in an improved CR rate. Again, in contrast to CLL, rituximab therapy does not appear to induce greater infusion-related toxicity in HCL than was observed in follicle center lymphoma. The efficacy of weekly rituximab therapy and decreased infusion toxicity in HCL, compared with CLL, might be due to the lower circulating tumor burden in HCL and the absence of soluble CD20 in HCL patients.

C. BL-22

As previously described, CD22 (Leu-14) is the ligand for CD45RO and is expressed on normal B lymphocytes and B-cell malignancies such as CLL and HCL. The murine IgG2 monoclonal antibody LL2 recognizes CD22 and has been conjugated to biological effectors in an attempt to target these toxins to HCL cells (Stein *et al.*, 1993). RFB4(dsFv)-PE38 (BL22) is a recombinant immunotoxin generated by fusion of the variable Fv portion of the anti-CD22 monoclonal antibody RFB4 to a truncated form of *Pseudomonas* exotoxin A. *Ex vivo* experiments with fresh tumor cells from patients with B-cell malignancies demonstrated that BL22 was cytotoxic to cells from 25 of 28 patients (89%), suggesting a potential clinical role for this immunotoxin (Kreitman *et al.*, 2000a). Sixteen patients with cladribine-resistant HCL received BL22 every other day for three doses in a Phase I dose escalation study (Kreitman *et al.*, 2001). Eleven patients achieved CR (69%), and two patients attained PR (13%). The three patients who failed to respond received low doses of BL22 or had preexisting antibodies that neutralized the toxin. Median follow-up was 16 months, and 3 of the 11 complete responders relapsed but then attained second CR after retreatment with BL22. Common toxicities were transient hypoalbuminemia and transaminitis, but the most serious toxicity was reversible hemolytic uremic syndrome in two patients.

D. LMB-2

As described previously, LMB-2 (anti-Tac(Fv)-PE38) is a recombinant immunotoxin derived by fusion of the variable Fv portion of the anti-CD25 monoclonal antibody anti-Tac to a truncated form of *Pseudomonas* exotoxin A (Kreitman *et al.*, 1999). CD25 (Tac) is the beta chain of the high-affinity IL-2 receptor and is expressed on the cell surface of T-cell malignancies, as well as HCL (Uchiyama *et al.*, 1981a,b). In an initial Phase I study, LMB-2

induced major responses, including 1 CR, in 4 of 4 patients with refractory HCL who had failed standard therapy (Kreitman *et al*., 1999). Minimal residual disease was detectable by flow cytometry of the bone marrow aspirate of the patient who achieved hematological CR. This initial study was expanded to a larger Phase I dose escalation trial, and 31 additional patients with refractory CD25+ lymphomas and leukemias received LMB-2 at dose levels ranging from 2 to 63 μg/kg IVB every other day for three doses (Kreitman *et al*., 2000b). In contrast to the 100% ORR (25% CR) in HCL, only 4 of the other 31 patients responded and no other patient achieved CR. Thus, refractory HCL is particularly amenable to therapy with LMB-2.

VI. Conclusions

Monoclonal antibody therapy for lymphoid leukemias, particularly CLL, is a rapidly expanding area of translational and clinical investigation. Although antibodies such as rituximab and Campath-1H have shown great promise in CLL, studies have clearly demonstrated that monoclonal antibodies as single agents will not produce long-term survival in patients with lymphoid leukemias. Thus, ongoing clinical trials are examining the optimal use of rituximab and Campath-1H in combination regimens in CLL. Results of initial studies combining monoclonal antibodies with fludarabine and other cytotoxic agents have been promising, and several trials are currently studying monoclonal antibody combinations. Although studies to date have been conducted primarily in CLL, monoclonal antibodies have also shown significant efficacy and promise in T-PLL and HCL. As new monoclonal antibodies are brought into clinical trials, critical challenges will be to (1) identify new antibodies with clinical efficacy, (2) determine the best way to administer these antibodies, and (3) study combination regimens incorporating these antibodies.

Although many monoclonal antibodies being studied in CLL and other lymphoid leukemias are also in clinical trials in lymphomas, each disease must be considered a separate entity. The results of the weekly rituximab trials serve as a reminder that agents and dosing schedules that are effective in lymphoma are not necessarily active in CLL. Similarly, the substantially higher activity of Campath-1H in T-PLL, compared to results in CLL, demonstrates that each monoclonal antibody should be evaluated separately in each disease. Each lymphoid malignancy is different, and investigators must resist the temptation to "lump" diseases together. Only by careful study of each monoclonal antibody in individual lymphoid cancers will the optimal use of each antibody be determined.

Acknowledgments

This work was supported by the National Cancer Institute (P01 CA95426-01A1, TL and JCB), the Sidney Kimmel Cancer Research Foundation (JCB), the Leukemia and Lymphoma Society of America (JCB), and the D. Warren Brown Foundation (JCB).

References

Abhyankar, V. V., Lucas, M. S., Stock, W., Aron, J. L., Briggs, B. R., and Mone, A. (2002). Phase I study of escalated thrice weekly dosing of Hu1D10 in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL): Minimal toxicity and early observation of *in vivo* tumor cell apoptosis. *Proc. Am. Soc. Clin. Oncol.* **21,** 268a.

Allebes, W., Knops, R., Herold, M., Huber, C., Haanen, C., and Capel, P. (1991). Immunotherapy with monoclonal anti-idiotypic antibodies: Tumour reduction and lymphokine production. *Leuk. Res.* **15,** 215–222.

Allebes, W. A., Preijers, F. W., Haanen, C., and Capel, P. J. (1988). The development of non-responsiveness to immunotherapy with monoclonal anti-idiotypic antibodies in a patient with B-CLL. *Br. J. Haematol.* **70,** 295–300.

Ansell, S. M., Witzig, T. E., Kurtin, P. J., Sloan, J. A., Jelinek, D. F., and Howell, K. G. (2002). Phase I study of interleukin-12 in combination with rituximab in patients with B-cell non-Hodgkin's lymphoma. *Blood* **99,** 67–74.

Bannerji, R., Kitada, S., Flinn, I. W., Pearson, M. D., Young, D., and Reed, J. C. (2003). Apoptotic-regulatory and complement-protecting protein expression in chronic lymphocytic leukemia: Relationship to *in vivo* rituximab resistance. *J. Clin. Oncol.* **21,** 1466–1471.

Baselga, J. M., Tripathy, D., Mendelsohn, J., Baughman, S. A., Benz, C. C., and Dantis, L. (1999). Phase II study of weekly intravenous trastuzumab (Herceptin) in patients with HER2/neu-overexpressing metastatic breast cancer. *Semin. Oncol.* **26**(4 Suppl. 12), 78–83.

Berinstein, N. L., Grillo-Lopez, A. J., White, C. A., Bence-Bruckler, I., Maloney, D., and Czuczman, M. S. (1998). Association of serum rituximab (IDEC-C2B8) concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma. *Ann. Oncol.* **9,** 995–1001.

Bikah, G., Lynd, F. M., Aruffo, A. A., Ledbetter, J. A., and Bondada, S. (1998). A role for CD5 in cognate interactions between T cells and B cells, and identification of a novel ligand for CD5. *Int. Immunol.* **101,** 1185–1196.

Boogaerts, M. A., Van Hoof, A., Catovsky, D., Kovacs, M., Montillo, M., and Zinzani, P. L. (2001). Activity of oral fludarabine phosphate in previously treated chronic lymphocytic leukemia. *J. Clin. Oncol.* **19,** 4252–4258.

Bowen, A. L., Zomas, A., Emmett, E., Matutes, E., Dyer, M. J., and Catovsky, D. (1997). Subcutaneous CAMPATH-1H in fludarabine-resistant/relapsed chronic lymphocytic and B-prolymphocytic leukaemia. *Br. J. Haematol.* **96,** 617–619.

Bregni, M., Siena, S., Formosa, A., Lappi, D. A., Martineau, D., and Malavasi, F. (1989). B-cell restricted saporin immunotoxins activity against B-cell lines and chronic lymphocytic leukemia cells. *Blood* **73,** 753–762.

Brett, S., Baxter, G., Cooper, H., Johnston, J. M., Tite, J., and Rapson, N. (1996). Repopulation of blood lymphocyte sub-populations in rheumatoid arthritis patients treated with the depleting humanized monoclonal antibody, CAMPATH-1H. *Immunology* **88,** 13–19.

Brown, S. L., Miller, R. A., Horning, S. J., Czerwinski, D. K., Hart, S. M., and McElderry, R. (1989a). Treatment of B-cell lymphomas with anti-idiotype antibodies alone and in combination with alpha interferon. *Blood* **73,** 651–661.

Brown, S. L., Miller, R. A., and Levy, R. (1989b). Antiidiotype antibody therapy of B-cell lymphoma. *Semin. Oncol.* **16,** 199–210.

Bubien, J. K., Zhou, L. J., Bell, P. D., Frizzell, R. A., and Tedder, T. F. (1993). Transfection of the CD20 cell surface molecule into ectopic cell types generates a Ca^{2+} conductance found constitutively in B lymphocytes. *J. Cell Biol.* **121,** 1121–1132.

Buggins, A. G. S., Mufti, G. J., Fishlock, K., Arno, M., Hale, G., and Waldmann, H. (2001). Peripheral blood dendritic cells express CD52 and are depleted *in vivo* by treatment with Campath-1H. *Blood* **98,** 366a.

Buschle, M., Campana, D., Carding, S. R., Richard, C., Hoffbrand, A. V., and Brenner, M. K. (1993). Interferon gamma inhibits apoptotic cell death in B cell chronic lymphocytic leukemia. *J. Exp. Med.* **177,** 213–218.

Byrd, J. C., Kitada, S., Flinn, I. W., Aron, J. L., Pearson, M. D., and Lucas, D. (2002). The mechanism of tumor cell clearance by rituximab *in vivo* in patients with B-cell chronic lymphocytic leukemia: Evidence of caspase activation and apoptosis induction. *Blood* **99,** 1038–1043.

Byrd, J. C., Murphy, T., Howard, R. S., Lucas, M. S., Goodrich, A., and Park, K. (2001). Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. *J. Clin. Oncol.* **19,** 2153–2164.

Byrd, J. C., Peterson, B. L., Morrison, V. A., Park, K., Jacobson, R. J., and Hoke, E. (2003). Randomized phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic, untreated patients with B-cell chronic lymphocytic leukemia: Results from Cancer and Leukemia Group B 9712 (CALGB 9712). *Blood* **101,** 6–14.

Byrd, J. C., Waselenko, J. K., Maneatis, T. J., Murphy, T., Ward, F. T., and Monahan, B. P. (1999). Rituximab therapy in hematologic malignancy patients with circulating blood tumor cells: association with increased infusion-related side effects and rapid blood tumor clearance. *J. Clin. Oncol.* **17,** 791–795.

Caulfield, M. J., Murthy, S., Tubbs, R. R., Sergi, J., and Bukowksi, R. M. (1989). Treatment of chronic lymphocytic leukemia with an anti-idiotypic monoclonal antibody. *Cleve. Clin. J. Med.* **56,** 182–188.

Chiang, K. Y., Abhyankar, S., Bridges, K., Godder, K., and Henslee-Downey, J. P. (2002). Recombinant human tumor necrosis factor receptor fusion protein as complementary treatment for chronic graft-versus-host disease. *Transplantation* **73,** 665–667.

Cioca, D. P., and Kitano, K. (2002). Apoptosis induction by hypercross-linking of the surface antigen CD5 with anti-CD5 monoclonal antibodies in B cell chronic lymphocytic leukemia. *Leukemia* **16,** 335–343.

Cleary, M. L., Meeker, T. C., Levy, S., Lee, E., Trela, M., and Sklar, J. (1986). Clustering of extensive somatic mutations in the variable region of an immunoglobulin heavy chain gene from a human B cell lymphoma. *Cell* **44,** 97–106.

Coiffier, B., Lepage, E., Briere, J., Herbrecht, R., Tilly, H., and Bouabdallah, R. (2002). CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large B-cell lymphoma. *N. Engl. J. Med.* **346,** 235–242.

Condiotti, R., and Nagler, A. (1996). Campath-1G impairs human natural killer (NK) cell-mediated cytotoxicity. *Bone Marrow Transplant.* **18,** 713–720.

Czuczman, M. S., Grillo-Lopez, A. J., White, C. A., Saleh, M., Gordon, L., and LoBuglio, A. F. (1999). Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. *J. Clin. Oncol.* **17,** 268–276.

Czuczman, M. S., Fallon, A., Scarpace, A., Stewart, C., Bernstein, Z. P., and McCarthy, P. (2000). Phase II study of rituximab in combination with fludarabine in patients (Pts) with low-grade or follicular B-cell lymphoma. *Blood* **96,** 729a.

Dearden, C. E., Matutes, E., Cazin, B., Tjonnfjord, G. E., Parreira, A., and Nomdedeu, B. (2001). High remission rate in T-cell prolymphocytic leukemia with Campath-1H. *Blood* **98,** 1721–1726.

Decker, T., Hipp, S., Kreitman, R. J., Pastan, I., Peschel, C., and Licht, T. (2002). Sensitization of B-cell chronic lymphocytic leukemia cells to recombinant immunotoxin by immunostimulatory phosphorothioate oligodeoxynucleotides. *Blood* **99,** 1320–1326.

Deeg, H. J., Gotlib, J., Beckham, C., Dugan, K., Holmberg, J., and Schubert, M. (2002). Soluble TNF receptor fusion protein (etanercept) for the treatment of myelodysplastic syndrome: a pilot study. *Leukemia* **16,** 162–164.

DeNardo, G. L., DeNardo, S. J., Lamborn, K. R., Goldstein, D. S., Levy, N. B., and Lewis, J. P. (1998). Low-dose, fractionated radioimmunotherapy for B-cell malignancies using 1311-Lym-1 antibody. *Cancer Biother. Radiopharm.* **13,** 239–254.

DeNardo, G. L., DeNardo, S. J., Shen, S., DeNardo, D. A., Mirick, G. R., and Macey, D. J. (1999a). Factors affecting 131I-Lym-1 pharmacokinetics and radiation dosimetry in patients with non-Hodgkin's lymphoma and chronic lymphocytic leukemia. *J. Nucl. Med.* **40,** 1317–1326.

DeNardo, G. L., Lamborn, K. R., Goldstein, D. S., Kroger, L. A., and DeNardo, S. J. (1997). Increased survival associated with radiolabeled Lym-1 therapy for non-Hodgkin's lymphoma and chronic lymphocytic leukemia. *Cancer* **80**(Suppl. 12), 2706–2711.

DeNardo, G. L., O'Donnell, R. T., Rose, L. M., Mirick, G. R., Kroger, I. A., and DeNardo, S. J. (1999b). Milestones in the development of Lym-1 therapy. *Hybridoma* **18,** 1–11.

di Celle, P. F., Carbone, A., Marchis, D., Zhou, D., Sozzani, S., and Zupo, S. (1994). Cytokine gene expression in B-cell chronic lymphocytic leukemia: Evidence of constitutive interleukin-8 (IL-8) mRNA expression and secretion of biologically active IL-8 protein. *Blood* **84,** 220–228.

di Celle, P. F., Mariani, S., Riera, L., Stacchini, A., Reato, G., and Foa, R. (1996). Interleukin-8 induces the accumulation of B-cell chronic lymphocytic leukemia cells by prolonging survival in an autocrine fashion. *Blood* **87,** 4382–4389.

Digel, W., Stefanic, M., Schoniger, W., Buck, C., Raghavachar, A., and Frickhofen, N. (1989). Tumor necrosis factor induces proliferation of neoplastic B cells from chronic lymphocytic leukemia. *Blood* **73,** 1242–1246.

Domagala, A., and Kurpisz, M. (2001). CD52 antigen: A review. *Med. Sci. Monit.* **7,** 325–331.

Elliott, M. J., Maini, R. N., Feldmann, M., Kalden, J. R., Antoni, C., and Smolen, J. S. (1994). Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. *Lancet* **344,** 1105–1110.

Fabian, J., Flidel, O., Gadish, M., Kletter, Y., Slavin, S., and Nagler, A. (1993). Effects of CAMPATH-1 antibodies on the functional activity of monocytes and polymorphonuclear neutrophils. *Exp. Hematol.* **21,** 1522–1527.

Faderl, S., Thomas, D. A., O'Brien, S., Garcia-Manero, G., Kantarjian, H. M., and Giles, F. J. (2003). Experience with alemtuzumab plus rituximab in patients with relapsed and refractory lymphoid malignancies. *Blood* **101,** 3413–3415.

Ferrajoli, A., O'Brien, S. M., Cortes, J. E., Giles, F. J., Thomas, D. A., and Faderl, S. (2003). Phase II study of alemtuzumab in chronic lymphoproliferative disorders. *Cancer* **98,** 773–778.

Ferrajoli, A., O'Brien, S. M., Williams, M. L., Fardel, S., Kantarjian, H., and Keating, M. J. (2001). Campath-1H in refractory hematological malignancies expressing CD-52: A phase II clinical trial of 68 patients. *Blood* **98,** 366a.

Flinn, I. W., Byrd, J. C., Morrison, C., Jamison, J., Diehl, I. E., and Murphy, T. (2000a). Fludarabine and cyclophosphamide with filgrastim support in patients with previously untreated indolent lymphoid malignancies. *Blood* **96,** 71–75.

Flinn, I. W., Sickler, J., Lucas, M., Buj, V., Waymer, S., and Shinn, C. (2000b). Randomized trial of early versus delayed GM-CSF with Campath-1H: Preliminary feasibility and correlative biologic studies results. *Blood* **96,** 838a.

Flynn, J. M., and Byrd, J. C. (2000). Campath-1H monoclonal antibody therapy. *Curr. Opin. Oncol.* **12,** 574–581.

Foa, R., Massaia, M., Cardona, S., Tos, A. G., Bianchi, A., and Attisano, C. (1990). Production of tumor necrosis factor-alpha by B-cell chronic lymphocytic leukemia cells: A possible regulatory role of TNF in the progression of the disease. *Blood* **76,** 393–400.

Foss, F. M., Raubitschek, A., Mulshine, J. L., Fleisher, T. A., Reynolds, J. C., and Paik, C. H. (1998). Phase I study of the pharmacokinetics of a radioimmunoconjugate, 90Y-T101, in patients with CD5-expressing leukemia and lymphoma. *Clin. Cancer Res.* **4,** 2691–2700.

Frankfurt, O. S., Byrnes, J. J., and Villa, L. (1997). Protection from apoptotic cell death by interleukin-4 is increased in previously treated chronic lymphocytic leukemia patients. *Leuk. Res.* **21,** 9–16.

Friedberg, J. W., Neuberg, D. S., Gribben, J. G., Canning, C., Daley, J. F., and Kuhlman, C. (2000). Phase II study of combination immunotherapy with interleukin-2 (IL-2) and rituximab in patients with relapsed or refractory follicular non-Hodgkin's lymphoma (NHL). *Blood* **96,** 730a.

Garcia-Manero, G., O'Brien, S., Cortes, J., Faderl, S., Giles, F. J., and Albitar, M. (2001). Update of results of the combination of fludarabine, cyclophosphamide and rituximab for previously treated patients with chronic lymphocytic leukemia (CLL). *Blood* **98,** 633a.

Ginaldi, L., De Martinis, M., Matutes, E., Farahat, N., Morilla, R., and Catovsky, D. (1998). Levels of expression of CD19 and CD20 in chronic B cell leukaemias. *J. Clin. Pathol.* **51,** 364–369.

Ginaldi, I., De Martinis, M., Matutes, E., Farahat, N., Morilla, R., and Dyer, M. J. (1998). Levels of expression of CD52 in normal and leukemic B and T cells: Correlation with in vivo therapeutic responses to Campath-1H. *Leuk. Res.* **22,** 185–191.

Gingrich, R. D., Dahle, C. E., Hoskins, K. F., and Senneff, M. J. (1990). Identification and characterization of a new surface membrane antigen found predominantly on malignant B lymphocytes. *Blood* **75,** 2375–2387.

Golay, J., Lazzari, M., Facchinetti, V., Bernasconi, S., Borleri, G. M., and Barbui, T. (2001). CD20 levels determine the *in vitro* susceptibility to rituximab and complement of B-cell chronic lymphocytic leukemia: Further regulation by CD55 and CD59. *Blood* **98,** 3383–3389.

Golay, J., Zaffaroni, L., Vaccari, T., Lazzari, M., Borleri, G. M., and Bernasconi, S. (2000). Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab *in vitro*: CD55 and CD59 regulate complement-mediated cell lysis. *Blood* **95,** 3900–3908.

Goldenberg, D. M., Horowitz, J. A., Sharkey, R. M., Hall, T. C., Murthy, S., and Goldenberg, H. (1991). Targeting, dosimetry, and radioimmunotherapy of B-cell lymphomas with iodine-131-labeled LL2 monoclonal antibody. *J. Clin. Oncol.* **9,** 548–564.

Goodman, G. E., Hellstrom, I., Brodzinsky, L., Nicaise, C., Kulander, B., and Hummel, D. (1990). Phase I trial of murine monoclonal antibody L6 in breast, colon, ovarian, and lung cancer. *J. Clin. Oncol.* **8,** 1083–1092.

Grever, M. R., Leiby, J., Kraut, E., Metz, E., Neidhart, J., and Balcerzak, S. (1990). A comprehensive phase I and II clinical investigation of fludarabine phosphate. *Semin. Oncol.* **17**(5 Suppl. 8), 39–48.

Grossbard, M. L., Freedman, A. S., Ritz, J., Coral, F., Goldmacher, V. S., and Eliseo, L. (1992). Serotherapy of B-cell neoplasms with anti-B4-blocked ricin: A phase I trial of daily bolus infusion. *Blood* **79,** 576–585.

Grossbard, M. L., Lambert, J. M., Goldmacher, V. S., Spector, N. L., Kinsella, J., and Eliseo, L. (1993). Anti-B4-blocked ricin: A phase I trial of 7-day continuous infusion in patients with B-cell neoplasms. *J. Clin. Oncol.* **11,** 726–737.

Gupta, N. K., Cao, T. M., French, J. N., Goris, M. L., Capizi, R., and Califano, J. (2001). Pilot study of Bexxar in advanced previously heavily treated refractory chronic lymphocytic leukemia (CLL). *Blood* **98,** 290b.

Hagberg, H., and Lundholm, L. (2001). Rituximab, a chimaeric anti-CD20 monoclonal antibody, in the treatment of hairy cell leukaemia. *Br. J. Haematol.* **115,** 609–611.

Hainsworth, J. D., Litchy, S., Barton, J. H., Houston, G. A., Hermann, R. C., and Bradof, J. E. (2003). Single-agent rituximab as first-line and maintenance treatment for patients with chronic lymphocytic leukemia or small lymphocytic lymphoma: A phase II trial of the Minnie Pearl Cancer Research Network. *J. Clin. Oncol.* **21,** 1746–1751.

Hale, G., Bright, S., Chumbley, G., Hoang, T., Metcalf, D., and Munro, A. (1983). Removal of T cells from bone marrow for transplantation: A monoclonal antilymphocyte antibody that fixes human complement. *Blood* **62,** 873–882.

Hale, G., Dyer, M. J., Clark, M. R., Phillips, J. M., Marcus, R., and Riechmann, L. (1988). Remission induction in non-Hodgkin' lymphoma with reshaped human monoclonal antibody CAMPATH-1H. *Lancet* **2**(8625), 1394–1399.

Hale, G., Jacobs, P., Wood, L., Fibbe, W. E., Barge, R., and Novitzky, N. (2000). CD52 antibodies for prevention of graft-versus-host disease and graft rejection following transplantation of allogeneic peripheral blood stem cells. *Bone Marrow Transplant.* **26,** 69–76.

Hale, G., Swirsky, D., Waldmann, H., and Chan, L. C. (1985). Reactivity of rat monoclonal antibody CAMPATH-1 with human leukaemia cells and its possible application for autologous bone marrow transplantation. *Br. J. Haematol.* **60,** 41–48.

Hale, G., Zhang, M. J., Bunjes, D., Prentice, H. G., Spence, D., and Horowitz, M. M. (1998). Improving the outcome of bone marrow transplantation by using CD52 monoclonal antibodies to prevent graft-versus-host disease and graft rejection. *Blood* **92,** 4581–4590.

Huh, Y. D., Keating, M. J., Saffer, H. L., Jilani, I., Lerner, S., and Albitar, M. (2001). Higher levels of surface CD20 expression on circulating lymphocytes compared with bone marrow and lymph nodes in B-cell chronic lymphocytic leukemia. *Am. J. Clin. Pathol.* **116,** 437–443.

Huhn, D., von Schilling, C., Wilhelm, M., Ho, A. D., Hallek, M., and Kuse, R. (2001). Rituximab therapy of patients with B-cell chronic lymphocytic leukemia. *Blood* **98,** 1326–1331.

Itala, M., Geisler, C. H., Kimby, E., Juvonen, E., Tjonnfjord, G., and Karlsson, K. (2002). Standard-dose anti-CD20 antibody rituximab has efficacy in chronic lymphocytic leukaemia: Results from a Nordic multicentre study. *Eur. J. Haematol.* **69,** 129–134.

Jensen, M., Winkler, U., Manzke, O., Diehl, V., and Engert, A. (1998). Rapid tumor lysis in a patient with B-cell chronic lymphocytic leukemia and lymphocytosis treated with an anti-CD20 monoclonal antibody (IDEC-C2B8, rituximab). *Ann. Hematol.* **77,** 89–91.

Juweid, M. E., Stadtmauer, E. A., Hajjar, G., Sharkey, R. M., Suleiman, S., and Luger, S. (1999). Pharmacokinetics, dosimetry, and initial therapeutic results with 131I- and (111)In-/90Y-labeled humanized LL2 anti-CD22 monoclonal antibody in patients with relapsed, refractory non-Hodgkin's lymphoma. *Clin. Cancer Res.* **5**(Suppl. 10), 3292s–3303s.

Kaminski, M. S., Zasadny, K. R., Francis, I. R., Fenner, M. C., Ross, C. W., and Milik, A. W. (1996). Iodine-131-anti-B1 radioimmunotherapy for B-cell lymphoma. *J. Clin. Oncol.* **14,** 1974–1981.

Kaminski, M. S., Zasadny, K. R., Francis, I. R., Milik, A. W., Ross, C. W., and Moon, S. D. (1993). Radioimmunotherapy of B-cell lymphoma with [1311]anti-B1 (anti-CD20) antibody *N. Engl. J. Med.* **329,** 459–465.

Kaminski, M. S., Zelenetz, A. D., Press, O. W., Saleh, M., Leonard, J., and Fehrenbacher, L. (2001). Pivotal study of iodine I131 tositumomab for chemotherapy-refractory low-grade or transformed low-grade B-cell non-Hodgkin's lymphomas. *J. Clin. Oncol.* **19,** 3918–3928.

Kay, N. E., Han, L., Bone, N., and Williams, G. (2001). Interleukin 4 content in chronic lymphocytic leukaemia (CLL) B cells and blood CD8+ T cells from B-CLL patients: Impact on clonal B-cell apoptosis. *Br. J. Haematol.* **112,** 760–767.

Keating, M. J., Byrd, J. C., Rai, K., Flinn, J., Jain, V., and Binet, L. (2000a). Multicenter study of Campath-1H in patients with chronic lymphocytic leukemia (B-CLL) refractory to fludarabine. *Blood* **96,** 722a.

Keating, M. J., Cazin, B., Coutre, S., Birhiray, R., Kovacsovics, T., and Langer, W. (2002). Campath-1H treatment of T-cell prolymphocytic leukemia in patients for whom at least one prior chemotherapy regimen has failed. *J. Clin. Oncol.* **20,** 205–213.

Keating, M. J., Flinn, I., Jain, V., Binet, J.-L., Hillmen, P., and Byrd, J. C. (2002c). Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: Results of a large international study. *Blood* **99,** 3554–3561.

Keating, M. J., Kantarjian, H., O'Brien, S., Koller, C. A., Talpaz, M., and Schachner, J. (1991). Fludarabine: A new agent with marked cytoreductive activity in untreated chronic lymphocytic leukemia. *J. Clin. Oncol.* **9,** 44–49.

Keating, M. J., Kantarjian, H., Talpaz, M., Redman, J., Koller, C. A., and Barlogie, B. (1989). Fludarabine: A new agent with major activity against chronic lymphocytic leukemia. *Blood* **74,** 19–25.

Keating, M. J., Manshouri, T., O'Brien, S., Wierda, W., Kantarjian, H., and Washington, J. (2002). A high proportion of molecular remission can be obtained with a fludarabine, cyclophosphamide, rituximab combination (FCR) in chronic lymphocytic leukemia (CLL). *Blood* **100,** 205a.

Keating, M. J., O'Brien, S., and Albitar, M. (2002d). Emerging information on the use of rituximab in chronic lymphocytic leukemia. *Semin. Oncol.* **29**(1, Suppl. 2), 70–74.

Keating, M. J., and O'Brien, S. (2000). High-dose rituximab therapy in chronic lymphocytic leukemia. *Semin. Oncol.* **27**(6, Suppl. 12), 86–90.

Keating, M., O'Brien, S., Lerner, S., Giles, F. J., Andreeff, M., and Cortes, J. (2000b). Combination chemo-antibody therapy with fludarabine (F), cyclophosphamide (C) and rituximab (R) achieves a high CR rate in previously untreated chronic lymphocytic leukemia (CLL). *Blood* **96,** 514a.

Keating, M. J., O'Brien, S., Lerner, S., Koller, C. A., Beran, M., and Robertson, L. E. (1998). Long-term follow-up of patients with chronic lymphocytic leukemia (CLL) receiving fludarabine regimens as initial therapy. *Blood* **92,** 1165–1171.

Keating, M. J., O'Brien, S., Kantarjian, H., Plunkett, W., Estey, E., and Koller, C. A. (1993). Long-term follow-up of patients with chronic lymphocytic leukemia treated with fludarabine as a single agent. *Blood* **81,** 2878–2884.

Keilholz, U., Szelenyi, H., Siehl, J., Foss, H. D., Knauf, W., and Thiel, E. (1999). Rapid regression of chemotherapy refractory lymphocyte predominant Hodgkin's disease after administration of rituximab (anti CD20 monoclonal antibody) and interleukin-2. *Leuk. Lymph.* **35,** 641–642.

Kennedy, B., Rawstron, A., Carter, C., Ryan, M., Speed, K., and Lucas, G. (2002). Campath-1H and fludarabine in combination are highly active in refractory chronic lymphocytic leukemia. *Blood* **99,** 2245–2247.

Kennedy, B., Rawstron, A., Richards, S., and Hillmen, P. (2000). Campath-1H in CLL: Immune reconstitution and viral infections during and after therapy. *Blood* **96,** 164a.

Khorana, A., Bunn, P., McLaughlin, P., Vose, J., Stewart, C., and Czuczman, M. S. (2001). A phase II multicenter study of Campath-1H antibody in previously treated patients with nonbulky non-Hodgkin's lymphoma. *Leuk. Lymp.* **41,** 77–87.

Kitada, S., Andersen, J., Akar, S., Zapata, J. M., Takayama, S., and Krajewski, S. (1998). Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: Correlations with *in vitro* and *in vivo* chemoresponses. *Blood* **91,** 3379–3389.

Kobbe, G., Schneider, P., Rohr, U., Fenk, R., Neumann, F., and Aivado, M. (2001). Treatment of severe steroid refractory acute graft-versus-host disease with infliximab, a chimeric human/mouse antiTNFalpha antibody. *Bone Marrow Transplant.* **28,** 47–49.

Kostelny, S. A., Link, B. K., Tso, J. Y., Vasquez, M., Jorgensen, B. H., and Wang, H. G. (2001). Humanization and characterization of the anti-HLA-DR antibody 1D10. *Int. J. Cancer* **93,** 556–565.

Koumenis, I. L., Shahrokh, Z., Leong, S., Hsei, V., Deforge, L., and Zapata, G. (2000). Modulating pharmacokinetics of an inter-leukin-8 F(ab')(2) by amine-specific PEGylation with preserved bioactivity. *Int. J. Pharm.* **198,** 83–95.

Kreitman, R. J., Margulies, I., Stetler-Stevenson, M., Wang, O. C., Fitzgerald, D. J., and Pastan, I. (2000). Cytotoxic activity of disulfide-stabilized recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) toward fresh malignant cells from patients with B-cell leukemias. *Clin. Cancer Res.* **6,** 1476–1487.

Kreitman, R. J., Wilson, W. H., Bergeron, K., Raggio, M., Stetler-Stevenson, M., and Fitzgerald, D. J. (2001). Efficacy of the anti-CD22 recombinant immunotoxin BL22 in chemotherapy-resistant hairy-cell leukemia. *N. Engl. J. Med.* **345,** 241–247.

Kreitman, R. J., Wilson, W. H., Robbins, D., Margulies, I., Stetler-Stevenson, M., and Waldmann, T. A. (1999). Responses in refractory hairy cell leukemia to a recombinant immunotoxin. *Blood* **94,** 3340–3348.

Kreitman, R. J., Wilson, W. H., White, J. D., Stetler-Stevenson, M., Jaffe, E. S., and Giardina, S. (2000b). Phase I trial of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. *J. Clin. Oncol.* **18,** 1622–1636.

Ladetto, M., Bergui, L., Ricca, I., Campana, S., Pileri, A., and Tarella, C. (2000). Rituximab anti-CD20 monoclonal antibody induces marked but transient reductions of peripheral blood lymphocytes in chronic lymphocytic leukaemia patients. *Med. Oncol.* **17,** 203–210.

Lauria, F., Lenoci, M., Annino, L., Raspadori, D., Marotta, G., and Bocchia, M. (2001). Efficacy of anti-CD20 monoclonal antibodies (Mabthera) in patients with progressed hairy cell leukemia. *Haematologica* **86,** 1046–1050.

Leung, S. O., Goldenberg, D. M., Dion, A. S., Pellegrini, M. C., Shevitz, J., and Shih, L. B. (1995). Construction and characterization of a humanized, internalizing, B-cell (CD22)-specific, leukemia/lymphoma antibody, LL2. *Mol. Immunol.* **32,** 1413–1427.

Leung, S. O., Shevitz, J., Pellegrini, M. C., Dion, A. S., Shih, L. B., and Goldenberg, D. M. (1994). Chimerization of LL2, a rapidly internalizing antibody specific for B cell lymphoma. *Hybridoma* **13,** 469–476.

Leveille, C., Al-Daccak, R., and Mourad, W. (1999). CD20 is physically and functionally coupled to MHC class II and CD40 on human B cell lines. *Eur. J. Immunol.* **29,** 65–74.

Levy, S., Mendel, E., Kon, S., Avnur, Z., and Levy, R. (1988). Mutational hot spots in Ig V region genes of human follicular lymphomas. *J. Exp. Med.* **168,** 475–489.

Linden, O., Tennvall, I., Cavallin-Stahl, E., Darte, I., Garkavij, M., and Lindner, K. J. (1999). Radioimmunotherapy using 131I-labeled anti-CD22 monoclonal antibody (LL2) in *patients* with previously treated B-cell lymphomas. *Clin. Cancer Res.* **5**(Suppl. 10), 3287s–3291s.

Linden, O., Tennvall, J., Cavallin-Stahl, E., Darte, L., Ohlsson, T., and Hindorf, C. (2001). Durable response to 90-ytrrium-epratuzumab (hLL2) in B-cell lymphoma failing chemotherapy by using dose-fractionation schedule. *Blood* **98,** 602a.

Link, B. K., Kahl, B., Czuczman, M. S., Powell, B. L., Bartlett, N., and Leonard, J. P. (2001a). A phase II study of Remitogen (Hu1D10), a humanized monoclonal antibody in patients with relapsed or refractory follicular, small lymphocytic, or marginal zone/MALT B-cell lymphoma. *Blood* **98,** 606a.

Link, B. K., Wang, H. G., Byrd, J. C., Leonard, J. P., Davis, T. A., and Flinn, I. (2001b). Phase I study of Hu1D10 monoclonal antibody in patients with B-cell lymphoma. *Proc. Am. Soc. Clin. Oncol.* **20,** 284a.

Lundin, J., Kimby, E., Bergmann, L., Karakas, T., Mellstedt, H., and Osterborg, A. (2001). Interleukin 4 therapy for patients with chronic lymphocytic leukaemia: A phase I/II study. *Br. J. Haematol.* **112,** 155–160.

Lundin, J., Kimby, E., Bjorkholm, M., Broliden, P. A.., Celsing, F., Hjalmar, V., Mollgard, L., Rebello, P., Hale, G., Waldmann, H., Mellstedt, H., and Osterborg, A. (2002). Phase II trial of subcutaneous anti-CD52 monoclonal antibody alemtuzumab (Campath-1H) as first-line treatment for patients with B-cell chronic lymphocytic leukemia (B-CLL). *Blood* **100,** 768–773.

Lundin, J., Osterborg, A., Brittinger, G., Crowther, D., Dombret, H., and Engert, A. (1998). CAMPATH-1H monoclonal antibody in therapy for previously treated low-grade non-Hodgkin's lymphoma: A phase II multicenter study. European Study Group of CAMPATH-1H Treatment in Low-Grade Non-Hodgkin's Lymphoma. *J. Clin. Oncol.* **16,** 3257–3263.

Mainou-Fowler, T., Miller, S., Proctor, S. J., and Dickinson, A. M. (2001). The levels of TNF alpha, IL4 and IL 10 production by T-cells in B-cell chronic lymphocytic leukaemia (B-CLL). *Leuk. Res.* **25,** 157–163.

Mainou-Fowler, T., Proctor, S. J., Miller, S., and Dickinson, A. M. (2001). Expression and production of interleukin 4 in B-cell chronic lymphocytic leukaemia. *Leuk Lymph.* **42,** 689–698.

Maloney, D. G., Brown, S., Czerwinski, D. K., Liles, T. M., Hart, S. M., and Miller, R. A. (1992). Monoclonal anti-idiotype antibody therapy of B-cell lymphoma: The addition of a short course of chemotherapy does not interfere with the antitumor effect nor prevent the emergence of idiotype-negative variant cells. *Blood* **80,** 1502–1510.

Maloney, D. G., Grillo-Lopez, A. J., White, C. A., Bodkin, D., Schilder, R. J., and Neidhart, J. A. (1997). IDEC-C2B8 (rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood* **90,** 2188–2195.

Manshouri, T., Do, K. A., Wang, X., Giles, F. J., O'Brien, S. M., and Saffer, H. L. (2003). Circulating CD20 is detectable in the plasma of patients with chronic lymphocytic leukemia and is of prognostic significance. *Blood* **101,** 2507–2513.

McLaughlin, P., Grillo-Lopez, A. J., Link, B. K., Levy, R., Czuczman, M. S., and Williams, M. E. (1998). Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: Half of patients respond to a four-dose treatment program. *J. Clin. Oncol.* **16,** 2825–2833.

McLaughlin, P., Hagemeister, F. B., Rodriguez, M. A., Sarris, A. H., Pate, O., and Younes, A. (2000). Safety of fludarabine, mitoxantrone, and dexamethasone combined with rituximab in the treatment of stage IV indolent lymphoma. *Semin. Oncol.* **27,** 37–41.

Meeker, T. C., Lowder, J., Cleary, M. L., Stewart, S., Warnke, R. A., and Sklar, J. (1985a). Emergence of idiotype variants during treatment of B-cell lymphoma with anti-idiotype antibodies. *N. Engl. J. Med.* **312,** 1658–1665.

Meeker, T. C., Lowder, J., Maloney, D. G., Miller, R. A., Thielemans, K., and Warnke, R. A. (1985b). A clinical trial of anti-idiotype therapy for B cell malignancy. *Blood* **65,** 1349–1363.

Miller, R. A., Maloney, D. G., Warnke, R. A., and Levy, R. (1982). Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. *N. Engl. J. Med.* **306,** 517–522.

Moberts, R., Hoogerbrugge, H., van Agthoven, T., Lowenberg, B., and Touw, I. (1989). Proliferative response of highly purified B chronic lymphocytic leukemia cells in serum free culture to interleukin-2 and tumor necrosis factors alpha and beta. *Leuk. Res.* **13,** 973–980.

Montserrat, E., Alcala, A., Parody, R., Domingo, A., Garcia-Conde, J., and Bueno, J. (1985). Treatment of chronic lymphocytic leukemia in advanced stages: A randomized trial comparing chlorambucil plus prednisone versus cyclophosphamide, vincristine, and prednisone. *Cancer* **56,** 2369–2375.

Moreland, L. W., Schiff, M. H., Baumgartner, S. W., Tindall, E. A., Fleischmann, R. M., and Bulpitt, K. J. (1999). Etanercept therapy in rheumatoid arthritis: A randomized, controlled trial. *Ann. Intern. Med.* **130,** 478–486.

Nabhan, C., Tallman, M. S., Riley, M. B., Fitzpatrick, J., Gordon, L. I., and Gartenhaus, R. (2001). Phase I study of rituximab and Campath-1H in patients with relapsed or refractory chronic lymphocytic leukemia. *Blood* **98,** 365a.

Newton, D. L., Hansen, H. J., Mikulski, S. M., Goldenberg, D. M., and Rybak, S. M. (2001). Potent and specific antitumor effects of an anti-CD22-targeted cytotoxic ribonuclease: Potential for the treatment of non-Hodgkin's lymphoma. *Blood* **97,** 528–535.

Nguyen, D. T., Amess, J. A., Doughty, H., Hendry, L., and Diamond, L. W. (1999). IDEC-C2B8 anti-CD20 (rituximab) immunotherapy in patients with low-grade non-Hodgkin's lymphoma and lymphoma and lymphoproliferative disorders: Evaluation of response on 48 patients. *Eur. J. Haematol.* **62,** 76–82.

O'Brien, S. M., Kantarjian, H. M., Cortes, J., Beran, M., Koller, C. A., and Giles, F. J. (2001a). Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. *J. Clin. Oncol.* **19,** 1414–1420.

O'Brien, S. M., Kantarjian, H., Thomas, D. A., Giles, F. J., Freireich, E. J., and Cortes, J. (2001b). Rituximab dose-escalation trial in chronic lymphocytic leukemia. *J. Clin. Oncol.* **19,** 2165–2170.

Olsen, E., Duvic, M., Frankel, A., Kim, Y., Martin, A., and Vonderheid, E. (2001). Pivotal phase III trial of two dose levels of denileukin diftitox for the treatment of cutaneous T-cell lymphoma. *J. Clin. Oncol.* **19,** 376–388.

Osterborg, A., Dyer, M. J., Bunjes, D., Pangalis, G. A., Bastion, Y., and Catovsky, D. (1997). Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia: European Study Group of CAMPATH-1H Treatment in Chronic Lymphocytic Leukemia. *J. Clin. Oncol.* **15,** 1567–1574.

Osterborg, A., Fassas, A. S., Anagnostopoulos, A., Dyer, M. J., Catovsky, D., and Mellstedt, H. (1996). Humanized CD52 monoclonal antibody Campath-1H as first-line treatment in chronic lymphocytic leukaemia. *Br. J. Haematol.* **93,** 151–153.

Pathan, N., Hopkins, M., Saven, A., Reff, M. E., Grint, P., and Hariharan, K. (2001). Induction of apoptosis by IDEC-152 (anti-CD23) in chronic lymphocytic leukemia. *Leuk. Lymph.* **42**(Suppl. 1), 133N.

Pawson, R., Dyer, M. J., Barge, R., Matutes, E., Thornton, P. D., and Emmett, E. (1997). Treatment of T-cell prolymphocytic leukemia with human CD52 antibody. *J. Clin. Oncol.* **15,** 2667–2672.

Pedersen, I. M., Buhl, A. M., Klausen, P., Geisler, C. H., and Jurlander, J. (2002). The chimeric anti-CD20 antibody rituximab induces apoptosis in B-cell chronic lymphocytic leukemia cells through a p38 mitogen activated protein-kinase-dependent mechanism. *Blood* **99,** 1314–1319.

Pegram, M. D., Lipton, A., Hayes, D. E., Weber, B. L., Baselga, J. M., and Tripathy, D. (1998). Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-over-expressing metastatic breast cancer refractory to chemotherapy treatment. *J. Clin. Oncol.* **6,** 2659–2671.

Pers, J. O., Berthou, C., Porakishvili, N., Burdjanadze, M., Le Calvez, G., and Abgrall, J. F. (2002). CD5-induced apoptosis of B cells in some patients with chronic lymphocytic leukemia. *Leukemia* **16,** 44–52.

Pers, J. O., Jamin, C., LeCorre, R., Lydyard, P. M., and Youinou, P. (1998). Ligation of CD5 on resting B cells, but not on resting T cells, results in apoptosis. *Eur. J. Immunol.* **28,** 4170–4176.

Perz, J., Topaly, J., Fruehauf, S., Hensel, M., and Ho, A. D. (2002). Level of CD20 expression and efficacy of rituximab treatment in patients with resistant or relapsing B-cell

prolymphocytic leukemia and B-cell chronic lymphocytic leukemia. *Leuk. Lymph.* **43,** 149–151.

Polliack, A., Cohen, Y., Daas, N., Libster, D., Shvidel, I., and Klepfish, A. (2001). Fludarabine (FLU)-containing regimen and rituximab (RI) as primary therapy with curative intent for younger patients with progressive and advanced B-CLL: High rate of initial response including molecular remissions. *Blood* **98,** 364a.

Press, O. W., Eary, J. F., Appelbaum, F. R., Martin, P. J., Badger, C. C., and Nelp, W. B. (1993). Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. *N. Engl. J. Med.* **329,** 1219–1224.

Pruzanski, W., Urowitz, M. B., Grouix, B., and Vadas, P. (1995). Induction of TNF-alpha and proinflammatory secretory phospholipase A2 by intravenous administration of CAMPATH-1H in patients with rheumatoid arthritis. *J. Rheumatol.* **22,** 1816–1819.

Pu, Q. Q., and Bezwoda, W. R. (1997). Interleukin-4 prevents spontaneous in-vitro apoptosis in chronic lymphatic leukaemia but sensitizes B-CLL cells to melphalan cytotoxicity. *Br. J. Haematol.* **98,** 413–417.

Rai, K. R., Coutre, S., Rizzieri, D., Gribben, J. G., Flinn, J., and Rabinowe, S. (2001). Efficacy and safety of alemtuzumab (Campath-1H) in refractory B-CLL patients treated on a compassionate basis. *Blood* **98,** 365a.

Raphael, B., Andersen, J. W., Silber, R., Oken, M., Moore, D., and Bennett, J. M. (1991). Comparison of chlorambucil and prednisone versus cyclophosphamide, vincristine, and prednisone as initial treatment for chronic lymphocytic leukemia: Long-term follow-up of an Eastern Cooperative Oncology Group randomized clinical trial. *J. Clin. Oncol.* **9,** 770–776.

Ravel, S., Colombatti, M., and Casellas, P. (1992). Internalization and intracellular fate of anti-CD5 monoclonal antibody and anti-CD5 ricin A-chain immunotoxin in human leukemic T cells. *Blood* **79,** 1511–1517.

Rezvany, M. R., Lundin, J., Edman, P., Porwit-McDonald, A., Mellstedt, H., and Osterborg, A. (2002). Long-term follow-up of lymphocyte subsets after subcutaneous alemtuzumab (MabCampath) treatment as primary therapy for B-cell chronic lymphocytic leukemia (B-CLL). *Blood* **100,** 207a.

Rowan, W., Tite, J., Topley, P., and Brett, S. J. (1998). Cross-linking of the CAMPATH-1 antigen (CD52) mediates growth inhibition in human B- and T-lymphoma cell lines, and subsequent emergence of CD52-deficient cells. *Immunology* **95,** 427–436.

Salisbury, J. R., Rapson, N. T., Codd, J. D., Rogers, M. V., and Nethersell, A. B. (1994). Immunohistochemical analysis of CDw52 antigen expression in non-Hodgkin's lymphomas. *J. Clin. Pathol.* **47,** 313–317.

Sawitsky, A., Rai, K. R., Glidewell, O., and Silver, R. T. (1977). Comparison of daily versus intermittent chlorambucil and prednisone therapy in the treatment of patients with chronic lymphocytic leukemia. *Blood* **50,** 1049–1059.

Scallon, B. J., Moore, M. A., Trinh, H., Knight, D. M., and Ghrayeb, J. (1995). Chimeric antiTNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions. *Cytokine* **7,** 251–259.

Scheinberg, D. A., Straus, D. J., Yeh, S. D., Divgi, C., Garin-Chesa, P., and Graham, M. (1990). A phase I toxicity, pharmacology, and dosimetry trial of monoclonal antibody OKB7 in patients with non-Hodgkin's lymphoma: Effects of tumor burden and antigen expression. *J. Clin. Oncol.* **8,** 792–803.

Schulz, H., Klein, S. K., Rehwald, U., Reiser, M., Hinke, A., and Knauf, W. U. (2002). Phase 2 study of a combined immunochemotherapy using rituximab and fludarabine in patients with chronic lymphocytic leukemia. *Blood* **100,** 115–120.

Shih, L. B., Lu, H. H., Xuan, H., and Goldenberg, D. M. (1994). Internalization and intracellular processing of an anti-B-cell lymphoma monoclonal antibody, LL2. *Int. J. Cancer* **56,** 538–545.

Siena, S., Bregni, M., Formosa, A., Brando, B., Marenco, P., and Lappi, D. A. (1989). Immunotoxin-mediated inhibition of chronic lymphocytic leukemia cell proliferation in humans. *Cancer Res.* **49,** 3328–3332.

Sievers, E. L., Larson, R. A., Stadtmauer, E. A., Estey, E., Lowenberg, B., and Dombret, H. (2001). Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J. Clin. Oncol.* **19,** 3244–3254.

Sigidin, Y. A., Loukina, G. V., Skurkovich, B., and Skurkovich, S. (2001). Randomized, double-blind trial of anti-interferon-gamma antibodies in rheumatoid arthritis. *Scand. J. Rheumatol.* **30,** 203–207.

Steensma, D. P., Mesa, R. A., Li, C. Y., Gray, L., and Tefferi, A. (2002). Etanercept, a soluble tumor necrosis factor receptor, palliates constitutional symptoms in patients with myelofibrosis with myeloid metaplasia: Results of a pilot study. *Blood* **99,** 2252–2254.

Stein, R., Belisle, E., Hansen, H. J., and Goldenberg, D. M. (1993). Epitope specificity of the anti-(B cell lymphoma) monoclonal antibody, LL2. *Cancer Immunol. Immunother.* **37,** 293–298.

Stone, M. J., Sausville, E. A., Fay, J. W., Headlee, D., Collins, R. H., and Figg, W. D. (1996). A phase I study of bolus versus continuous infusion of the anti-CD19 immunotoxin, IgG-HD37-dgA, in patients with B-cell lymphoma. *Blood* **88,** 1188–1197.

Tang, S. C., Hewitt, K., Reis, M. D., and Berinstein, N. L. (1996). Immunosuppressive toxicity of CAMPATH1H monoclonal antibody in the treatment of patients with recurrent low grade lymphoma. *Leuk. Lymph.* **24,** 93–101.

Targan, S. R., Hanauer, S. B., van Deventer, S. J., Mayer, L., Present, D. H., and Braakman, T. (1997). A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease: Crohn's Disease cA2 Study Group. *N. Engl. J. Med. J.* **337,** 1029–1035.

Thomas, D. A., O'Brien, S., Bueso-Ramos, C., Faderl, S., Keating, M. J., Giles, F. J., Cortes, J., and Kantarjian, H. M. (2003). Rituximab in relapsed or refractory hairy cell leukemia. *Blood* **102,** 3906–3911.

Thomas, D. A., O'Brien, S., Giles, F. J., Cortes, J., Faderl, S., and Kantarjian, H. (2001). Single agent rituxan in early stage chronic lymphocytic leukemia (CLL). *Blood* **98,** 364a.

Treon, S. P., Mitsiades, C., Mitsiades, N., Young, G., Doss, D., and Schlossman, R. (2001). Tumor cell expression of CD59 is associated with resistance to CD20 serotherapy in patients with B-cell malignancies. *J. Immunother.* **24,** 263–271.

Treumann, A., Lifely, M. R., Schneider, P., and Ferguson, M. A. (1995). Primary structure of CD52. *J. Biol. Chem.* **270,** 6088–6099.

Uchiyama, T., Broder, S., and Waldmann, T. A. (1981a). A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells I: Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J. Immunol.* **126,** 1393–1397.

Uchiyama, T., Nelson, D. L., Fleisher, T. A., and Waldmann, T. A. (1981b). A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells II: Expression of Tac antigen on activated cytotoxic killer T cells, suppressor cells, and on one of two types of helper T cells. *J. Immunol.* **126,** 1398–1403.

van Kooten, C., Rensink, I., Aarden, L., and van Oers, R. (1992). Interleukin-4 inhibits both paracrine and autocrine tumor necrosis factor-alpha-induced proliferation of B chronic lymphocytic leukemia cells. *Blood* **80,** 1299–1306.

van Kooten, C., Rensink, J., Aarden, I., and van Oers, R. (1993). Effect of IL-4 and IL-6 on the proliferation and differentiation of B-chronic lymphocytic leukemia cells. *Leukemia* **7,** 618–624.

van Dullemen, H. M., van Deventer, S. J., Hommes, D. W., Bijl, H. A., Jansen, J., and Tytgat, G. N. (1995). Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology* **109,** 129–135.

Vose, J. M., Giles, F. J., Manshouri, T., Bociek, G., Bierman, P. J., and Armitage, J. O. (2001a). High levels of soluble CD20 (sCD20) in patients with non-Hodgkin's lymphoma (NHL): Correlation with clinical behavior and contrast with patients with Hodgkin's disease (HD). *Blood* **98,** 767a.

Vose, J. M., Link, B. K., Grossbard, M. L., Czuczman, M. S., Grillo-Lopez, A. J., and Gilman, P. (2001b). Phase II study of rituximab in combination with CHOP chemotherapy in patients with previously untreated, aggressive non-Hodgkin's lymphoma. *J. Clin. Oncol.* **19,** 389–397.

Vose, J. M., Colcher, D., Gobar, L., Bierman, P. J., Augustine, S., and Tempero, M. (2000a). Phase I/II trial of multiple dose 131Iodine-MAb LL2 (CD22) in patients with recurrent non-Hodgkin's lymphoma. *Leuk Lymph.* **38,** 91–101.

Vose, J. M., Wahl, R. L., Saleh, M., Rohatiner, A. Z., Knox, S. J., and Radford, J. A. (2000b). Multicenter phase II study of iodine-131 tositumomab for chemotherapy-relapsed/refractory low-grade and transformed low-grade B-cell non-Hodgkin's lymphomas. *J. Clin. Oncol.* **181,** 1316–1323.

Weinblatt, M. F., Kremer, J. M., Bankhurst, A. D., Bulpitt, K. J., Fleischmann, R. M., and Fox, R. I. (1999). A trial of etanercept, a recombinant tumor necrosis factor receptor: Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N. Engl. J. Med.* **340,** 253–259.

Wierda, W., O'Brien, S., Albitar, M., Lerner, S., Plunkett, W., and Giles, F. J. (2001). Combined fludarabine, cyclophosphamide, and rituximab achieves a high complete remission rate as initial treatment for chronic lymphocytic leukemia. *Blood* **98,** 771a.

Wing, M. G., Moreau, T., Greenwood, J., Smith, R. M., Hale, G., and Isaacs, J. D. (1996). Mechanism of first-dose cytokine-release syndrome by CAMPATH 1-H: Involvement of CD16 (Fcgamma RIII) and CD11a/CD18 (LFA-1) on NK cells. *J. Clin. Invest.* **98,** 2819–2826.

Winkler, U., Jensen, M., Manzke, O., Schulz, H., Diehl, V., and Engert, A. (1999). Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8). *Blood* **94,** 2217–2224.

Wiseman, G. A., White, C. A., Witzig, T. E., Gordon, I. I., Emmanouilides, C., and Raubitschek, A. (1999). Radioimmunotherapy of relapsed non-Hodgkin's lymphoma with zevalin a 90Y-labeled anti-CD20 monoclonal antibody. *Clin. Cancer Res.* **5**(Suppl. 10), 3281s–3286s.

Witzig, T. E., White, C. A., Wiseman, G. A., Gordon, I. I., Emmanouilides, C., and Raubitschek, A. (1999). Phase I/II trial of IDEC-Y2B8 radioimmunotherapy for treatment of relapsed or refractory CD20(+) B-cell non-Hodgkin's lymphoma. *J. Clin. Oncol.* **17,** 3793–3803.

Yabuuchi, S., Nakamura, T., Kloetzer, W. S., and Reff, M. E. (2002). Anti-CD23 monoclonal antibody inhibits germline Cepsilon transcription in B cells. *Int. Immunopharmacol.* **2,** 453–461.

Yang, X. D., Corvalan, J. R., Wang, P., Roy, C. M., and Davis, C. G. (1999). Fully human anti-interleukin-8 monoclonal antibodies: Potential therapeutics for the treatment of inflammatory disease states. *J. Leukoc. Biol.* **66,** 401–410.

Zaki, M., Douglas, R., Patten, N., Bachinsky, M., Lamb, R., and Nowell, P. (2000). Disruption of the IFN-gamma cytokine network in chronic lymphocytic leukemia contributes to resistance of leukemic B cells to apoptosis. *Leuk. Res.* **24,** 611–621.

Eric L. Sievers

Clinical Research Division
Fred Hutchinson Cancer Research Center
Seattle, Washington 98109; and
Department of Pediatrics
University of Washington
Seattle, Washington 98105

Native Antibody and Antibody-Targeted Chemotherapy for Acute Myeloid Leukemia

I. Chapter Overview

CD33 is a normal myeloid surface antigen that is expressed by leukemic blast cells from the vast majority of patients with acute myeloid leukemia (AML). Early clinical studies performed in New York and Seattle demonstrated that unconjugated antibodies directed against CD33 specifically target sites of normal and abnormal hematopoiesis. These findings provided a rationale for the development of a second generation of antibodies capable of delivering cytotoxic agents to leukemic blast cells. One such agent, Mylotarg^{TM} (gemtuzumab ozogamicin) was approved in 2000 by the U.S. Food and Drug Administration (FDA) for the treatment of patients with CD33-positive AML in first relapse who are 60 years of age or older and who are not considered candidates for other types of cytotoxic chemotherapy. Among 277 adult patients with CD33-positive AML in first relapse, 26% experienced an overall response after Mylotarg monotherapy. Despite

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

myelosuppression, hyperbilirubinemia, and elevated hepatic transminases being commonly observed, the agent was reasonably well tolerated by adult patients with advanced AML. Newer treatment regimens combining Mylotarg and conventional chemotherapy have yielded a surprisingly high remission induction rate in *de novo* AML patients. These preliminary findings have prompted the planning of prospective, randomized studies in the U.S. and the U.K. that should help us refine our use of this novel immunoconjugate.

II. Introduction

Almost 50 years have passed since Pressman and Korngold showed that antibodies could target tumor cells, and 2 decades have elapsed since Kohler and Milstein made large-scale production of monoclonal antibodies feasible. Why is antibody treatment of AML uncommon? Despite numerous attempts to identify them, leukemia cells rarely express novel antigenic targets that are not otherwise expressed by normal tissues. For this reason, normal cell surface antigens with expression restricted to the hematopoietic system have been selected as targets. With the stunning exception of rituximab in CD20-positive lymphomas, most antibodies targeting various normal hematopoietic antigens have proven clinically ineffective as therapeutic agents against hematologic malignancies. In the last few years, however, strategies employing antibodies for the treatment of patients with AML have begun to bear fruit.

A. CD33: A Normal Antigen Expressed During Myeloid Differentiation

Investigators on opposite coasts at the Memorial Sloan-Kettering Cancer Center (MSKCC) and the Fred Hutchinson Cancer Research Center (FHCRC) have both selected the normal myeloid antigen CD33 as an attractive target for antibody-based therapy. Targeting CD33 makes sense for a variety of reasons. First, leukemic blast cells from more than 80–90% of AML patients express the antigen at high levels (Dinndorf *et al.*, 1986; Griffin *et al.*, 1984). Second, because nonhematopoietic tissues and normal primitive hematopoietic precursors both lack CD33 expression, relatively selective targeting of a malignant population of cells can be achieved. Because primitive precursor cells remain unscathed, hematopoietic recovery readily occurs over a several-week period. Finally, antibody and any conjugated cytotoxic agent are internalized after CD33 cell surface engagement by antibody. This modulation of the antigen–antibody complex enables the targeted delivery of a radionucleotide, protein toxin, or other cytotoxic substance into the cytoplasm of leukemic cells.

In a tightly regulated manner, CD33 is expressed as pluripotent hematopoietic stem cells mature and give rise to progenitors with diminished self-renewal capacity and a greater degree of differentiation (Andrews *et al*., 1983; Dinndorf *et al*., 1986; Griffin *et al*., 1984). CD33 is expressed by maturing normal hematopoietic cells, but stem cells lack surface expression of CD33 (Andrews *et al*., 1989). In marrow long-term culture experiments from some patients with AML, selective ablation of CD33-positive cells from leukemic marrow aspirates resulted in the growth of normal nonclonal granulocytes and monocytes (Bernstein *et al*., 1987, 1992). Although these findings suggested that selectively targeting and eliminating CD33-positive cells might enable patients with AML to achieve clinical remissions, other investigators have also provided compelling data suggesting that the more rare, clonogenic leukemic cell does not express CD33 and other lineage-associated antigens. Bonnet and Dick (1997) demonstrated growth of AML in an immunodeficient mouse model after infusion of isolated primitive (CD34+ CD38−) precursors from human marrow specimens obtained from AML patients. In reconciling these apparently conflicting data, it is conceivable that selective ablation of CD33-positive cells using antibody might rid the body of large numbers of mature leukemic cells without fully deleting the rare progenitor cells from which the leukemia arises. This hypothesis is buttressed by the clinical observation that AML remissions induced by antibody-targeted ablation of CD33-positive cells (described later) were relatively brief if further definitive therapy was not subsequently administered.

B. Unconjugated Anti-CD33 Antibody

Cytotoxicity from unconjugated monoclonal antibodies occurs by several mechanisms. In antibody-dependent cellular cytotoxicity (ADCC), granulocytes and tissue macrophages eliminate target cells coated with antibody through binding of the antibody Fc receptor. In complement-dependent cellular cytotoxicity (CDC), the Fc portion of immunoglobulin bound to tumor cells induces cell death by complement fixation. ADCC is likely the mechanism associated with the impressive non-Hodgkin's lymphoma tumor regressions seen in association with anti-CD20 antibodies (Buchsbaum *et al*., 1992). Data also suggest that ligation of CD20 by antibody interferes with normal signal transduction, directly leading to apoptosis without a significant component of ADCC (Shan *et al*., 1998). Whereas rituximab has been shown to be effective therapy in certain types of non-Hodgkin's lymphoma, unconjugated antibody approaches targeting CD33 expressed by AML cells have shown limited efficacy for patients with large tumor burdens. However, some benefit might exist for patients with acute promyelocytic leukemia (APL) who harbor minimal residual disease (Jurcic *et al*., 2000).

Investigators at MSKCC and FHCRC first employed trace radioiodinated anti-CD33 antibodies in patients with advanced AML. Intravenous administration of approximately 5 mg/m^2 of antibody resulted in selective targeting and rapid saturation of leukemic blast cells in patients' peripheral blood and marrow (Appelbaum *et al*., 1992; Scheinberg *et al*., 1991). Although no significant clinical efficacy was observed by using these early strategies, investigators from Protein Design Labs and MSKCC performed several clinical evaluations of HuM195, a humanized monoclonal antibody created by grafting the CDR regions of the M195 anti-CD33 murine monoclonal antibody onto the Eu human IgG1 antibody (Co *et al*., 1992).

In a pilot monotherapy study conducted by MSKCC, patients received supersaturating doses of HuM195 at doses of 12 or 36 mg/m^2/day on Days 1 through 4, with repeat doses provided on Days 15 through 18 (Caron *et al*., 1998). Among 10 patients with advanced myeloid leukemias (9 AML and 1 CML) treated, 1 achieved a complete remission. In a larger randomized study, 50 patients with advanced leukemia (median age of 62 years) received either 12 or 36 mg/m^2 of HuM195 daily for 4 consecutive days weekly for a total of four courses (Feldman *et al*., 2003). Two complete remissions and one partial remission were observed among 49 evaluable patients. Nine additional patients experienced decreases in blast counts ranging from 30–74%. The antibody treatments were extremely well tolerated. Infusion-related fevers and chills were commonly observed, but serious organ toxicity was uncommon. No immune responses to HuM195 were detected. Overall, only patients with minimal tumor burden experienced clinical benefit from HuM195 monotherapy.

Because HuM195 monotherapy was associated with little toxicity, the unconjugated antibody was evaluated in combination with conventional chemotherapy in a prospective randomized study (Feldman *et al*., 2002). The primary endpoint of the study was response rate. Treatment consisted of mitoxantrone, cytarabine, and etoposide plus or minus HuM195 given in two courses at the completion of the induction chemotherapy regimen. The study enrolled 191 patients with a median age of 57 years who had AML that was initially refractory to therapy or had relapsed with remission duration of less than 1 year. One quarter of enrolled patients had a history of an antecedent hematologic disorder. Although the two randomized cohorts were reasonably well matched for demographic features, the antibody treatment group included a disproportionately high portion with active infections, primary refractory AML, or a prior antecedent hematological disorder. Inclusion of HuM195 was well tolerated by study patients. Although the overall response rate observed with HuM195 was 36% compared with 28% among those not treated with antibody, this difference was not statistically significant at $p = 0.28$. Unfortunately, this suggestion of improved response rate did not translate into improved clinical outcome as

no survival difference was observed between the two treated populations with extended follow-up.

Although results from the antibody chemotherapy combination trial for advanced AML patients were disappointing, mature data in patients with APL in complete remission using HuM195 monotherapy as maintenance are encouraging, particularly among patients harboring minimal residual disease. Of 27 APL patients induced into first remission with all-*trans* retinoic acid (ATRA), followed by idarubicin and cytarabine consolidation therapy, 25 had evidence of residual leukemia by reverse transcription-polymerase chain reaction (RT-PCR) before HuM195 treatment (Jurcic *et al.*, 2000). Subsequently, they received an additional 6 months of maintenance therapy with HuM195 given monthly in two doses separated by 3 or 4 days. Bone marrow aspirates were evaluated serially for the PML/RAR-α mRNA by RT-PCR. Among 22 patients evaluable, HuM195 monotherapy appeared to result in the conversion to RT-PCR negativity in 11 patients. Overall, 25 of 27 (93%) patients with *de novo* APL remained in clinical complete remission for 7+ to 58+ months, with a median follow-up of 60+ months. Taken together, these results suggest that HuM195 might have reasonable efficacy for APL patients who harbor evidence of residual disease after induction therapy. Because the leukemia progenitor cell in APL is more likely to express CD33, APL might be uniquely amenable to antibody strategies targeting CD33.

C. Anti-CD33 Conjugated with Calicheamicin: Gemtuzumab Ozogamicin

Clinical studies of p67.6, an anti-CD33 antibody developed in the laboratory of Dr. Irwin Bernstein in Seattle, demonstrated that rapid and specific targeting of CD33-positive cells could be achieved *in vivo* (Appelbaum *et al.*, 1992). Unfortunately, a short marrow residence time was observed with conventionally radiolabeled anti-CD33, thus limiting potential clinical efficacy with this approach. Hence, a cytotoxic agent was sought for conjugation to the p67.6 anti-CD33 antibody. Because the original p67.6 murine anti-CD33 antibody was immunogenic, a humanized monoclonal antibody containing approximately 98% human amino acid sequences was created. Gemtuzumab was synthesized by grafting the p67 anti-CD33 murine monoclonal CDR sequences onto a human IgG4 isotype antibody. The IgG4 antibody isotype was selected because it was associated with fewer Fc-dependent functions and a relatively long half-life in circulation. The humanized antibody gemtuzumab had CD33-binding affinity similar to that of the precursor murine p67 antibody.

Administration of anti-CD33 antibody results in rapid saturation of CD33 sites throughout the body. The antigen–antibody complex is then rapidly internalized into the cell, enabling antibody-targeted delivery of

a cytotoxic agent into the intracellular space. Calicheamicin, a potent antitumor antibiotic that cleaves double-stranded DNA, was conjugated to a humanized anti-CD33 antibody to create gemtuzumab ozogamicin (Mylotarg). As illustrated in Fig. 1, assays of cell surface binding of gemtuzumab suggest that the antigen–antibody complexes are rapidly internalized (van der Velden *et al*., 2001). From studies that follow the fate of internalized antibodies, it is suggested that the endocytosed anti-CD33 complexes translocate to lysosomes, where hydrolytic release of calicheamicin from the linker occurs. Unlike conventional chemotherapy agents that cause single- or double-strand lesions through radical intermediates or topoisomerases, the extremely reactive calicheamicin behaves like ionizing radiation by cleaving both DNA strands simultaneously. These site-specific double-stranded DNA breaks result in apoptotic cell death (Ellestad *et al*., 1995; Sissi *et al*., 1999; Zein *et al*., 1988).

Gemtuzumab was evaluated in three *in vitro* tests for specific targeting and killing of leukemia cells: cultured HL-60 leukemia cells, HL-60 human xenograft tumors, and marrow specimens from AML patients in colony-forming assays. Uniformly, leukemia cells were ablated with high specificity compared with that of calicheamicin linked to antibodies directed against nonspecific antigens or unconjugated anti-CD33 antibody.

D. Clinical Studies of Gemtuzumab Ozogamicin

In collaboration with Wyeth-Ayerst Research, investigators at FHCRC and the City of Hope National Medical Center conducted a Phase I study of gemtuzumab in which patients with relapsed or refractory CD33-positive AML were treated with escalating doses of drug every 2 weeks for three doses (Sievers *et al*., 1999). Leukemia was ablated from the blood and marrow of 8 of 40 (20%) patients and blood counts normalized in three (8%) patients. Figure 2 shows the relationship between hematologic parameters and time for a patient who received gemtuzumab at 4 mg/m^2 per dose. Gemtuzumab doses up to 9 mg/m^2 were generally well tolerated, and a postinfusion syndrome of fever and chills was the most common side effect. Modest and reversible hepatic transaminase elevations and hyperbilirubinemia was observed in several patients who received gemtuzumab at higher dose levels.

Subsequent prospective international Phase II studies evaluated gemtuzumab in 142 patients with CD33-positive AML in first untreated relapse (Sievers *et al*., 2001). Three similar concurrent Phase II studies evaluated safety and efficacy of gemtuzumab in patients with CD33-positive AML in first relapse, lacking history of an antecedent hematologic disorder. The initial report described here detailed findings from 142 adults with a median age of 61 years. Among those in whom cytogenetics were documented, 39% had abnormalities known to be associated with unfavorable outcomes.

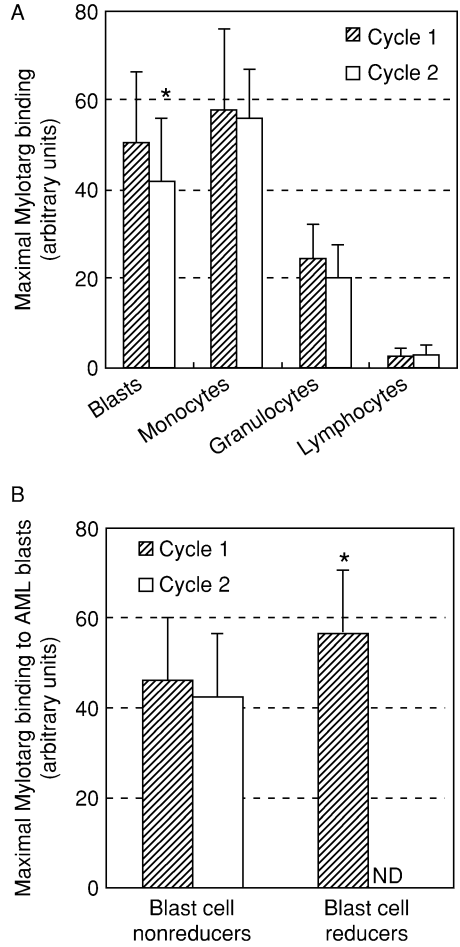

FIGURE 1 Maximal Mylotarg binding to leukocyte subsets. Maximal Mylotarg binding to different leukocyte subsets was analyzed prior to start of the first (hatched bars) and second (open bars) Mylotarg treatment cycle by incubating PB with an excess of Mylotarg in vitro, followed by detection with biotin-conjugated antihuman IgG4 and streptavidin FITC. (A) Maximal Mylotarg binding to AML blast cells (cycle 1: $n = 86$; cycle 2: $n = 35$), monocytes ($n = 33$; $n = 45$), granulocytes ($n = 55$; $n = 32$), and lymphocytes ($n = 61$; $n = 43$). Patient numbers differ between leukocyte subsets and between cycle 1 and cycle 2 because not all patients received a second treatment cycle, in some patients the PB sample was not available or of too low quality, or too few events were available for a reliable analysis of all leukocyte subsets. (B) Maximal Mylotarg binding to AML blast cells from patients showing less than 5% blast cells in their PB just prior to the start of the second treatment cycle (blast cell reducers; $n = 27$) or 5% or more blasts in PB (blast cell nonreducers; $n = 31$ and $n = 26$ for cycle 1 and cycle 2, respectively). Maximal Mylotarg binding data for cycle 2 could not be obtained in the blast cell reducers because the number of blast cells was too low to perform a reliable analysis. ND indicates no data. Data are expressed as mean ± SD. Significant differences ($P < .05$; indicated by the asterisks [unpaired t test]) were observed in maximal Mylotarg binding to blast cells between cycle 1 and cycle 2 and between cycle 1 data of the blast cell reducers and blast cell nonreducers.

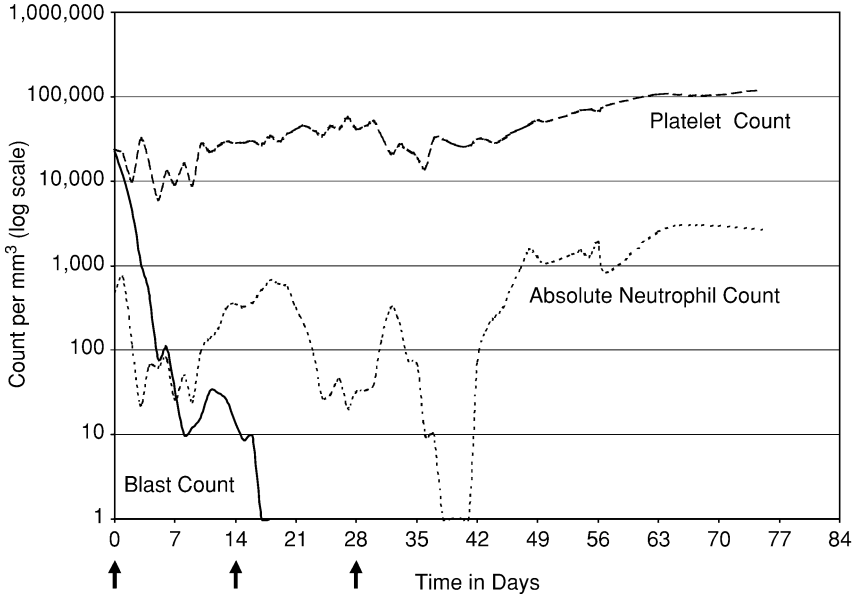

FIGURE 2 Relationship between hematologic parameters and time for a representative patient (FH-012) who received CMA-676 at 4 mg/m^2 per dose. Arrows denote infusions of CMA-676. All counts refer to peripheral blood counts.

Patients received gemtuzumab as a 2-h intravenous infusion at a dose of 9 mg/m^2 at 2-week intervals for two doses. Before therapy, patients with elevated peripheral white blood cell counts were given hydroxyurea to reduce these counts to <30,000/ml.

Among the 142 patients, 46% had fewer than 5% blasts in the bone marrow after one dose of gemtuzumab, based on morphologic analysis of bone marrow aspirates. Thirty percent achieved an overall remission (OR) characterized by <5% blasts in the bone marrow, >1500 neutrophils/ml, and RBC and platelet transfusion independence. Twenty-three patients (16%) achieved CR (complete remission), and 19 (13%) obtained CRp (complete remission with incomplete platelet recovery to 100,000/ml) to produce the OR rate of 30%. Surprisingly, poor prognostic features, including advanced age and short duration of first CR, did not appear to appreciably influence the likelihood of remission induction using gemtuzumab. A 26% OR rate was seen in patients aged 60 or more, compared with 34% in younger patients. Correspondingly, the OR rate was 28% for patients who had a CR1 of less than 1 year compared with an OR rate of 32% for patients whose first remissions were longer. In addition, similar remission induction rates were observed among favorable, intermediate, and unfavorable risk cytogenetic groups as well. Based on these data, gemtuzumab

was approved by the FDA in May 2000 as monotherapy for the treatment of patients with CD33-positive AML in first relapse who are >60 years of age and not considered candidates for cytotoxic chemotherapy (Bross *et al.*, 2001).

Remission durations after gemtuzumab monotherapy were short lived unless patients received consolidation with hematopoietic stem cell transplantation (HSCT) or further chemotherapy. Relapse-free survival (RFS) was measured from the date of first documented OR to relapse, death, or data cutoff. A considerable number of patients who achieved OR were sufficiently healthy to tolerate subsequent HSCT. In the published report of 142 patients with recurrent AML (Fig. 3), the median RFS was at least 8.9 months among OR patients who received allogeneic ($n = 10$) or autologous ($n = 5$) HSCT (Sievers *et al.*, 2001). In contrast, the median RFS was only 2.1 months for the 23 OR patients who received no further therapy. These data suggest that postremission therapy, particularly in the form of allogeneic hematopoietic stem cell transplant, enables the majority of patients who achieve gemtuzumab monotherapy responses to remain in extended remissions.

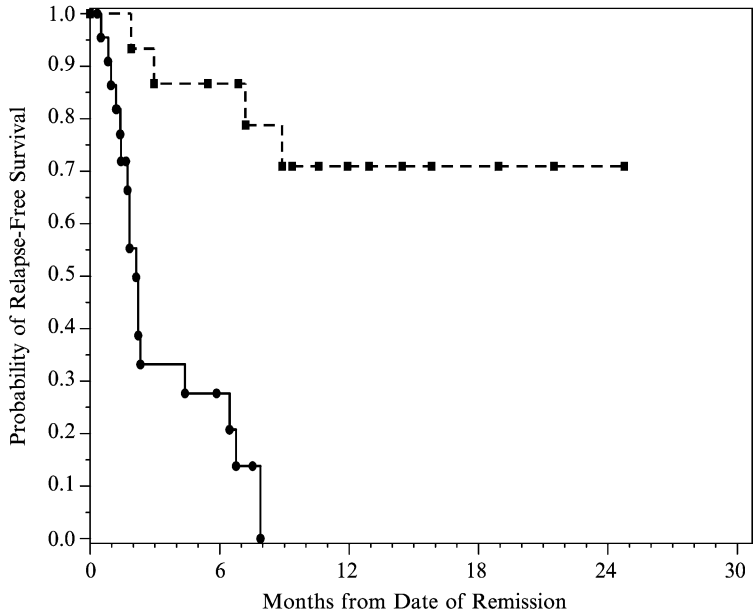

FIGURE 3 Relapse-free survival for OR patients who received HSCT (●) and for OR patients who received no further therapy (■) as postremission therapy. Fifteen OR patients received HSCT (median > 8.9 months), and 23 OR patients received no further therapy (median 2.1 months).

Most gemtuzumab-treated patients experienced a postinfusion syndrome of fevers and chills. Because hypotension rarely developed several hours after administration of gemtuzumab, close medical monitoring for several hours following infusion is suggested. Hypotension did not occur in any patients after the second dose of gemtuzumab. Severe neutropenia and thrombocytopenia were regularly observed because gemtuzumab ablates normal myeloid and megakaryocytic precursors. Twenty-eight percent of patients developed serious infection of grade 3 or 4. Mucositis was rarely observed in only 4% of patients. No treatment-related cardiotoxicity, cerebellar toxicity, or alopecia was seen. No patients in the Phase II studies developed antiglobulin or anticonjugate immune responses. For reasons that are not entirely clear, gemtuzumab can induce hepatic dysfunction. Moderate but typically reversible hepatic transaminase and bilirubin elevations were commonly seen. Among 142 patients, 1 patient died of liver failure on Day 22 of study and another died on Day 156 of study with persistent ascites and hepatic splenomegaly.

A recent report summarized gemtuzumab monotherapy in a population of 101 patients with first untreated relapse of AML, including 80 treated on the previous studies, who were 60 years and older (Larson *et al.*, 2002). The overall remission rate was 28%. CR was observed in 13% of patients and CRp in 15%. The median survival was 5.4 months for all enrolled patients and 14.5 months and 11.8 months for patients achieving CR and CRp, respectively.

III. Lingering Questions Regarding Immunoconjugate Therapies

A. Is Acute Promyelocytic Leukemia Unusually Sensitive to Gemtuzumab?

Because primitive hematopoietic stem cells as defined by CD34+/CD38− antigens do not appear to be involved in the neoplastic process in APL (Turhan *et al.*, 1995), it has been hypothesized that gemtuzumab might have a high likelihood of ablating APL progenitor cells. Petti *et al.* (2001) reported results from a patient with APL who was unusually refractory to conventional approaches and achieved prolonged hematological and molecular remission after two doses of gemtuzumab. In a prospective trial of 19 patients with *de novo* APL, investigators at the M. D. Anderson Cancer Center evaluated ATRA in combination with gemtuzumab as a possible replacement for anthracycline that is typically used for this disease (Estey *et al.*, 2002a). Once patients achieved CR, eight additional courses of gemtuzumab and ATRA were delivered every 4–5 weeks. Of 19 evaluable patients, 16 (84%) achieved CR and most remained PCR-negative 2–4

months from the date of achieving remission. Patients in remission tolerated maintenance doses of gemtuzumab quite well. A median of five post-CR courses was given; three patients received eight and four patients received seven post-CR courses of treatment. These findings demonstrate that gemtuzumab is safe in repeated doses, and suggest that it has clinical activity in APL.

B. Is Gemtuzumab Effective in the Treatment of Older Patients with *De Novo* AML?

Investigators at the M. D. Anderson Cancer Center also evaluated gemtuzumab monotherapy in 51 patients aged 65 years or more with newly diagnosed AML and advanced myelodysplastic syndrome (Estey *et al*., 2002b). Gemtuzumab was given in a compressed dose schedule on Days 1 and 8, or as directed by the product label on Days 1 and 15. Interleukin 11 (IL-11) was administered by random treatment assignment to half of enrolled patients. Among patients who received gemtuzumab monotherapy, only 2 of 26 (8%) entered remission. Among those who also received IL-11, 9 of 25 (36%) achieved CR. After comparing these data with historical results obtained at their center with idarubicin plus cytarabine, the authors concluded that survival with gemtuzumab monotherapy (with or without IL-11) appeared to be inferior.

C. Why Do Liver Toxicities Occasionally Occur with Gemtuzumab Ozogamicin?

Moderately severe elevations in hepatic transaminases and bilirubin occurred at a median of 8 days after treatment in about a quarter of 142 treated patients treated in the Phase II studies (Sievers *et al*., 2001). Although laboratory abnormalities were usually transient and reversible, one patient experienced liver failure and died. A second patient died with persistent ascites and hepatosplenomegaly. A venoocclusive-like disease (VOD) characterized by ascites, weight gain, and moderate elevations in bilirubin was observed in 11 of 271 (4%) patients treated in the collective clinical trials dataset, and in 6 of 120 (5%) patients in the compassionate-use program (Sievers *et al*., 2000). These collective clinical features have recently been termed *sinusoidal obstruction syndrome* by McDonald *et al*. (2002). Among 36 patients who received Mylotarg *before* transplant, 3 patients (8%) died of VOD (Sievers *et al*., 2000). Conversely, among 23 consecutive patients who were treated with Mylotarg *after* transplant, 8 patients (35%) developed fatal liver disease (Rajvanshi *et al*., 2002). In instances in which hepatic tissue was obtained from patients manifesting signs of sinusoidal occlusion syndrome, a consistent pattern of hepatic endothelial cell damage with marked increase in collagen deposition was observed. Although hepatic Kupffer cells express CD33 and are thus targeted by gemtuzumab, the pathophysiology of

hepatic toxicity remains enigmatic. Gemtuzumab should be given with great caution in patients who have preexisting hepatic injury or a history of allogeneic HSCT.

D. Why Are Many Patients Resistant to Gemtuzumab?

Although the overall response rate observed with gemtuzumab is comparable with that of conventional agents, most relapsed AML patients treated on the Phase II studies failed to enter remission. Several biological features of leukemic cells could interfere with Mylotarg-induced cytotoxicity and be manifested as drug resistance. For instance, insufficient levels of CD33 might be expressed by a subpopulation of leukemic blast cells, rendering the cells resistant to cytotoxicity. However, among patients in the Phase II trials with 80% CD33-positive leukemic blasts that stained at four times above background, no correlation between CD33 expression and clinical response was observed (Sievers *et al*., 2001). Gemtuzumab treatment also does not appear to select for antigen-negative subclones. In instances in which a subsequent relapse occurred after an initial response to gemtuzumab, leukemic cells typically express CD33 at high levels. Several clinical observations suggest that MDR transporter proteins extrude free calicheamicin from leukemic cells. Clinical remissions with gemtuzumab were also associated with low blast cell MDR function (Linenberger *et al*., 2001). Because elevated blast cell drug efflux might be blocked by cyclosporine A (CSA), induction therapy with gemtuzumab and CSA might target the cytotoxic agent to the leukemic cells and toxicity to normal tissues might be limited. Clinical trials to explore this hypothesis are being designed.

IV. Summary

Some efficacy has been observed in a small number of AML patients harboring a low disease burden at the time of relapse and others with APL using the unconjugated antibody HuM195. In the setting of antibody–drug conjugates, approximately 25% of patients with AML in first relapse achieved remission after gemtuzumab monotherapy. Because blast cell expression of the MDR phenotype is associated with an inferior response with gemtuzumab, trials exploring MDR inhibition are planned. Without subsequent consolidation treatment, patients who respond to gemtuzumab will likely experience remission durations that average only 2 months. In comparison, gemtuzumab responders who are consolidated with HSCT sustain remissions that exceeded 18 months. Despite data suggesting that gemtuzumab is reasonably well tolerated by adults and children, no randomized trials have compared gemtuzumab monotherapy with combination chemotherapy. Neutropenia and thrombocytopenia were universally

observed, but mucositis was rarely seen. Gemtuzumab is targeted to CD33-expressing cells using an antibody, but liver injury appeared with moderate frequency. New clinical trials should shed light on the etiology of hepatic injury and possibly elucidate what clinical scenarios demand greater caution with gemtuzumab. Although emerging data suggest that combinations of conventional chemotherapy and gemtuzumab result in a relatively high remission induction rate for newly diagnosed AML patients, these findings must be regarded as highly preliminary. Nonetheless, a first generation of antibody-targeted chemotherapy has made its way to the clinics, certainly to be followed by second-generation strategies that improve antileukemic efficacy while simultaneously limiting damage to normal tissues.

References

Andrews, R. G., Singer, J. W., and Bernstein, I. D. (1989). "Precursors of colony-forming cells in humans can be distinguished from colony-forming cells by expression of the CD33 and CD34 antigens and light scatter properties." *J. Exp. Med.* **169,** 1721–1731.

Andrews, R. G., Torok-Storb, B., and Bernstein, I. D. (1983). "Myeloid-associated differentiation antigens on stem cells and their progeny identified by monoclonal antibodies." *Blood* **62**(1), 124–132.

Appelbaum, F. R., Matthews, D. C., Eary, J. F., Badger, C. C., Kellogg, M., Press, O. W., Martin, P. J., Fisher, D. R., Nelp, W. B., Thomas, E. D., and Bernstein, I. D. (1992). "The use of radiolabeled anti-CD33 antibody to augment marrow irradiation prior to marrow transplantation for acute myelogenous leukemia." *Transplantation* **54**(5), 829–833.

Bernstein, I. D., Singer, J. W., Andrews, R. G., Keating, A., Powell, J. S., Bjornson, B. H., Cuttner, J., Najfeld, V., Reaman, G., Raskind, W., Sutton, D. M. C., and Fialkow, P. J. (1987). "Treatment of acute myeloid leukemia cells in vitro with a monoclonal antibody recognizing a myeloid differentiation antigen allows normal progenitor cells to be expressed." *J. Clin. Invest.* **79**(4), 1153–1159.

Bernstein, I. D., Singer, J. W., Smith, F. O., Andrews, R. G., Flowers, D. A., Petersens, J., Steinmann, L., Najfeld, V., Savage, D., Fruchtman, S., Arlin, Z., and Fialkow, P. J. (1992). "Differences in the frequency of normal and clonal precursors of colony-forming cells in chronic myelogenous leukemia and acute myelogenous leukemia." *Blood* **79**(7), 1811–1816.

Bonnet, D., and Dick, J. E. (1997). "Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell." *Nature Med.* **3**(7), 730–737.

Bross, P. F., Beitz, J., Chen, G., Chen, X. H., Duffy, E., Kieffer, L., Roy, S., Sridhara, R., Rahman, A., Williams, G., and Pazdur, R. (2001). "Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia." *Clin Cancer Res.* **7**(6), 1490–1496.

Buchsbaum, D. J., Wahl, R. L., Normolle, D. P., and Kaminski, M. S. (1992). "Therapy with unlabeled and 131I-labeled pan-B-cell monoclonal antibodies in nude mice bearing Raji Burkitt's lymphoma xenografts." *Cancer Res.* **52**(23), 6476–6481.

Caron, P. C., Dumont, L., and Scheinberg, D. A. (1998). "Supersaturating infusional humanized anti-CD33 monoclonal antibody HuM195 in myelogenous leukemia." *Clin Cancer Res.* **4**(6), 1421–1428.

Co, M. S., Avdalovic, N. M., Caron, P. C., Avdalovic, M. V., Scheinberg, D. A., and Queen, C. (1992). "Chimeric and humanized antibodies with specificity for the CD33 antigen." *J. Immunol.* **148**(4), 1149–1154.

Dinndorf, P. A., Andrews, R. G., Benjamin, D., Ridgway, D., Wolff, L., and Bernstein, I. D. (1986). "Expression of normal myeloid-associated antigens by acute leukemia cells." *Blood* **67**(4), 1048–1053.

Ellestad, G. A., Ding, W.-D., Zein, N., and Townsend, C. A. (1995). DNA-Cleaving Properties of Calicheamicin Gamma1(I). *In* "Enediyne Antibiotics as Antitumor Agents" (D. B. Borders and T. W. Doyle, Eds.), pp. 137–160. Marcel Dekker, New York.

Estey, E. H., Giles, F. J., Beran, M., O'Brien, S, Pierce, S. A., Faderl, S. H., Cortes, J. E., and Kantarjian, H. M. (2002a). "Experience with gemtuzumab ozogamycin ("mylotarg") and all-trans retinoic acid in untreated acute promyelocytic leukemia." *Blood* **99**(11), 4222–4224.

Estey, E. H., Thall, P. F., Giles, F. J., Wang, X. M., Cortes, J. E., Beran, M., Pierce, S. A., Thomas, D. A., and Kantarjian, H. M. (2002b). "Gemtuzumab ozogamicin with or without interleukin 11 in patients 65 years of age or older with untreated acute myeloid leukemia and high-risk myelodysplastic syndrome: comparison with idarubicin plus continuous-infusion, high-dose cytosine arabinoside." *Blood* **99**(12), 4343–4349.

Feldman, E., Kalaycio, M., Weiner, G., Frankel, S., Schulman, P., Schwartzberg, L., Jurcic, J., Velez-Garcia, E., Seiter, K., Scheinberg, D., Levitt, D., and Wedel, N. (2003). "Treatment of relapsed or refractory acute myeloid leukemia with humanized anti-CD33 monoclonal antibody HuM195." *Leukemia* **17**(2), 314–318.

Feldman, E., Stone, R., Brandwein, J., Kalaycio, M., Moore, J., Chopra, R., Jurcic, J., Miller, C., Roboz, G., Levitt, D., Young, D., and O'Connor, J. (2002). "Phase III randomized trial of an anti-CD33 monoclonal antibody (HuM195) in combination with chemotherapy compared to chemotherapy alone in adults with refractory or first-relapse acute myeloid leukemia (AML)." *Proceedings of ASCO* **21,** 261a.

Griffin, J. D., Linch, D., Sabbath, K., Larcom, P., and Schlossman, S. F. (1984). "A monoclonal antibody reactive with normal and leukemic human myeloid progenitor cells." *Leuk Res.* **8**(4), 521–534.

Jurcic, J. G., DeBlasio, T., Dumont, L., Yao, T. J., and Scheinberg, D. A. (2000). "Molecular remission induction with retinoic acid and anti-CD33 monoclonal antibody HuM195 in acute promyelocytic leukemia." *Clin. Cancer Res.* **6**(2), 372–380.

Larson, R. A., Boogaerts, M., Estey, E., Karanes, C., Stadtmauer, E. A., Sievers, E. L., Mineur, P., Bennett, J. M., Berger, M. S., Eten, C. B., Munteanu, M., Loken, M. R., Van Dongen, J. J., Bernstein, I. D., and Appelbaum, F. R. (2002). "Antibody-targeted chemotherapy of older patients with acute myeloid leukemia in first relapse using Mylotarg (gemtuzumab ozogamicin)." *Leukemia* **16**(9), 1627–1636.

Linenberger, M. L., Hong, T., Flowers, D., Sievers, E. L., Gooley, T. A., Bennett, J. M., Berger, M. S., Appelbaum, F. R., and Bernstein, I. D. (2001). "Multidrug-resistance phenotype and clinical responses to gemtuzumab ozogamicin." *Blood* **98**(4), 988–994.

McDonald, G. B. (2002). "Management of hepatic sinusoidal obstruction syndrome following treatment with gemtuzumab ozogamicin (mylotarg(r))." *Clin Lymphoma* **2**(Suppl. 1), S35–539.

Petti, M. C., Pinazzi, M. B., Diverio, D., Romano, A., Petrucci, M. T., De Santis, S., Meloni, G., Tafuri, A., Mandelli, F., and Lo Coco, F. (2001). "Prolonged molecular remission in advanced acute promyelocytic leukaemia after treatment with gemtuzumab ozogamicin (Mylotarg CMA-676)." *Br. J. Haematol.* **115**(1), 63–65.

Rajvanshi, P. S. H., Sievers, E. L., and McDonald, G. B. (2002). "Hepatic sinusoidal obstruction after gemtuzumab ozogamicin (Mylotarg) therapy." *Blood* **99**(7), 2310–2314.

Scheinberg, D. A., Lovett, D., Divgi, C. R., Graham, M. C., Berman, E., Pentlow, K., Feirt, N., Finn, R. D., Clarkson, B. D., Gee, T. S., Larson, S., Oettgen, H., and Old, L. (1991). "A phase I trial of monoclonal antibody M195 in acute myelogenous leukemia: Specific bone marrow targeting and internalization of radionuclide." *J. Clin. Oncol.* **9**(3), 478–490.

Shan, D., Ledbetter, J. A., and Press, O. W. (1998). "Apoptosis of malignant human B cells by ligation of CD20 with monoclonal antibodies." *Blood* **91**(5), 1644–1652.

Sievers, E. L., Appelbaum, F. A., Spielberger, R. T., Forman, S. J., Flowers, D., Smith, F. O., Shannon-Dorcy, K., Berger, M. S., and Bernstein, I. D. (1999). "Selective ablation of acute myeloid leukemia using antibody-targeted chemotherapy: A phase I study of an anti-CD33 calicheamicin immunoconjugate." *Blood* **93**(11), 3678–3684.

Sievers, E. L., Larson, R. A., Estey, E., Stadmauer, E. A., Castaigne, S., Berger, M. S., Leopold, L. H., and Appelbaum, F. R. (2000). "Low incidence of hepatic veno-occlusive disease after treatment with gemtuzumab ozogamicin (Mylotarg, CMA-676): Relationship to hematopoietic stem cell transplantation (Abstract)." *Blood* **96,** 206b.

Sievers, E. L., Larson, R. A., Stadtmauer, E. A., Estey, E., Lowenberg, B. L., Dombret, H., Karanes, C., Theobald, M., Bennett, J. M., Sherman, M. L., Berger, M. S., Eten, C. B., Loken, M. R., van Dongen, J. J. M., Bernstein, I. D., and Appelbaum, F. R. (2001). "Efficacy and Safety of Gemtuzumab Ozogamicin in Patients with CD33-Positive Acute Myeloid Leukemia in First Relapse." *J. Clin. Oncol.* **19**(13), 3244–3254.

Sissi, C., Aiyar, J., Boyer, S., Depew, K., Danishefsky, S., and Crothers, D. M. (1999). "Interaction of calicheamicin gamma1(I) and its related carbohydrates with DNA-protein complexes." *Proc. Natl. Acad. Sci. USA* **96**(19), 10643–10648.

Turhan, A. G., Lemoine, F. M., Debert, C., Bonnet, M. L., Baillou, C., Picard, F., Macintyre, E. A., and Varet, B. (1995). "Highly purified primitive hematopoietic stem cells are PML-RARA negative and generate nonclonal progenitors in acute promyelocytic leukemia." *Blood* **85**(8), 2154–2161.

van der Velden, V. H. J., te Marvelde, J. G., Hoogeveen, P. G., Bernstein, I. D., Houtsmuller, A. B., Berger, M. S., and van Dongen, J. J. M. (2001). "Targeting of the CD33-calicheamicin immunoconjugate Mylotarg (CMA-676) in acute myeloid leukemia: *in vivo* and *in vitro* saturation and internalization by leukemic and normal myeloid cells." *Blood* **97,** 3197–3204.

Zein, N., Sinha, A. M., McGahren, W. J., and Ellestad, G. A. (1988). "Calicheamicin gamma 1I: An antitumor antibiotic that cleaves double-stranded DNA site specifically." *Science* **240**(4856), 1198–1201.

John M. Burke* **and Joseph G. Jurcic**[†]

*Department of Medicine and
[†]Leukemia Service, Department of Medicine
Memorial Sloan-Kettering Cancer Center
New York, New York 10021

Radioimmunotherapy of Leukemia

I. Chapter Overview

The use of monoclonal antibodies to deliver radioisotopes directly to tumor cells has become a promising strategy to enhance the antitumor effects of native antibodies. Because of the easy accessibility of tumor cells and the well-defined differentiation antigens, leukemias are ideal diseases in which to study radioimmunotherapy. Since the α and β particles emitted during the decay of radioisotopes differ in significant ways, proper selection of particular isotope and antibody combinations is crucial to making radioimmunotherapy a standard therapeutic modality. Clinical trials have demonstrated that anti-CD33, anti-CD45, and anti-CD66 antibodies, labeled with β-emitting radioisotopes such as iodine-131 (^{131}I), yttrium-90 (^{90}Y), and rhenium-188 (^{188}Re), can deliver significant doses of radiation to target organs with acceptable toxicity. These β-emitting radioimmunoconjugates can be particularly useful as a means to intensify conditioning

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

before hematopoietic stem cell transplantation. Radioimmunotherapy using shorter-ranged α-particle emitters such as bismuth-213 (^{213}Bi), astatine-211 (^{211}At), and actinium-225 (^{225}Ac) has been studied less extensively, but shows promise in the treatment of small-volume and cytoreduced disease. Further advances in the radioimmunotherapy of leukemia will require investigation of more potent isotopes, new methods of isotope delivery, treatment of patients with less advanced disease, and eventually randomized trials comparing radioimmunotherapy to standard approaches.

II. Introduction

The use of unlabeled monoclonal antibodies in the treatment of leukemias has met with mixed success. The anti-CD52 antibody alemtuzumab (Keating *et al*., 2002) and the anti-CD20 antibody rituximab (O'Brien *et al*., 2001) have displayed significant activity in chronic lymphocytic leukemia (CLL). Because of the lack of potency of many unconjugated monoclonal antibodies, however, investigators have used antibodies to deliver cytotoxic agents directly to tumor cells. Significant antileukemic effects have been observed with the anti-CD33-calcheamicin conjugate gemtuzumab ozogamicin in acute myeloid leukemia (AML) (Sievers *et al*., 2001) and the anti-CD22-*Pseudomonas* exotoxin construct BL22 in hairy cell leukemia (Kreitman *et al*., 2001). In an alternative strategy, antibodies can be used to target radioisotopes directly to tumor cells. Radioactive isotopes decay by emitting charged particles that can traverse cells and damage DNA or other cellular components, resulting in cell death by necrosis or apoptosis (Jonathan *et al*., 1999).

The leukemias are ideally suited for radioimmunotherapy for several reasons. First, because of their location in the blood, bone marrow, spleen, and lymph nodes, leukemic blasts are easily accessible to circulating antibodies. Second, target antigens on blasts and other hematopoietic cells are well known and can be characterized for individual patients using flow cytometry. Finally, leukemias are radiosensitive tumors. This chapter focuses on issues of target antigen and isotope selection, radiolabeling, antibody pharmacokinetics, and dosimetry. We also review the results of recent preclinical and clinical trials in the radioimmunotherapy of leukemia.

III. Antigenic Targets

Immunophenotypic characterization of the lineages and stages of hematopoietic differentiation provides the rationale for the selection of antigenic targets and associated carrier molecules for radioimmunotherapy. Most of these antigens, however, are neither lineage nor tumor specific. For example,

CD10, found on pre-B-cell acute lymphoblastic leukemia (ALL), is also found on follicular lymphomas and T-cell ALL. The only markers generally considered to be lineage specific are myeloperoxidase for myeloid cells, cytoplasmic CD3 for T lymphocytes, and cytoplasmic CD22 and cytoplasmic CD79 for B lymphocytes (Todd, 2002).

AML is characterized by the expression of the myeloid-associated antigens myeloperoxidase, CD13, CD15, CD33, and CD117 (Todd, 2002). HLA-DR is typically found on all subtypes of AML except acute promyelocytic leukemia (APL). Monocytic leukemias express antigens associated with more mature granulocytes and monocytes, including CD11a/18, CD11c, CD14, and CD15. Antigen expression in early phase chronic myeloid leukemia (CML) resembles that of mature granulocytes but is heterogeneous in blast crisis.

ALLs of B-cell origin are derived from the earliest stages of B-cell differentiation. CD10, CD19, and CD34 are found on most cases, and a small proportion express CD20. CLL is typically characterized by coexpression of CD5 and CD19, as well as by expression of CD23 and surface immunoglobulin. In addition, CLL weakly expresses CD20 (Rozman and Montserrat, 1995). Hairy cell leukemia generally expresses CD11c, CD19, CD20, CD22, CD25, CD103, and surface immunoglobulin (Jennings and Foon, 1997).

T-cell ALL and lymphoblastic lymphoma are the malignant counterparts of the earliest T-cells and express CD2, cytoplasmic CD3, CD5, CD7, and CD10 (Huh and Ibrahim, 2000). Adult T-cell leukemia/lymphoma (ATL) is characterized by expression of CD3, CD4, CD5, CD25, and HLA-DR (Jennings and Foon, 1997). Antibodies that target some of these antigens and have been studied clinically are listed in Table I. The antibodies investigated most extensively for the radioimmunotherapy of leukemia have been M195 and HuM195 (anti-CD33), the pan-leukocyte antibody BC8 (anti-CD45), anti-Tac (anti-CD25), and BW250/183 (anti-CD66) directed against granulocytes.

IV. Radioisotope Selection

The choice of an appropriate isotope for radioimmunotherapy depends on various factors, including the emission characteristics of the radionuclide, its physical and biological half-life, the stability of the immunoconjugate *in vivo*, the disease burden, and the clinical setting for which the therapy is intended. The α and β particles emitted by these isotopes have different physical properties that confer theoretical advantages and disadvantages to each. Alpha particles are positively charged helium nuclei that have a short range in tissue (50–80 μm) and a high linear energy transfer (LET; $\sim$100 keV/μm), whereas β particles are electrons that have a longer range

TABLE I Selected Target Antigens and Antibodies for Immunotherapy of Leukemia

Antigen	*Disease*	*Antibody*
CD5	ALL, CLL	T101, Tp67
CD7	ALL	Tp41
CD14	AML	AML2-23
CD15	AML	PM81
CD19	ALL, CLL	Anti-B4
CD20	CLL	Tositumomab (B1), rituximab, ibritumomab (2B8), 1F5
CD22	HCL, CLL	LL2, epratuzumab (hLL2), RFB4
CD23	CLL	IDEC-152
CD25	ATL	Anti-Tac
CD33	AML, CML	MY9, p67, M195, lintuzumab (HuM195)
CD45	AML, MDS, ALL	BC8
CD52	CLL	Alemtuzumab (Campath-1H)
CD66	AML, ALL	BW 250/183
HLA-DR	CLL	Lym-1, apolizumab (Hu1D10)

Note: ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; HCL, hairy cell leukemia; ATL, adult T-cell leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome.

(1–5 mm) with a lower LET (~0.2 keV/μm) (Zalutsky and Bigner, 1996). Because the range of β emissions extends for several millimeters, radioimmunotherapy with β emitters can create a crossfire effect, destroying tumor cells to which the radioimmunoconjugate is not directly bound. Because of the large size and high molecular weight of most antibodies, their diffusion into sites of bulky disease can be limited. Therefore, therapy with β emitters can potentially overcome resistance of antigen-negative tumor cells and is likely to be most useful in the setting of bulky disease and marrow transplantation, in which the preparative regimen should eliminate both malignant and nonmalignant hematopoietic cells.

The crossfire effect of β particles, however, might damage normal "bystander" cells, producing unwanted toxicity. Because the range of α particles measures only a few cell diameters, radioimmunotherapy with α-emitters might result in more specific tumor cell kill and less damage to surrounding normal tissue. Additionally, because α particles have a high LET, only one to five traversals through the nucleus are needed to kill a targeted cell, whereas up to 10,000 traversals by β particles might be required for cell death (Humm, 1987). These properties make α particles ideal for the treatment of small-volume or minimal residual disease.

Most studies of radioimmunotherapy trials for leukemia have used β-emitting isotopes such as iodine-131 (^{131}I), yttrium-90 (^{90}Y), and rhenium-188 (^{188}Re) (Table II). ^{131}I has a relatively long half-life of 8.1 days and emits a low-energy β particle. The β emissions from ^{131}I travel several centimeters within tissue and can be detected by gamma imaging,

TABLE II Characteristics of Selected β-Emitting Radioisotopes

Isotope	*Particle(s) emitted*	*Half-life*	*Particulate energy (keV)*	*Mean range of β emission (mm)*
Iodine-131	β, γ	8.0 days	970	0.8
Rhenium-188	β, γ	17 h	2120	2.4
Yttrium-90	β	64 h	2280	2.7

thereby facilitating biodistribution and dosimetry studies. Despite these benefits, a number of limitations are associated with the use of ^{131}I. First, treatment at high doses requires patient isolation and can result in significant exposure to hospital staff. Second, because approximately one third of the tyrosine residues, to which ^{131}I binds, are located in the hypervariable region of most monoclonal antibodies, radioiodination at high specific activities impairs the ability of these antibodies to bind to their target antigen (Nikula *et al.*, 1995). Finally, when used in conjunction with hematopoietic stem cell transplantation, sufficient time must separate ^{131}I treatment from stem cell infusion in order to prevent injury to the grafted cells from retained long-lived ^{131}I within the marrow. In transplantation trials with ^{131}I-labeled M195 and HuM195, these factors added up to 16 days to the preparative regimen (Burke *et al.*, 2003).

Many of these limitations of ^{131}I can be overcome by the use of radiometals such as ^{90}Y and ^{188}Re. Unlike ^{131}I, antibody labeling of most radiometals requires bifunctional chelators, thereby permitting labeling at higher specific activities and ensuring greater quality control. Because ^{90}Y does not emit γ rays, hospitalization and patient isolation are not necessary. Imaging for biodistribution and dosimetry studies, however, requires administration of antibody labeled with trace amounts of a second isotope, usually indium-111 (^{111}In), the biodistribution of which is not identical to that of ^{90}Y (Carrasquillo *et al.*, 1999). Positron emission tomography (PET) of ^{86}Y-labeled constructs is one strategy that might improve radiation dosimetry estimates for radioimmunotherapy with ^{90}Y (Lovqvist *et al.*, 2001).

Like β emitters, the various γ emitters have different properties that must be considered when selecting an isotope for radioimmunotherapy (Table III; McDevitt *et al.*, 1998). The 7.2-h half-life of astatine-211 (^{211}At) allows time for multistep labeling procedures and theoretically should enable ^{211}At-labeled constructs to be used even when the targeting molecule does not gain immediate access to tumor cells. The polonium-211 (^{211}Po) daughter of ^{211}At emits X-rays that permit external imaging for biodistribution studies. Bismuth-213 (^{213}Bi) has a half-life of only 46 min; therefore, this isotope is likely to be most useful in systems in which carrier

TABLE III Characteristics of Selected α-Emitting Radioisotopes

Isotope	*Particle(s) emitted*	*Half-life*	*Energy of α particle (keV)*
Astatine-211	1α	7.2 h	6800
Actinium-225	4α, 2β	10.0 days	5935
Bismuth-212	1α, 1β	60.6 min	7800
Bismuth-213	1α, 2β	46 min	5982
Lead-212	1α, 2β	10.6 h	7800

molecules can rapidly target disease sites. Preparation for clinical use requires a generator consisting of its parent isotope actinium-225 (^{225}Ac) dispersed onto a cation-exchange resin from which ^{213}Bi can be eluted (Ma *et al.*, 2001; McDevitt *et al.*, 1999a,b). Unlike many α emitters, ^{225}Ac has a long half-life (10 days) and decays by α emission through three atoms, each of which also emits an α particle (Fig. 1). As a result of these properties, ^{225}Ac-containing immunoconjugates are approximately 1000 times more potent than ^{213}Bi-containing conjugates (McDevitt *et al.*, 2001). Although this increased potency could make ^{225}Ac more effective than other α emitters, the possibility of free daughter radioisotopes in circulation after decay of ^{225}Ac raises concerns about the potential toxicity of this isotope.

V. Radiolabeling

A variety of methods are used to conjugate radioisotopes to antibodies, depending primarily on the nature of the radioisotope. Because ^{131}I binds to tyrosine residues, it can be conjugated directly to antibodies by using the chloramine-T method. Tumor resistance due to internalization of the antigen–antibody complex, followed by rapid degradation of the radioimmunoconjugate and expulsion of isotope metabolites, represents a significant disadvantage to therapy with some ^{131}I-labeled antibody constructs. This problem could potentially be overcome by the use of radiometals, which are better retained after catabolism than is ^{131}I (Scheinberg and Strand, 1983), or by novel iodination methods, such as tyramine cellobiose, resulting in more stable radioimmunoconjugates (Ali *et al.*, 1990). ^{211}At is a halogen like ^{131}I and is usually labeled to antibodies by incorporation of an aryl carbon-astatine bond into the antibody (Zalutsky and Vaidyanathan, 2000). Methods used to create the aryl carbon-astatine bond usually involve an astatodemetallation reaction using a tin, silicon, or mercury precursor (Zalutsky and Narula, 1988; Zalutsky and Vaidyanathan, 2000). ^{188}Re has

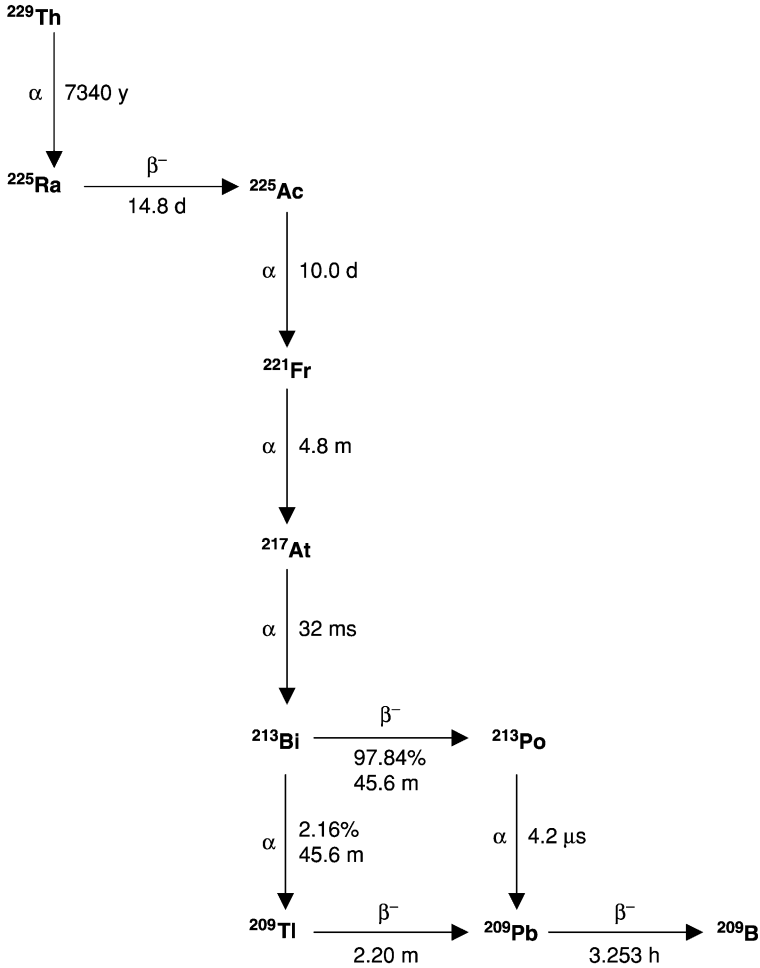

FIGURE 1 The ^{229}Th decay scheme. ^{225}Ac is isolated from ^{229}Th sources and decays by α emission through ^{221}Fr, ^{217}At, and ^{213}Bi, each of which also emits an α particle.

been directly labeled to the anti-CD66 antibody BW250/183 using tris-(2-carboxyethyl) phosphine as a reducing agent (Seitz *et al.*, 1999).

Other radioisotopes require bifunctional chelators for linkage to antibodies (Fig. 2). The macrocyclic ligand 1,4,7,10-tetraazacyclododecane tetraacetic acid (DOTA) and its derivatives have been used effectively for labeling antibodies with ^{90}Y (Deshpande *et al.*, 1990), ^{212}Bi (Junghans *et al.*, 1993), and ^{225}Ac (McDevitt *et al.*, 2002). However, in some experimental systems, DOTA might be immunogenic, and the formation of bismuth–DOTA complexes is slow. Alternative chelators derived from

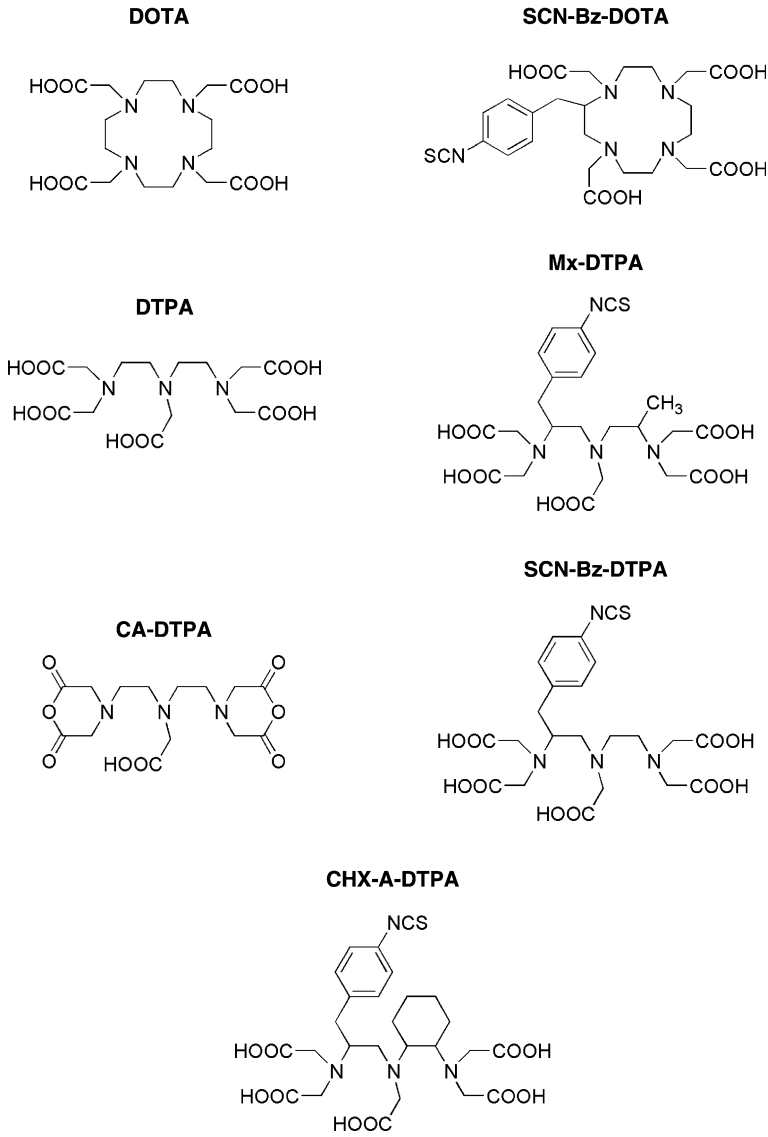

FIGURE 2 Chemical structures of selected chelators derived from 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and diethylenetriaminepentaacetic acid (DTPA).

diethylenetriaminepentaacetic acid (DTPA) have been developed. One of these is tiuxetan, or 2-(*p*-isothiocyanatobenzyl)-5(6)-methyl-DTPA (Mx-DTPA), which is used to label ibritumomab with ^{90}Y and ^{111}In for use in lymphoma (Roselli *et al.*, 1991). Other derivatives of DTPA include

the cyclic dianhydride derivative (Macklis *et al.*, 1988), 2-(*p*-isothiocyanatobenzyl)-DTPA (SCN-Bz-DTPA) (Ruegg *et al.*, 1990), and the cyclohexylbenzyl derivative (CHX-A-DTPA) (Huneke *et al.*, 1992; McDevitt *et al.*, 1999a). CHX-A-DTPA is effective at chelating yttrium and bismuth to antibodies (Camera *et al.*, 1994; McDevitt *et al.*, 1999a). The resulting immunoconjugates are stable (Huneke *et al.*, 1992; Nikula *et al.*, 1999) and have been used effectively in clinical trials (Burke *et al.*, 2002; Jurcic *et al.*, 2000b, 2002).

VI. Pharmacokinetics and Dosimetry

Factors such as variability in tumor burden and number of binding sites per cell for individual patients, antibody specificity and binding avidity, immunoreactivity, antigen–antibody internalization after binding, immunogenicity, and isotope half-life contribute to the difficult pharmacokinetics of radioimmunoconjugates. Careful biodistribution and dosimetry studies have led to greater insights on the pharmacology of antibodies. For example, the influence of the number of available antigen sites on antibody biodistribution was observed in a dose-escalation trial of trace-labeled ^{131}I-M195, in which superior targeting to sites of disease as determined by gamma camera imaging was seen with a comparatively small dose (Scheinberg *et al.*, 1991). This might be explained in part by the relatively low number of binding sites (approximately 10,000–20,000) on each leukemia cell. Furthermore, in a Phase I study of ^{213}Bi-HuM195, decreased activity in the liver and spleen was noted after multiple injections of small antibody doses, suggesting first-pass binding to leukemia cells and CD33-positive monocytes at these sites (Jurcic *et al.*, 2002).

Serial gamma camera imaging and measurements of plasma, urine, bone marrow, and tissue biopsy radioactivity are used to estimate absorbed radiation doses to different organs and tumor sites based on the Medical Internal Radiation Dose model (Society of Nuclear Medicine, 1988). Contours around regions of interest, such as the liver, spleen, and vertebrae, are used to calculate the activity at these sites. Kinetic curves can then be generated and converted to percentage injected dose for each region. Cumulated activity within each region, $\tilde{A}$, is estimated by fitting a sum or difference of two exponential expressions to time-activity data and integrating these expressions. The estimated absorbed dose over an organ volume, D_{ORG}, is given by the equation $D_{\mathrm{ORG}} = \tilde{A} \times \Delta / M_{\mathrm{ORG}}$, where Δ is the mean energy emitted per nuclear transition and M_{ORG} is the mass of the organ.

The validity of these predictions, however, is limited by the accuracy in measuring activity using gamma camera imaging and by the inability to visualize all sites of disease in patients. Single-photon emission computed tomography (SPECT) can increase the accuracy of planar scintigraphy,

especially when used in conjunction with computed tomography (Koral *et al*., 1994; Sgouros *et al*., 1993a). Nevertheless, the quantitative value of SPECT remains unknown. Models based on this dosimetric data can provide information about radiation doses delivered to tissues not directly sampled and also be used to estimate total tumor burden and tumor burden in individual organs (Hamacher and Sgouros, 2001; Sgouros *et al*., 1993b).

Radioimmunotherapy with short-lived α-emitters such as ^{213}Bi results in markedly different pharmacology than with longer-lived β-emitters. With longer-lived isotopes, pharmacokinetics are determined predominantly by the biologic clearance of the antibody. The distribution of the antibody within the first several minutes to hours after administration yields residence times that are negligible in proportion to the overall residence times achieved in target and normal organs. In contrast, for ^{213}Bi, with its 46-min half-life, 20% of the total α emissions occur within the first 15 min of injection, and after 3 h only 6% of the total emissions remain. Additionally, the higher LET of α particles compared with that of β particles results in an relative biological effectiveness (RBE) for cell sterilization of 3–7, which must be considered in dosimetry estimates for α-particle immunotherapy (Sgouros *et al*., 1999).

Given the high energy of α particles delivered over a short range, conventional methodologies that estimate mean absorbed dose over a specific organ volume might not always yield biologically meaningful information. Although targeted cells might receive high absorbed radiation doses, adjacent cells might receive no radiation at all. Therefore, microdosimetric or stochastic analyses that account for the spatial distribution of various cell types and the distribution of α decays within the organ will be necessary to estimate the absorbed dose to tumor cells and normal tissues more accurately. Because the geometric relationship between the radionuclide and the target cell is not uniform, α particle hits cannot be assumed to be a Poisson distribution. Several distributions have been modeled, and microdosimetric spectra, expressed as specific energy probability densities, have been calculated. Based on this work, methods have been developed to perform basic microdosimetric assessments that account for the probability of the number of hits and the mean specific energy from a single hit (Humm *et al*., 1993).

VII. Radioimmunotherapy with β-Particle Emitters

Most clinical radioimmunotherapy trials to date have used isotopes that emit β particles; however, α-particle immunotherapy has been studied in patients with myeloid leukemias more recently. The results of selected radioimmunotherapy trials for leukemia are summarized in Table IV.

TABLE IV Selected Clinical Trials of Radiolabeled Antibodies for Leukemia

Radiolabeled antibody	*Disease*	*Isotope dose*	*No. of patients*	*Results*	*Comments*	*Reference*
^{131}I-M195	Advanced AML, MDS, blastic CML	50–210 mCi/m^2	24	CR in 3 of 8 patients receiving BMT	5 Patients received autologous BMT; 3 received allogeneic BMT	Schwartz *et al.*, 1993
^{131}I-M195, ^{131}I-HuM195	Advanced AML, MDS, blastic CML	120–230 mCi/m^2	30	24/25 Evaluable patients had no evidence of leukemia; long-term DFS in 3 patients	Used with Bu/Cy before allogeneic BMT	Burke *et al.*, 2003
^{90}Y-HuM195	Advanced AML	0.1–0.3 mCi/kg	19	13 Patients had reductions in marrow blasts; 1 CR	Higher doses result in prolonged myelosuppression	Jurcic *et al.*, 2000b
^{213}Bi-HuM195	Advanced AML, CMML	0.28–1 mCi/kg	18	14 Patients had reduction in marrow blasts; no CRs	First demonstration of safety of α-particle therapy	Jurcic *et al.*, 2002
^{213}Bi-HuM195	AML	0.5–1.25 mCi/kg	15	2 CRs, 2 PRs, 2 marrow remissions	Given after partial cytoreduction with Ara-C	Burke *et al.*, 2002
^{131}I-p67	AML	110–330 mCi	9	3 of 4 patients treated with therapeutic doses relapsed	Given with Cy/TBI before BMT; many patients had unfavorable biodistribution	Appelbaum *et al.*, 1992
^{131}I-BC8	Advanced AML, ALL	76–612 mCi	44	7 of 25 patients with AML or MDS and 3 of 9 patients with ALL had long-term DFS	Given with Cy/TBI before BMT	Matthews *et al.*, 1999
^{131}I-BC8	AML in first remission	101–263 mCi	24	18 Patients with long-term DFS	Given with Bu/Cy prior to allogeneic BMT	Matthews *et al.*, 1996
^{188}Re-BW 250/183	High-risk AML, MDS	11.1 GBq (mean)	36	45% DFS at median 18 months	Given as part of preparative regimen prior to BMT	Bunjes *et al.*, 2001
^{90}Y-anti-Tac	ATL	5–15 mCi	18	2 CRs, 7 PRs	6 Patients developed HAMA	Waldmann *et al.*, 1995

Note: AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; CMML, chronic myelomonocytic leukemia; ALL, acute lymphoblastic leukemia; ATL, adult T-cell leukemia/lymphoma; CR, complete remission; BMT, bone marrow transplantation; DFS, disease-free survival; PR, partial remission; Bu, busulfan; Cy, cyclophosphamide; Ara-C, cytarabine; TBI, total body irradiation; HAMA, human antimouse antibodies.

A. ^{131}I-M195 and ^{131}I-HuM195

The murine antibody M195 and its humanized counterpart HuM195 target CD33, a glycoprotein expressed by most myeloid leukemia cells as well as committed myelomonocytic and erythroid progenitor cells (Co *et al.*, 1992; Tanimoto *et al.*, 1989). Although murine M195 rapidly targets leukemia cells in patients with AML, it does not have antileukemic activity in humans and produces human antimouse antibody (HAMA) responses in approximately 40% of patients (Scheinberg *et al.*, 1991). Unlike M195, HuM195 mediates complement-dependent and antibody-dependent cellular cytotoxicity *in vitro* (Caron *et al.*, 1992) and is not immunogenic *in vivo* (Caron *et al.*, 1994). In patients with APL, HuM195 can eliminate minimal residual disease detectable by reverse transcription-polymerase chain reaction (RT-PCR) amplification in 50% of patients (Jurcic *et al.*, 2000a). In patients with other subtypes of AML, however, HuM195 produces only occasional complete remissions in patients with low leukemic burdens (Caron *et al.*, 1994, 1998; Feldman *et al.*, 2003).

In a series of early studies, these antibodies were therapeutically labeled with ^{131}I to increase their antileukemic activity. In a Phase I trial, 24 patients with relapsed or refractory myeloid leukemias were treated with escalating doses (50–210 mCi/m^2) of ^{131}I-M195 (Schwartz *et al.*, 1993). Gamma camera images of the whole body demonstrated rapid uptake of the ^{131}I-M195 into the bone marrow, liver, and spleen. The radioisotope was retained at these sites for at least 3 days. The maximum tolerated dose was not reached, but profound myelosuppression occurred at ^{131}I doses more than 135 mCi/m^2, necessitating bone marrow transplantation in eight patients. Twenty-two patients had reductions in the percentage of bone marrow blasts, and three achieved complete remissions. This study demonstrated that ^{131}I-M195 can deliver high radiation doses to the marrow with limited extramedullary toxicity and significant antileukemic effects.

Based on these results, ^{131}I-M195 and ^{131}I-HuM195 were investigated as part of a preparative regimen for bone marrow transplantation (Burke *et al.*, 2003). Thirty-one patients with overt relapsed or refractory AML, accelerated or myeloblastic CML, or advanced myelodysplastic syndrome (MDS) were treated with ^{131}I-M195 or ^{131}I-HuM195 (122-437 mCi) followed by busulfan (16 mg/kg), cyclophosphamide (90 or 120 mg/kg), and infusion of related-donor bone marrow. Estimated absorbed radiation doses to the marrow ranged between 272 and 1470 cGy. Toxicities beyond those observed with the busulfan/cyclophosphamide conditioning regimen alone did not occur. Eight of 13 evaluable patients had decreases in bone marrow blasts immediately after treatment with ^{131}I-labeled anti-CD33 antibodies, and three patients remain in complete remission for 5+ to 8+ years following transplant.

B. ^{90}Y-HuM195

^{90}Y offers several potential advantages to overcome the limitations associated with ^{131}I-labeled anti-CD33 antibodies. The higher energy, longer-ranged β emissions of ^{90}Y permit a lower effective dose than ^{131}I. The absence of γ emissions allows large doses of ^{90}Y to be given safely in the outpatient setting. In a Phase I trial, 19 patients with relapsed or refractory AML were treated with escalating doses of ^{90}Y-HuM195 (0.1–0.3 mCi/kg), given as a single infusion without marrow support (Jurcic *et al.*, 2000b). Biodistribution and dosimetry studies were performed by coadministration of trace-labeled ^{111}In-HuM195. Up to 560, 880, and 750 cGy were delivered to the marrow, liver, and spleen, respectively. Despite these modest estimated absorbed doses, myelosuppression was the dose-limiting toxicity. Transient low-grade liver function test abnormalities were also seen in 11 patients. The maximum tolerated dose of ^{90}Y-HuM195 without stem cell rescue was 0.275 mCi/kg. Of 19 patients, 13 had reductions in bone marrow blasts. All patients treated at the highest dose level had markedly hypocellular bone marrow without evidence of leukemia up to 4 weeks after treatment. One patient treated at the maximum tolerated dose achieved complete remission lasting 5 months. Currently, ^{90}Y-HuM195 is being investigated as part of a reduced-intensity preparative regimen before allogeneic stem cell transplantation in patients with CD33-positive leukemias.

C. ^{131}I-p67

Investigators at the Fred Hutchinson Cancer Research Center studied another ^{131}I-labeled murine anti-CD33 antibody, p67, in patients with AML. In a Phase I trial, nine patients were initially treated with trace-labeled doses of ^{131}I-p67 (Appelbaum *et al.*, 1992). While the isotope localized to the marrow in most patients, residence times were relatively short (9–41 h), likely due to rapid catabolism of the radioimmunoconjugate following internalization. Only four patients had favorable biodistribution with greater uptake of ^{131}I in the marrow and spleen than in nonhematopoietic organs. Those patients subsequently received therapeutic doses of ^{131}I-p67 (110–330 mCi), cyclophosphamide (120 mg/kg), and total body irradiation (TBI; 12 Gy), followed by allogeneic bone marrow transplantation. Although the therapy was well tolerated, three of the four patients eventually relapsed (Ruffner and Matthews, 2000). Because of the unfavorable pharmacology and biodistribution of this construct, these investigators have since focused on the anti-CD45 antibody BC8.

D. ^{131}I-BC8

BC8 is a murine IgG1 antibody that targets CD45, a tyrosine phosphatase expressed by virtually all leukocytes, including myeloid and lymphoid

precursors, mature lymphocytes, and myeloid and lymphoid blasts. Unlike anti-CD33 antibodies, BC8 is not internalized after binding to cells (Matthews *et al.*, 1995). Radioimmunoconjugates targeting CD45 should eliminate not only leukemic blasts but also normal leukocytes in the marrow, limiting applications to the bone marrow transplant setting.

In a Phase I trial, 44 patients with advanced acute leukemia or MDS initially received BC8 labeled with trace doses of ^{131}I (Matthews *et al.*, 1999). Favorable biodistribution occurred in 37 patients (84%). Of these patients, 34 then received escalating therapeutic doses of ^{131}I-BC8 (76–612 mCi) followed by cyclophosphamide (120 mg/kg), TBI (12 Gy), and allogeneic or autologous transplantation. Therapeutic doses of ^{131}I-BC8 were calculated to deliver a specific absorbed dose to the normal organ that received the highest dose in the dosimetry studies performed after the trace-labeled injection. The maximum tolerated dose was an estimated absorbed dose of 10.5 Gy to the liver. At this dose, an average of 24 Gy and 50 Gy was delivered to the marrow and spleen, respectively. Of the 25 patients with AML or MDS, 7 remained alive and disease-free at a median follow-up of 65 months. Of the nine patients with ALL, three remained alive and disease-free at 19, 54, and 66 months. Based on these results, a Phase I/II trial using the preparative regimen of ^{131}I-BC8, busulfan, and cyclophosphamide in patients with AML in first remission is underway. In an encouraging preliminary report, favorable biodistribution occurred in 90% of patients (Matthews *et al.*, 1996). Of 24 patients treated with therapeutic doses, 18 were alive and disease-free at a median follow-up of 42 months.

E. ^{188}Re-Anti-CD66

A group at the Ulm University Hospital in Germany has investigated the use of a murine anti-CD66 antibody labeled with ^{188}Re as part of a preparative regimen before stem cell transplantation. ^{188}Re (17-h half-life) is a radiometal that emits both β particles and γ rays, which facilitate biodistribution and dosimetry studies. Because the glycoprotein CD66 is expressed on myeloid cells but not on leukemic blasts, any antileukemic effect of anti-CD66 must rely on "crossfire" resulting from the long range of the β particles emitted by the decay of ^{188}Re. In a pilot dosimetry trial, 12 patients with advanced leukemias received 6.5–12.4 GBq (175–335 mCi) of ^{188}Re-anti-CD66 followed by a standard preparative regimen and T-cell-depleted allogeneic transplantation (Seitz *et al.*, 1999). Favorable biodistribution occurred in most patients, and a median of 14 Gy were delivered to the bone marrow (Kotzerke *et al.*, 2000).

Subsequently, 36 patients with high-risk AML or MDS were treated with ^{188}Re-anti-CD66 followed by one of three preparative regimens: TBI (12 Gy) plus cyclophosphamide (120 mg/kg), busulfan (12.8 mg/kg) plus cyclophosphamide (120 mg/kg), or TBI plus thiotepa (10 mg/kg) and cyclophosphamide

(120 mg/kg) (Bunjes *et al.*, 2001). Of the patients, 31 received allogeneic grafts, 1 received a syngeneic graft, and 4 received autologous grafts. Favorable biodistribution occurred in all patients. The mean therapeutic dose of ^{188}Re was 11.1 GBq (300 mCi), and the median dose delivered to the bone marrow was 14.9 Gy (range 8.1–28 Gy). In contrast to studies with ^{131}I-anti-CD45, in which the liver was the dose-limiting normal organ, the normal organ receiving the highest dose of radiation after ^{188}Re-anti-CD66 was the kidney (median dose 7.2 Gy). Clinically, the administration of ^{188}Re-anti-CD66 did not result in additional toxicities beyond those associated with conventional preparative regimens; however, nephrotoxicity, possibly due to the radiation, occurred in six patients (17%) between 6 and 12 months after transplantation. At a median follow-up of 18 months, disease-free survival was 45%. This study demonstrated that ^{188}Re-anti-CD66, like ^{131}I- and ^{90}Y-labeled anti-CD33 and ^{131}I-anti-CD45 antibodies, can deliver significant doses of radiation to the bone marrow with acceptable toxicity. Whether preparative regimens that incorporate β particle radioimmunotherapy improve outcomes compared with standard preparative regimens remains to be determined by randomized clinical trials.

F. ^{90}Y-Anti-CD25

The receptor for interleukin-2 (IL-2) consists of at least three subunits called α (also known as Tac or CD25), β, and γ. Anti-Tac is a murine antibody that binds CD25, which is expressed on malignant cells in human T-cell leukemia virus I (HTLV)-associated ATL. Eighteen patients with ATL were treated with ^{90}Y-anti-Tac (5–15 mCi) in a Phase I/II trial (Waldmann *et al.*, 1995). Patients who attained remission were eligible to receive additional courses of therapy. Toxicities of ^{90}Y-anti-Tac included myelosuppression, transient hepatic toxicity, and transient proteinuria; in addition, one patient died from unexplained cardiac asystole 23 days after treatment. HAMA responses developed in six patients. Partial remissions, lasting a mean of 9 months, occurred in 7 patients, and complete remissions were seen in 2 of the 16 evaluable patients. One of the two patients in complete remission died from secondary AML 3 years after treatment with ^{90}Y-anti-Tac. At autopsy, evidence of persistent ATL was found in the skin.

VIII. Radioimmunotherapy with α-Particle Emitters

The high energy and short range of α particles offer the possibility of more efficient and selective killing of tumor cells. Therefore, to increase the antitumor activity of native monoclonal antibodies but avoid the nonspecific cytotoxicity of β-emitting radionuclides, α-particle immunotherapy has been investigated.

A. Preclinical Studies

In a number of different rodent xenograft models, treatment with monoclonal antibodies labeled with α particle emitters has prolonged survival compared with relevant controls (Horak *et al.*, 1997; Macklis *et al.*, 1988; McDevitt *et al.*, 2000, 2001; Zalutsky *et al.*, 1994). In one of the first reports suggesting the feasibility of this approach, ^{212}Bi conjugated to the tumor-specific antibody 103A demonstrated activity against murine erythroleukemia (Huneke *et al.*, 1992). The results of many of these studies support the hypothesis that α-particle radioimmunotherapy might be more effective in the treatment of small-volume disease than in the treatment of bulky tumors. For example, administration of ^{212}Bi-anti-Tac after inoculation of nude mice with a CD25-expressing lymphoma cell line led to prolonged tumor-free survival and prevented development of leukemia in some animals, whereas treatment of established tumors failed to produce responses (Hartmann *et al.*, 1994). Similarly, in spheroid models, α-particle therapy has been more effective in reducing the volume of smaller spheroids compared with larger ones (Ballangrud *et al.*, 2001; Kennel *et al.*, 1999; Langmuir *et al.*, 1990). In most of the animal models in which α-emitters and β-emitters have been directly compared, α-emitters have been more effective in preventing tumor growth and prolonging survival (Andersson *et al.*, 2001; Behr *et al.*, 1999).

^{213}Bi-labeled antibodies to CD45 (Sandmaier *et al.*, 2002) and the T-cell receptor (TCR) $\alpha\beta$ (Bethge *et al.*, 2003) have been used for immunosuppression before nonmyeloablative bone marrow transplantation in a canine model. Both ^{213}Bi-labeled antibodies, when given alone prior to transplantation and followed by additional immunosuppression with mycophenoloate mofetil and cyclosporine, allowed for prompt engraftment of transplanted marrow and resulted in stable mixed chimerism after transplantation. Toxicities included transient myelosuppression and liver function abnormalities. The high activities of ^{213}Bi (at least 2 mCi/kg) required for engraftment, however, might limit the use of this treatment in humans.

B. ^{213}Bi-HuM195

In vitro, ^{213}Bi-HuM195 killed cells expressing CD33 in a dose-dependent and specific-activity-dependent fashion (McDevitt *et al.*, 1999a). Up to 10 mCi/kg of ^{213}Bi-HuM195 could be injected intravenously into BALB/c mice without significant toxicity (Nikula *et al.*, 1999). Based on these preclinical studies, a Phase I clinical trial of ^{213}Bi-labeled HuM195 was performed in patients with advanced myeloid leukemias (Jurcic *et al.*, 2002). Eighteen patients with relapsed or refractory AML or chronic myelomonocytic leukemia were treated with 0.28–1.0 mCi/kg of ^{213}Bi-HuM195 in three to seven fractions over 2–4 days. Myelosuppression occurred in all

patients, and transient minor liver function abnormalities occurred in six patients. Gamma camera images demonstrated uptake of ^{213}Bi in the bone marrow, liver, and spleen within 10 min of administration without significant uptake in any other organs, including the kidneys, which are known to be avid for free bismuth. Because of low whole-body radiation doses, absorbed dose ratios between the marrow, liver, and spleen and the whole body were 1000-fold higher than those seen with β-emitting HuM195 constructs in similar patients. Of 15 evaluable patients, 14 (93%) had reductions in circulating blasts, and 14 of 18 (78%) evaluable patients had reductions in the percentage of bone marrow blasts. No complete remissions occurred, indicating the difficulty of targeting one to two ^{213}Bi atoms to each leukemic blast at the specific activities used in this trial.

Because α particle immunotherapy is likely to be most useful in the treatment of small-volume disease, a subsequent Phase I/II study was undertaken in which patients were first treated with chemotherapy to achieve partial cytoreduction of the leukemic burden followed by ^{213}Bi-HuM195 (Burke *et al*., 2002). To date, 15 patients with AML have been treated with sequential cytarabine (200 mg/m^2/day) and ^{213}Bi-HuM195 (0.5–1.25 mCi/kg). Prolonged myelosuppression was dose limiting, and the maximum tolerated dose was 1 mCi/kg. Among the nine patients who received doses of 1 mCi/kg or higher, two patients achieved complete remissions, two had partial remissions, and two had reductions in bone marrow blasts to less than 5% with incomplete recovery of peripheral blood counts. Although these results are preliminary, this study demonstrates that sequential cytarabine and ^{213}Bi-HuM195 can be given safely and can lead to complete remissions in patients with advanced AML.

C. ^{225}Ac Atomic Nanogenerators

Over its decay, ^{225}Ac emits four α particles and can be conjugated to a variety of antibodies by using derivates of DOTA. Therefore, ^{225}Ac-DOTA can act as an atomic nanogenerator, delivering an α-particle cascade to an individual cancer cell when coupled to an internalizing antibody. In initial toxicity studies, up to 15 μCi/kg could safely to given to BALB/c mice by intraperitoneal injection. In nude mice bearing prostate carcinoma xenografts, single nanocurie doses of ^{225}Ac-J591 directed against prostate-specific membrane antigen decreased prostate-specific antigen levels, prolonged survival compared with controls, and cured a substantial proportion of animals. Similarly, in a disseminated lymphoma mouse model, treatment with ^{225}Ac-anti-CD19 improved survival compared with controls (McDevitt *et al*., 2001). Based on these preclinical results, a Phase I trial of ^{225}Ac-labeled HuM195 in patients with advanced myeloid leukemias is planned.

D. Pretargeted Approaches

To improve tumor-to-normal tissue dose ratios, a novel pretargeting strategy for radioimmunotherapy has been developed that takes advantage of the rapid, high-affinity, and specific binding between streptavidin and biotin (Axworthy *et al.*, 2000). A monoclonal antibody or fusion protein is first conjugated to the tetravalent streptavidin molecule and infused intravenously. Then, a biotinylated *N*-acetylgalactosamine-containing clearing agent is given to remove excess antibody–streptavidin conjugate from the bloodstream. In this step, the biotin component of the clearing agent binds to the streptavidin portion of the antibody construct, and galactose receptors on hepatocytes remove the complexes from the circulation. Finally, therapeutically radiolabeled biotin is administered and binds to the pretargeted antibody–streptavidin conjugate on target cells. Unbound radiolabeled biotin is rapidly excreted in the urine.

Such a pretargeting approach has been applied to a mouse model of ATL (Zhang *et al.*, 2002). After treatment with humanized anti-Tac-streptavidin and the clearing agent, immunodeficient mice with human ATL received DOTA-biotin labeled with the α-emitter ^{213}Bi or the β-emitter ^{90}Y. Treatment with ^{213}Bi resulted in reductions in the concentrations of surrogate tumor markers human β_2-microglobulin and soluble CD25 and improved survival compared with controls. Treatment with ^{90}Y, however, did not improve survival compared with controls. Mice treated with ^{213}Bi by the pretargeting approach survived longer than those treated with ^{213}Bi labeled directly to anti-Tac. Despite these promising results, no animals were cured by a single course of therapy. This approach was also studied using an anti-Tac single-chain Fv-streptavidin fusion protein followed by radiolabeled biotin to treat ATL in xenografted mice (Zhang *et al.*, 2003). With ^{90}Y-DOTA-biotin, all 10 lymphoma-bearing mice were cured. Significant antitumor effects were also seen after administration of ^{213}Bi-DOTA-biotin to leukemic mice, and when combined with immunotherapy using unconjugated humanized anti-Tac, 7 of 10 mice were cured.

IX. Summary

Unlabeled monoclonal antibodies have become essential components of the therapeutic arsenal for cancer. Many unlabeled monoclonal antibodies, however, lack sufficient antitumor activity to provide meaningful responses. To increase their efficacy, antibodies can be used to deliver radioisotopes to target cells. To date, most studies in leukemia have used the β-emitters ^{131}I, ^{90}Y, and ^{188}Re labeled to anti-CD33, anti-CD45, anti-CD66, and anti-CD25 antibodies. These radioimmunoconjugates can eliminate large burdens of leukemia and can be given safely in conjunction with standard preparative

regimens prior to marrow or stem cell transplantation. Whether they can improve outcomes compared with conventional preparative regimens remains to be determined by randomized trials. α Emitters have promise in the treatment of small-volume disease. ^{213}Bi-labeled anti-CD33 has antileukemic activity and can produce complete remissions following treatment with single-agent cytarabine in some patients with advanced AML. Further advances in radioimmunotherapy will require investigation of more potent isotopes such as ^{225}Ac, new methods of isotope delivery such as pretargeting, treatment of patients with less advanced disease, and eventually randomized trials comparing radioimmunotherapy to more standard approaches.

References

Ali, S. A., Warren, S. D., Richter, K. Y., Badger, C. C., Eary, J. F., Press, O. W., Krohn, K. A., Bernstein, I. D., and Nelp, W. B. (1990). Improving tumor retention of radioiodinated antibody: Aryl carbohydrate adducts. *Cancer Res.* **50**(Suppl.), 783s–788s.

Andersson, H., Palm, S., Lindegren, S., Back, T., Jacobsson, L., Leser, G., and Horvath, G. (2001). Comparison of the therapeutic efficacy of ^{211}At- and ^{131}I-labelled monoclonal antibody MOv18 in nude mice with intraperitoneal growth of human ovarian cancer. *Anticancer Res.* **21,** 409–412.

Appelbaum, F. R., Matthews, D. C., Eary, J. F., Badger, C. C., Kellogg, M., Press, O. W., Martin, P. J., Fisher, D. R., Nelp, W. B., Thomas, E. D., and Bernstein, I. D. (1992). The use of radiolabeled anti-CD33 antibody to augment marrow irradiation prior to marrow transplantation for acute myelogenous leukemia. *Transplantation* **54,** 829–833.

Axworthy, D. B., Reno, J. M., Hylarides, M. D., Mallett, R. W., Theodore, L. J., Gustavson, L. M., Su, F., Hobson, L. J., Beaumier, P. L., and Fritzberg, A. R. (2000). Cure of human carcinoma xenografts by a single dose of pretargeted yttrium-90 with negligible toxicity. *Proc. Natl. Acad. Sci. USA* **97,** 1802–1807.

Ballangrud, Å. M., Yang, W. H., Charlton, D. E., McDevitt, M. R., Hamacher, K. A., Panageas, K. S., Ma, D., Bander, N. H., Scheinberg, D. A., and Sgouros, G. (2001). Response of LNCaP spheroids after treatment with an alpha-particle emitter (^{213}Bi)-labeled anti-prostate-specific membrane antigen antibody (J591). *Cancer Res.* **61,** 2008–2014.

Behr, T. M., Behe, M., Stabin, M. G., Wehrmann, E., Apostolidis, C., Molinet, R., Strutz, F., Fayyazi, A., Wieland, E., Gratz, S., Koch, L., Goldenberg, D. M., and Becker, W. (1999). High-linear energy transfer (LET) alpha versus low-LET beta emitters in radioimmunotherapy of solid tumors: Therapeutic efficacy and dose-limiting toxicity of 213Bi-versus 90Y-labeled CO17-1A Fab′ fragments in a human colonic cancer model. *Cancer Res.* **59,** 2635–2643.

Bethge, W. A., Wilbur, D. S., Storb, R., Hamlin, D. K., Santos, E. B., Brechbiel, M. W., Fisher, D. R., and Sandmaier, B. M. (2003). Selective T-cell ablation with bismuth-213 labeled anti-TCR $\alpha\beta$ as nonmyeloablative conditioning for allogeneic canine marrow transplantation. *Blood* **101,** 5068–5075.

Bunjes, D., Buchmann, I., Duncker, C., Seitz, U., Kotzerke, J., Wiesneth, M., Dohr, D., Stefanic, M., Buck, A., Harsdorf, S. V., Glatting, G., Grimminger, W., Karakas, T., Munzert, G., Dohner, H., Bergmann, L., and Reske, S. N. (2001). Rhenium 188-labeled anti-CD66 (a, b, c, e) monoclonal antibody to intensify the conditioning regimen prior to

stem cell transplantation for patients with high-risk acute myeloid leukemia or myelodysplastic syndrome: results of a phase I–II study. *Blood* **98,** 565–572.

Burke, J. M., Caron, P. C., Papadopoulos, E. B., Divgi, C. R., Sgouros, G., Panageas, K. S., Finn, R. D., Larson, S. M., O'Reilly, R. J., Scheinberg, D. A., and Jurcic, J. G. (2003). Cytoreduction with iodine-131-anti-CD33 antibodies before bone marrow transplantation for advanced myeloid leukemias. *Bone Marrow Transplant* **32,** 549–556.

Burke, J. M., Jurcic, J. G., Divgi, C. R., McDevitt, M. R., Sgouros, G., Finn, R. D., Larson, S. M., and Scheinberg, D. A. (2002). Sequential cytarabine and alpha-particle immunotherapy with bismuth-213-labeled anti-CD33 monoclonal antibody HuM195 in acute myeloid leukemia (AML). *Blood* **100**(Suppl.), 339a (abstract #1314).

Camera, L., Kinuya, S., Garmestani, K., Wu, C., Brechbiel, M. W., Pai, L. H., McMurry, T. J., Gansow, O. A., Pastan, I., and Paik, C. H. (1994). Evaluation of the serum stability and *in vivo* biodistribution of CHX-DTPA and other ligands for yttrium labeling of monoclonal antibodies. *J. Nucl. Med.* **35,** 882–889.

Caron, P. C., Co, M. S., Bull, M. K., Avdalovic, N. M., Queen, C., and Scheinberg, D. A. (1992). Biological and immunological features of humanized M195 (anti-CD33) monoclonal antibodies. *Cancer Res.* **52,** 6761–6767.

Caron, P. C., Dumont, L., and Scheinberg, D. A. (1998). Supersaturating infusional humanized anti-CD33 monoclonal antibody HuM195 in myelogenous leukemia. *Clin. Cancer Res.* **4,** 1421–1428.

Caron, P. C., Jurcic, J. G., Scott, A. M., Finn, R. D., Divgi, C. R., Graham, M. C., Jureidini, I. M., Sgouros, G., Tyson, D., Old, L. J., Larson, S. M., and Scheinberg, D. A. (1994). A phase 1B trial of humanized monoclonal antibody M195 (anti-CD33) in myeloid leukemia: Specific targeting without immunogenicity. *Blood* **83,** 1760–1768.

Carrasquillo, J. A., White, J. D., Paik, C. H., Raubitschek, A., Le, N., Rotman, M., Brechbiel, M. W., Gansow, O. A., Top, L. E., Perentesis, P., Reynolds, J. C., Nelson, D. L., and Waldmann, T. A. (1999). Similarities and differences in 111In- and 90Y-labeled 1B4M-DTPA antiTac monoclonal antibody distribution. *J. Nucl. Med.* **40,** 268–276.

Co, M. S., Avdalovic, N. M., Caron, P. C., Avdalovic, M. V., Scheinberg, D. A., and Queen, C. (1992). Chimeric and humanized antibodies with specificity for the CD33 antigen. *J. Immunol.* **148,** 1149–1154.

Deshpande, S. V., DeNardo, S. J., Kukis, D. L., Moi, M. K., McCall, M. J., DeNardo, G. L., and Meares, C. F. (1990). Yttrium-90-labeled monoclonal antibody for therapy: Labeling by a new macrocyclic bifunctional chelating agent. *J. Nucl. Med.* **31,** 473–479.

Feldman, E., Kalaycio, M., Weiner, G., Franke, S., Schulman, P., Schwartzberg, L., Jurcic, J., Velez-Garcia, E., Seiter, K., Scheinberg, D. A., Levitt, D., and Wedel, N. (2003). Treatment of relapsed or refractory acute myeloid leukemia with humanized anti-CD33 monoclonal antibody HuM195. *Leukemia* **17,** 314–318.

Hamacher, K. A., and Sgouros, G. (2001). Theoretical estimation of absorbed dose to organs in radioimmunotherapy using radionuclides with multiple unstable daughters. *Med. Phys.* **28,** 1857–1874.

Hartmann, F., Horak, E. M., Garmestani, K., Wu, C., Brechbiel, M. W., Kozak, R. W., Tso, J., Kosteiny, S. A., Gansow, O. A., Nelson, D. L., and Waldmann, T. A. (1994). Radioimmunotherapy of nude mice bearing a human interleukin 2 receptor alpha-expressing lymphoma utilizing the alpha-emitting radionuclide-conjugated monoclonal antibody 212Bi-anti-Tac. *Cancer Res.* **54,** 4362–4370.

Horak, E., Hartmann, F., Garmestani, K., Wu, C., Brechbiel, M., Gansow, O. A., Landolfi, N. F., and Waldmann, T. A. (1997). Radioimmunotherapy targeting of HER2/neu oncoprotein on ovarian tumor using lead-212-DOTA-AE1. *J. Nucl. Med.* **38,** 1944–1950.

Huh, Y. O., and Ibrahim, S. (2000). Immunophenotypes in adult acute lymphocytic leukemia. Role of flow cytometry in diagnosis and monitoring of disease. *Hematol. Oncol. Clin. North Am.* **14,** 1251–1265.

Humm, J. L. (1987). A microdosimetric model of astatine-211 labeled antibodies for radioimmunotherapy. *Int. J. Radiat. Oncol. Biol. Phys.* **13,** 1767–1773.

Humm, J. L., Roeske, J. C., Fisher, D. R., and Chen, G. T. (1993). Microdosimetric concepts in radioimmunotherapy. *Med. Phys.* **20,** 535–541.

Huneke, R. B., Pippin, C. G., Squire, R. A., Brechbiel, M. W., Gansow, O. A., and Strand, M. (1992). Effective alpha-particle-mediated radioimmunotherapy of murine leukemia. *Cancer Res.* **52,** 5818–5820.

Jennings, C. D., and Foon, K. A. (1997). Recent advances in flow cytometry: Application to the diagnosis of hematologic malignancy. *Blood* **90,** 2863–2892.

Jonathan, E. C., Bernhard, E. J., and McKenna, W. G. (1999). How does radiation kill cells? *Curr. Opin. Chem. Biol.* **3,** 77–83.

Junghans, R. P., Dobbs, D., Brechbiel, M. W., Mirzadeh, S., Raubitschek, A. A., Gansow, O. A., and Waldmann, T. A. (1993). Pharmacokinetics and bioactivity of 1,4,7,10-tetra-azacylododecane off′,N″,N‴-tetraacetic acid (DOTA)-bismuth-conjugated anti-Tac antibody for alpha-emitter (^{212}Bi) therapy. *Cancer Res.* **53,** 5683–5689.

Jurcic, J. G., DeBlasio, T., Dumont, L., Yao, T.-J., and Scheinberg, D. A. (2000a). Molecular remission induction with retinoic acid and anti-CD33 monoclonal antibody HuM195 in acute promyelocytic leukemia. *Clin. Cancer Res.* **6,** 372–380.

Jurcic, J. G., Divgi, C. R., McDevitt, M. R., Ma, D., Sgouros, G., Finn, R. D., Jimenez, J., Larson, S. M., and Scheinberg, D. A. (2000b). Potential for myeloablation with yttrium-90-HuM195 (anti-CD33) in myeloid leukemia. *Proc. Am. Soc. Clin. Oncol.* **19,** 8a (abstract #24).

Jurcic, J. G., Larson, S. M., Sgouros, G., McDevitt, M. R., Finn, R. D., Divgi, C. R., Ballangrud, Å. M., Hamacher, K. A., Ma, D., Humm, J. L., Brechbiel, M. W., Molinet, R., and Scheinberg, D. A. (2002). Targeted alpha particle immunotherapy for myeloid leukemia. *Blood* **100,** 1233–1239.

Keating, M. J., Flinn, I., Jain, V., Binet, J.-L., Hillmen, P., Byrd, J., Albitar, M., Brettman, L., Santabarbara, P., Wacker, B., and Rai, K. R. (2002). Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. *Blood* **99,** 3554–3561.

Kennel, S. J., Stabin, M., Roeske, J. C., Foote, L. J., Lankford, P. K., Terzaghi-Howe, M., Patterson, H., Barkenbus, J., Popp, D. M., Boll, R., and Mirzadeh, S. (1999). Radiotoxicity of bismuth-213 bound to membranes of monolayer and spheroid cultures of tumor cells. *Radiat. Res.* **151,** 244–256.

Koral, K. F., Zasadny, K. R., Kessler, M. L., Luo, J. Q., Buchbinder, S. F., Kaminski, M. S., Francis, I., and Wahl, R. L. (1994). CT-SPECT fusion plus conjugate views for determining dosimetry in iodine-131-monoclonal antibody therapy of lymphoma patients. *J. Nucl. Med.* **35,** 1714–1720.

Kotzerke, J., Glatting, G., Seitz, U., Rentschler, M., Neumaier, B., Bunjes, D., Duncker, C., Dohr, D., Bergmann, L., and Reske, S. N. (2000). Radioimmunotherapy for the intensification of conditioning before stem cell transplantation: Differences in dosimetry and biokinetics of 188Re- and 99mTc-labeled anti-NCA-95 MAbs. *J. Nucl. Med.* **41,** 531–537.

Kreitman, R. J., Wilson, W. H., Bergeron, K., Raggio, M., Stetler-Stevenson, M., FitzGerald, D. J., and Pastan, I. (2001). Efficacy of the anti-CD22 recombinant immunotoxin BL22 in chemotherapy-resistant hairy-cell leukemia. *N. Engl. J. Med.* **345,** 241–247.

Langmuir, V. K., Atcher, R. W., Hines, J. J., and Brechbiel, M. W. (1990). Iodine-125-NRLU-10 kinetic studies and bismuth-212-NRLU-10 toxicity in LS174T multicell spheroids. *J. Nucl. Med.* **31,** 1527–1533.

Lovqvist, A., Humm, J. L., Sheikh, A., Finn, R. D., Koziorowski, J., Ruan, S., Pentlow, K. S., Jungbluth, A., Welt, S., Lee, F. T., Brechbiel, M. W., and Larson, S. M. (2001). PET imaging of ^{86}Y-labeled anti-Lewis Y monoclonal antibodies in a nude mouse model: Comparison between ^{86}Y and ^{111}In radiolabels. *J. Nucl. Med.* **42,** 1281–1287.

Ma, D., McDevitt, M. R., Finn, R. D., and Scheinberg, D. A. (2001). Rapid preparation of short-lived alpha particle emitting radioimmunopharmaceuticals. *Appl. Radiat. Isot.* **55,** 463–470.

Macklis, R. M., Kinsey, B. M., Kassis, A. I., Ferrara, J. L., Atcher, R. W., Hines, J. J., Coleman, C. N., Adelstein, S. J., and Burakoff, S. J. (1988). Radioimmunotherapy with alpha-particle-emitting immunoconjugates. *Science* **240,** 1024–1026.

Matthews, D. C., Appelbaum, F. R., Eary, J. F., Fisher, D. R., Durack, L. D., Bush, S. A., Hui, T. E., Martin, P. J., Mitchell, D., Press, O. W., Badger, C. C., Storb, R., Nelp, W. B., and Bernstein, I. D. (1995). Development of a marrow transplant regimen for acute leukemia using targeted hematopoietic irradiation delivered by ^{131}I-labeled anti-CD45 antibody, combined with cyclophosphamide and total body irradiation. *Blood* **85,** 1122–1131.

Matthews, D. C., Appelbaum, F. R., Eary, J. F., Fisher, D. R., Durack, L. D., Hui, T. E., Martin, P. J., Mitchell, D., Press, O. W., Storb, R., and Bernstein, I. D. (1999). Phase I study of ^{131}I-anti-CD45 antibody plus cyclophosphamide and total body irradiation for advanced acute leukemia and myelodysplastic syndrome. *Blood* **94,** 1237–1247.

Matthews, D. C., Appelbaum, F. R., Eary, J. F., Mitchell, D., Press, O. W., and Bernstein, I. D. (1996). ^{131}I-anti-CD45 antibody plus busulfan/cyclophosphamide in matched related transplants for AML in first remission [Abstract 556]. *Blood* **88**(Suppl.), 142a.

McDevitt, M. R., Barendswaard, E., Ma, D., Lai, L., Curcio, M. J., Sgouros, G., Ballangrud, A. M., Yang, W. H., Finn, R. D., Pellegrini, V., Geerlings, M. W., Jr., Lee, M., Brechbiel, M. W., Bander, N. H., Cordon-Cardo, C., and Scheinberg, D. A. (2000). An alpha-particle emitting antibody ([^{213}Bi]J591) for radioimmunotherapy of prostate cancer. *Cancer. Res.* **60,** 6095–6100.

McDevitt, M. R., Finn, R. D., Ma, D., Larson, S. M., and Scheinberg, D. A. (1999a). Preparation of alpha-emitting ^{213}Bi-labeled antibody constructs for clinical use. *J. Nucl. Med.* **40,** 1722–1727.

McDevitt, M. R., Finn, R. D., Sgouros, G., Ma, D., and Scheinberg, D. A. (1999b). An ^{225}Ac/^{213}Bi generator system for therapeutic clinical applications: Construction and operation. *Appl. Radiat. Isot.* **50,** 895–904.

McDevitt, M. R., Ma, D., Lai, L. T., Simon, J., Borchardt, P., Frank, R. K., Wu, K., Pellegrini, V., Curcio, M. J., Miederer, M., Bander, N. H., and Scheinberg, D. A. (2001). Tumor therapy with targeted atomic nanogenerators. *Science* **294,** 1537–1540.

McDevitt, M. R., Ma, D., Simon, J., Frank, R. K., and Scheinberg, D. A. (2002). Design and synthesis of ^{225}Ac radioimmunopharmaceuticals. *Appl. Radiat. Isot.* **57,** 841–847.

McDevitt, M. R., Sgouros, G., Finn, R. D., Humm, J. L., Jurcic, J. G., Larson, S. M., and Scheinberg, D. A. (1998). Radioimmunotherapy with alpha-emitting nuclides. *Eur. J. Nucl. Med.* **25,** 1341–1351.

Nikula, T. K., Bocchia, M., Curcio, M. J., Sgouros, G., Ma, Y., Finn, R. D., and Scheinberg, D. A. (1995). Impact of the high tyrosine fraction in complementarity determining regions: Measured and predicted effects of radioiodination on IgG immunoreactivity. *Mol. Immunol.* **32,** 865–872.

Nikula, T. K., McDevitt, M. R., Finn, R. D., Wu, C., Kozak, R. W., Garmestani, K., Brechbiel, M. W., Curcio, M. J., Pippin, C. G., Tiffany-Jones, L., Geerlings, M. W., Sr., Apostolidis, C., Molinet, R., Geerlings, M. W., Jr., Gansow, O. A., and Scheinberg, D. A. (1999). Alpha-emitting bismuth cyclohexylbenzyl DTPA constructs of recombinant humanized anti-CD33 antibodies: Pharmacokinetics, bioactivity, toxicity and chemistry. *J. Nucl. Med.* **40,** 166–176.

O'Brien, S. M., Kantarjian, H., Thomas, D. A., Giles, F. J., Freireich, E. J., Cortes, J., Lerner, S., and Keating, M. J. (2001). Rituximab dose-escalation trial in chronic lymphocytic leukemia. *J. Clin. Oncol.* **19,** 2165–2170.

Roselli, M., Schlom, J., Gansow, O. A., Brechbiel, M. W., Mirzadeh, S., Pippin, C. G., Milenic, D. E., and Colcher, D. (1991). Comparative biodistribution studies of DTPA-derivative

bifunctional chelates for radiometal labeled monoclonal antibodies. *Int. J. Radiat. Applic. Instrument. Part B* **18,** 389–394.

Rozman, C., and Montserrat, E. (1995). Chronic lymphocytic leukemia. *N. Engl. J. Med.* **333,** 1052–1057.

Ruegg, C. L., Anderson-Berg, W. T., Brechbiel, M. W., Mirzadeh, S., Gansow, O. A., and Strand, M. (1990). Improved *in vivo* stability and tumor targeting of bismuth-labeled antibody. *Cancer Res.* **50,** 4221–4226.

Ruffner, K. L., and Matthews, D. C. (2000). Current uses of monoclonal antibodies in the treatment of acute leukemia. *Semin. Oncol.* **27,** 531–539.

Sandmaier, B. M., Bethge, W. A., Wilbur, D. S., Hamlin, D. K., Santos, E. B., Brechbiel, M. W., Fisher, D. R., and Storb, R. (2002). Bismuth 213-labeled anti-CD45 radioimmunoconjugate to condition dogs for nonmyeloablative allogeneic marrow grafts. *Blood* **100,** 318–326.

Scheinberg, D. A., Lovett, D., Divgi, C. R., Graham, M. C., Berman, E., Pentlow, K., Feirt, N., Finn, R. D., Clarkson, B. D., Gee, T. S., Larson, S. M., Oettgen, H. F., and Old, L. J. (1991). A phase I trial of monoclonal antibody M195 in acute myelogenous leukemia: Specific bone marrow targeting and internalization of radionuclide. *J. Clin. Oncol.* **9,** 478–490.

Scheinberg, D. A., and Strand, M. (1983). Kinetic and catabolic considerations of monoclonal antibody targeting in erythroleukemic mice. *Cancer Res.* **43,** 265–272.

Schwartz, M. A., Lovett, D. R., Redner, A., Finn, R. D., Graham, M. C., Divgi, C. R., Dantis, L., Gee, T. S., Andreeff, M., Old, L. J., Larson, S. M., and Scheinberg, D. A. (1993). Dose-escalation trial of M195 labeled with iodine 131 for cytoreduction and marrow ablation in relapsed or refractory myeloid leukemias. *J. Clin. Oncol.* **11,** 294–303.

Seitz, U., Neumaier, B., Glatting, G., Kotzerke, J., Bunjes, D., and Reske, S. N. (1999). Preparation and evaluation of the rhenium-188-labelled anti-NCA antigen monoclonal antibody BW 250/183 for radioimmunotherapy of leukaemia. *Eur. J. Nucl. Med.* **26,** 1265–1273.

Sgouros, G., Ballangrud, Å. M., Jurcic, J. G., McDevitt, M. R., Humm, J. L., Erdi, Y. E., Mehta, B. M., Finn, R. D., Larson, S. M., and Scheinberg, D. A. (1999). Pharmacokinetics and dosimetry of an alpha-particle emitter labeled antibody: ^{213}Bi-HuM195 (anti-CD33) in patients with leukemia. *J. Nucl. Med.* **40,** 1935–1946.

Sgouros, G., Chiu, S., Pentlow, K. S., Brewster, L. J., Kalaigian, H., Baldwin, B., Daghighian, F., Graham, M. C., Larson, S. M., and Mohan, R. (1993a). Three-dimensional dosimetry for radioimmunotherapy treatment planning. *J. Nucl. Med.* **34,** 1595–1601.

Sgouros, G., Graham, M. C., Divgi, C. R., Larson, S. M., and Scheinberg, D. A. (1993b). Modeling and dosimetry of monoclonal antibody M195 (anti-CD33) in acute myelogenous leukemia. *J. Nucl. Med.* **34,** 422–430.

Sievers, E. L., Larson, R. A., Stadtmauer, E. A., Estey, E., Löwenberg, B., Dombret, H., Karanes, C., Theobald, M., Bennett, J. M., Sherman, M. L., Berger, M. S., Eten, C. B., Loken, M. R., van Dongen, J. J. M., Bernstein, I. D., and Appelbaum, F. R. (2001). Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J. Clin. Oncol.* **19,** 3244–3254.

Society of Nuclear Medicine (1988). "MIRD Primer for Absorbed Dose Calculations." Society of Nuclear Medicine, Washington, D.C.

Tanimoto, M., Scheinberg, D. A., Cordon-Cardo, C., Huie, D., Clarkson, B. D., and Old, L. J. (1989). Restricted expression of an early myeloid and monocytic cell surface antigen defined by monoclonal antibody M195. *Leukemia* **3,** 339–348.

Todd, W. M. (2002). Acute myeloid leukemia and related conditions. *Hematol. Oncol. Clin. North Am.* **16,** 301–319.

Waldmann, T. E., White, J. D., Carrasquillo, J. A., Reynolds, J. C., Paik, C. H., Gansow, O. A., Brechbiel, M. W., Jaffe, E. S., Fleisher, T. A., Goldman, C. K., Top, L. E., Bamford, R.,

Zaknoen, S., Roessler, E., Kasten-Sportes, C., England, R., Litou, H., Johnson, J. A., Jackson-White, T., Manns, A., Hanchard, B., Junghans, R. P., and Nelson, D. L. (1995). Radioimmunotherapy of interleukin-2Rα-expressing adult T-cell leukemia with yttrium-90-labeled anti-Tac. *Blood* **86,** 4063–4075.

Zalutsky, M. R., and Bigner, D. D. (1996). Radioimmunotherapy with alpha-particle emitting radioimmunoconjugates. *Acta Oncol.* **35,** 373–379.

Zalutsky, M. R., and Narula, A. S. (1988). Astatination of proteins using an N-succinimidyl tri-*n*-butylstannyl benzoate intermediate. *Int. J. Rad. Appl. Instrum. [A]* **39,** 227–232.

Zalutsky, M. R., McLendon, R. E., Garg, P. K., Archer, G. E., Schuster, J. M., and Bigner, D. D. (1994). Radioimmunotherapy of neoplastic meningitis in rats using an alpha-particle-emitting immunoconjugate. *Cancer Res.* **54,** 4719–4725.

Zalutsky, M. R., and Vaidyanathan, G. (2000). Astatine-211-labeled radiotherapeutics: An emerging approach to targeted alpha-particle radiotherapy. *Curr. Pharm. Des.* **6,** 1433–1455.

Zhang, M., Yao, Z., Garmestani, K., Axworthy, D. B., Zhang, Z., Mallett, R. W., Theodore, L. J., Goldman, C. K., Brechbiel, M. W., Carrasquillo, J. A., and Waldmann, T. A. (2002). Pretargeting radioimmunotherapy of a murine model of adult T-cell leukemia with the alpha-emitting radionuclide, bismuth 213. *Blood* **100,** 208–216.

Zhang, M., Zhang, Z., Garmestani, K., Schultz, J., Axworthy, D. B., Goldman, C. K., Brechbiel, M. W., Carrasquillo, J. A., and Waldmann, T. A. (2003). Pretarget radiotherapy with an atni-CD25 antibody-stretavidin fusion protein was effective in therapy of leukemia/lymphoma xenografts. *Proc. Natl. Acad. Sci. USA* **100,** 1891–1895.

Michael Rosenblum

Immunopharmacology and Targeted Therapy Section
Department of Bioimmunotherapy
M. D. Anderson Cancer Center
Houston, Texas 77030

Immunotoxins and Toxin Constructs in the Treatment of Leukemia and Lymphoma

I. Chapter Overview

In the past few years, clinical trials have demonstrated the therapeutic benefit of a new generation of agents that use monoclonal antibodies and growth factors to target tumor cells, providing unprecedented specificity. The recent approval by the Food and Drug Administration (FDA) of several targeted therapeutic agents has encouraged preclinical and clinical development of novel targeted therapeutic approaches by numerous commercial and academic groups. This review highlights several of these promising approaches specifically targeting leukemia and lymphomas.

II. Introduction

Almost 25 years after the discovery of monoclonal antibodies, targeted therapeutics based on these agents are finally beginning to realize their initial promise with the approval of numerous agents and with many more agents

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

currently under development. Although initially developed to much fanfare in the early 1980s with the advent of murine monoclonal antibodies and hybridoma technology, the clinical use of murine antibodies never really demonstrated clinical significance for imaging and therapy because of problems associated with their immunogenicity, which limited repeated administration, and because of technological limitations, which limited the design of these agents. Nonetheless, the numerous early clinical studies attempting to use antibodies as cell-targeting carriers for isotopes, drugs, and toxins were extremely useful in that they provided an insight into and an understanding of the problems facing targeted delivery approaches (soluble antigen, immunogenicity, *in vivo* stability, *in vivo* delivery characteristics). The fairly recent application of molecular biology techniques to the antibody field has resulted in next-generation therapeutic agents such as recombinant, chimeric, human, and humanized antibodies and led to the eventual development of the current class of therapeutic agents.

Numerous radioisotopic, drug, and toxin payloads have been employed in the construction of therapeutic agents targeting leukemia-associated or hematologically restricted antigens. There are at present several agents approved and under development containing radioisotopes for radioimmunotherapeutic applications for leukemic and lymphoma applications (reviewed in this volume). In addition, groups have used other payloads containing small molecules such as doxorubicin (Messinger *et al*., 1996; Oldham *et al*., 1988; Trail *et al*., 2003) calichiemaicin (reviewed later), and protein toxins such as diptheria toxin (DI) (Alexander *et al*., 2000; Feuring-Buske *et al*., 2000; Frankel *et al*., 2003a; Nichols *et al*., 1997; Sweeney *et al*., 1998; Thorburn *et al*., 2003), pokeweed antiviral protein (PAP) (Chu *et al*., 1990; Gunther *et al*., 1993a; Myers and Uckun, 1995), saporin (Bregni *et al*., 1989; Flavell *et al*., 2001; Siena *et al*., 1989; Tazzari *et al*., 1993; Terenzi *et al*., 1996) ricin A chain (Amlot *et al*., 1993; Conry *et al*., 1995; Engert *et al*., 1990, 1995; Ghetie *et al*., 1992; Huang *et al*., 1993; Kreitman, 2001), and gelonin (reviewed later; Harris *et al*., 1991; Ishiguro *et al*., 1992; Shin *et al*., 2003). In addition to these approaches, other investigators have used proapoptotic enzymatic payloads, such as human RNase (Huhn *et al*., 2001; Psarras *et al*., 2000), for developing constructs with reduced potential for immunogenicity.

III. "Targets of Opportunity" in Leukemia and Lymphoma

A. CD22

The CD22 antigen is expressed on the surface of normal human B cells and some neoplastic B-cell lines and tumors. Previous cross-blocking studies using a panel of monoclonal anti-CD22 antibodies have defined four epitope

groups, termed A–D (Engel *et al*., 1995; Mason *et al*., 1990; Toba *et al*., 2002; Zola *et al*., 1987). Several groups initially identified immunotoxins composed of anti-CD22 antibodies chemically conjugated to ricin A-chain (RTA) (Ghetie *et al*., 1988; May *et al*., 1986; Shen *et al*., 1988). These agents demonstrated impressive cytotoxicity against target cells at IC_{50} concentrations well in the picomolar range. Phase I clinical trials of anti-CD22-RTA immunotoxins demonstrated evidence of both toxicity and clinically relevant antitumor effects in resistant patients (Amlot *et al*., 1993; Sausville *et al*. 1995; Vitetta *et al*., 1991). In addition to immunotoxins containing RTA, other investigators have generated and tested anti-CD22 immunotoxins containing the toxins gelonin (French *et al*., 1995) and saporin (Bérgamaschi *et al*., 1996; Bonardi *et al*., 1993). More recently, Hursey *et al*. (2002) demonstrated that conjugation of anti-CD22 antibodies with human RNase can cause specific and potent cytotoxicity to lymphocytes *in vitro* and might represent a novel class of immunotoxin platforms that potentially avoid problems of toxicity and immunogenicity associated with some plant or bacterial toxins.

A group under the direction of Dr. Ira Pastan at the National Cancer Institute (NCI) has generated numerous immunotoxins and fusion constructs targeting a variety of cell-surface antigens that contain a recombinant version of the toxin *Pseudomonas* exotoxin (PE) (Husain *et al*., 1999; Joshi *et al*., 2002; Lorimer *et al*., 1995; Pastan, 2003; Rozemuller *et al*., 2001; Shinohara *et al*., 2002). Studies from this group have demonstrated that fusion toxins targeting CD22 display impressive cytotoxicity *in vitro* and *in vivo* in human tumor models (Kreitman *et al*., 1999, 2000; Mansfield *et al*., 1996, 1997a,b). In addition, clinical trials of CD22 fusion toxins have demonstrated clinical responses in chemotherapy-refractory patients with hairy cell leukemia (Kreitman and Pastan, 2003; Kreitman *et al*., 2001).

B. CD33

The CD33 antigen is expressed on most early myeloid cells (Kristensen and Hokland, 1991; Liu *et al*., 1991; Scheinberg *et al*., 1989; Simmons and Seed, 1988; Tanimoto *et al*., 1989) and by more than 90% of cases of acute myeloid leukemia (AML) and in virtually all cases of chronic myeloid leukemia (CML). This antigen is found on myeloid leukemia blasts as well as on myeloid progenitor cells, but it is not expressed in detectable amounts on the ultimate hematopoietic progenitor stem cell. The CD33 antigen appears to be expressed at a density of approximately 10,000–100,000 sites per cell (Griffin *et al*., 1984; Robertson *et al*., 1992; Scheinberg *et al*., 1989; Tanimoto *et al*., 1989), but does not appear to be expressed by cells outside the hematopoietic system (Feuring-Buske *et al*., 2000). Clinical studies by Scheinberg *et al*. (1989) with an unmodified recombinant murine anti-CD33 antibody in patients with AML demonstrated that the antibody

quickly bound to leukemia cells and that the antigen–antibody complex rapidly internalized following cell binding. However, when administered to patients with overt leukemia, unmodified antibody resulted in transient decreases in peripheral blast counts but not in sustained response (Caron *et al*., 1998; Feldman *et al*., 2003; Jurcic *et al*., 2000). As reviewed in another chapter, Scheinberg *et al*. (1989) are developing radioimmunotherapeutic agents targeting the CD33 antigen and that have significant clinical promise. In addition to this approach, several other agents are under development and approved that target the CD33 antigen, as described next.

1. Gemtuzumab Ozogamicin

Gemtuzumab ozogamicin (Mylotarg®) is a classical conjugate of a humanized anti-CD33 antibody and the DNA-damaging agent calicheamicin. Calicheamicin is a potent cytotoxic agent that causes double-strand DNA breaks, resulting in cell death (Dedon *et al*., 1993; Drak *et al*., 1991; Zein *et al*., 1988). When conjugated to monoclonal antibodies specific for tumor-associated antigens, calicheamicin exerts strong antigen-specific antitumor effects *in vitro* and against human tumor xenografts in preclinical models (Bernstein, 2002; Nabhan and Tallman, 2002; Voutsadakis, 2002). Numerous clinical trials with this agent have demonstrated efficacy in CD33-positive leukemic patients (Douer, 2002; Roboz *et al*., 2002; Stadtmauer, 2002; Viele, 2002; Voutsadakis, 2002).

2. HuM195/rGel

Gelonin toxin, originally isolated from the seeds of *Gelonium multiflorum*, is a single polypeptide chain and is in a class of molecules designated as ribosome-inhibitory proteins (RIPs) that have *N*-glycosidase activity. Another molecule in this class of proteins, ricin, is composed of an enzymatically active A chain (RTA) linked to a lectin-binding B chain (RTB), which serves as an indiscriminate cell-binding and internalization vehicle for the A-chain component. In contrast, gelonin contains no lectin-binding component (Falasca *et al*., 1982; Stirpe *et al*., 1980; Thorpe *et al*., 1981). The gelonin molecule is, therefore, relatively nontoxic to intact cells, and numerous chemical conjugates of gelonin have been reported to have impressive antitumor activities both *in vitro* and *in vivo* (Atkinson *et al*., 2001; Bolognesi *et al*., 2000; Delprino *et al*., 1993; Gosselaar *et al*., 2002; Marcil *et al*., 1993; Rosenblum *et al*., 1996; Yazdi and Murphy, 1994). Several years ago, our laboratory reported on the cloning, expression, and biologic activity of recombinant gelonin (rGel) (Rosenblum *et al*., 1995), which has cytotoxic activity equivalent to that of natural gelonin (nGel) and improved pharmacokinetics *in vivo* probably due to the absence of high-level carbohydrate structures that can result in mistargeting *in vivo* (Rosenblum *et al*., 1999). In addition, fusion constructs and chemical conjugates of rGel have preserved *N*-glycosidase activity without the necessity

of release from the cell-targeting protein carrier. This is in contrast to other toxins such as RTA, which requires intracellular release from its protein carrier to become enzymatically active (O'Hare *et al.*, 1990). Although both RTA and rGel both operate as *N*-glycosidases, there is only ~30% sequence homology between these two molecules and there are other subtle biological differences in the intracellular behavior of these two proteins (McGrath *et al.*, 2003).

We constructed a leukemia-selective immunotoxin by linking rGel to the recombinant humanized anti-CD33 antibody HuM195 (McGrath *et al.*, 2003; McGraw *et al.*, 1994; Pagliaro *et al.*, 1998; Xu *et al.*, 1996). The schematic representation for this conjugate is shown in Fig. 1. This is a covalent, chemical conjugate of the antibody and the toxin enabled using a disulfide linkage. The resulting conjugate material is a 1:1 molar ratio of antibody to toxin (~190 kDa). ELISA studies demonstrate that the antibody-binding activity of the immunoconjugate was essentially unchanged compared to that of unmodified antibody. Analysis of the biologic activity of the rGel component in a cell-free protein synthesis assay demonstrated that the activity of the toxin component of the immunotoxin was also essentially unchanged and fully functional (Fig. 2). Confocal imaging that examines the internalization of the rGel toxin into HL-60 cells (Fig. 3) demonstrated that by 30 min of exposure, a significant proportion of cells internalized this agent into the cytoplasm. Within 4 h, internalization of the toxin into the cellular cytoplasm appeared to be maximal. We then evaluated the ability of this conjugate to inhibit cellular protein synthesis. Figure 4 demonstrates that the inhibitory concentration for the HuM195–rGel immunotoxin on

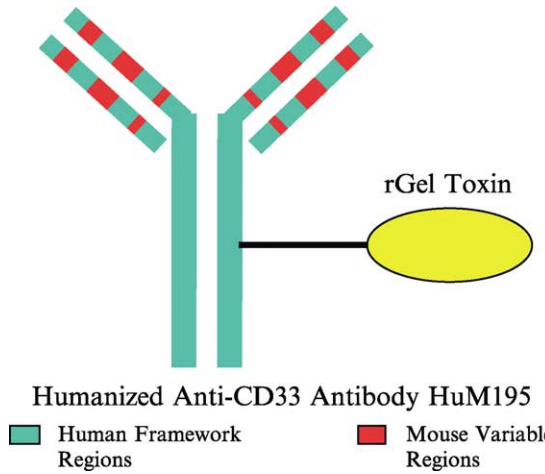

FIGURE 1 Humanized anti-CD33 antibody HuM195. Schematic representation of HuM195–rGel chemical conjugate. This is a 1:1 molar ratio of antibody to toxin.

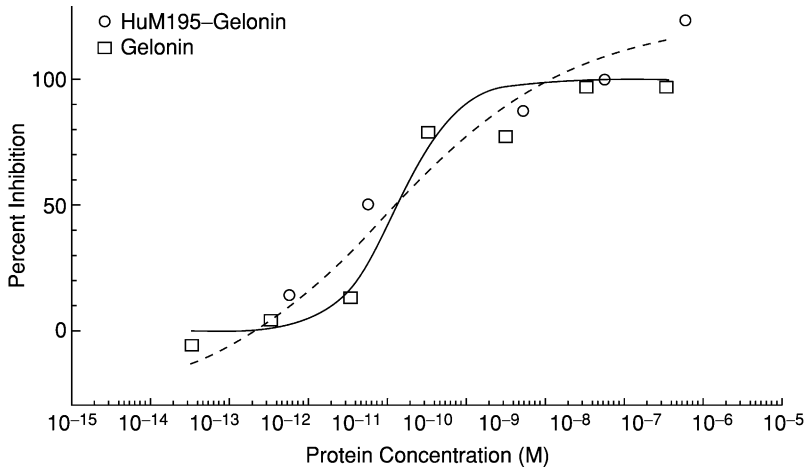

FIGURE 2 Inhibition of cell-free protein synthesis by gelonin and HuM195–rGel. A rabbit reticulocyte protein synthesis assay, both gelonin toxin and the HuM195–rGel conjugate were able to inhibit protein synthesis at concentrations of $\sim 10^{-11}$M. This suggests that the gelonin component of the HuM195–rGel conjugate has preserved biological activity compared with that of free gelonin.

HL60 cells was approximately four logs lower (2×10^{-10} M) than that of free gelonin toxin (IC_{50} of 5×10^{-6} M). We next examined the cytotoxicity of various immunotoxin concentrations on fresh tumor isolates from a variety of patients. As shown in Figs. 5 and 6, there was a wide variation in the dose–response curves of tumor cells isolated from different patients. On further analysis (Fig. 6), the IC_{50} values appeared to be proportional to the number of CD33-positive cells in the isolate. Surprisingly, the IC_{50} values also appeared to be within 20- to 30-fold of that found for the HL60 cell line.

We also evaluated HuM195–rGel *in vivo* in a nude mouse model of human myeloid leukemia (HL60). Nude mice were injected intraperitoneally with 10^7 log-phase HL60 human leukemia cells (Fig. 7 and Table I) 10 days prior to the start of intraperitoneal HuM195–rGel treatments. HuM195–rGel demonstrated significant tumor-suppressive activity in this model. All mice treated with saline, rGel alone, or HuM195 plus unconjugated rGel (at 10 or 14 days after transplantation) had rapid tumor growth or early deaths, but 50% of mice treated with HuM195–rGel failed to develop leukemic tumors for 5 months and the 50% had significantly retarded tumor growth after treatment with HuM195–rGel. Mice treated at later times (28 days after transplantation of leukemia cells) also showed delayed leukemia cell growth, but none was cured. These data show that HuM195–rGel can target leukemia cells *in vivo* and can result in pronounced antileukemic effects.

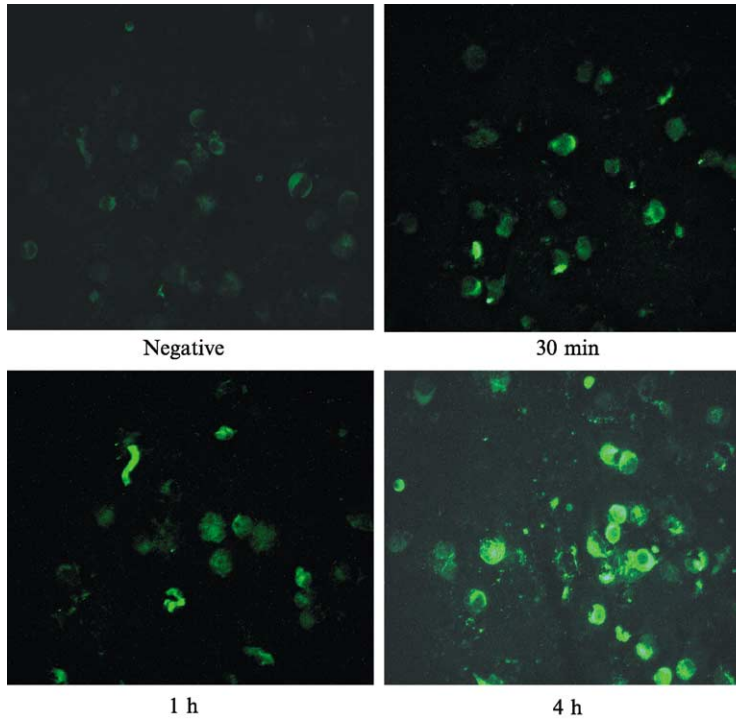

FIGURE 3 Internalization of HuM195–rGel on HL60 cells. Log-phase HL60 cells were treated with HuM195–rGel, saline, or gelonin itself. After various time periods, the cells were washed and then acid treated to remove cell-surface bound immunotoxin. The cells were fixed, permeabilized, and rabbit antigelonin polyclonal antibodies were employed to visualize the internalized toxin. Internalized toxin was observed as early as 30 min after exposure to the HuM195–rGel. Internalization appeared maximal 4 h after cell exposure. There was no internalization of gelonin observed after incubation for up to 24 h.

Phase I clinical studies of the HuM195–rGel immunotoxin are currently ongoing at the M. D. Anderson Cancer Center. The Phase I design for the intravenous administration study is a 1-h infusion of the drug every 72 h (twice per week) for 14 days (four doses total) followed by a 14-day observation period. The total dose levels are 10, 12, 18, 28, 40, and 60 mg/m^2. To date, 22 patients have been treated at doses up to 40 mg/m^2. Fever and chills have been noted with infusion of HuM195–rGel (Talpaz *et al.*, 2003). Pharmacokinetic studies (Fig. 8) demonstrate that the intact immunotoxin can reach levels of $\sim$1 μg/ml in serum immediately after the end of the drug infusion (at the 28 mg/m^2 dose level). Drug levels for the immunotoxin appeared to be within or exceed the IC_{50} value range for the immunotoxin against cell lines and patient isolates. Thus far, we have neither encountered major dose-limiting toxicity nor noted any vascular leak

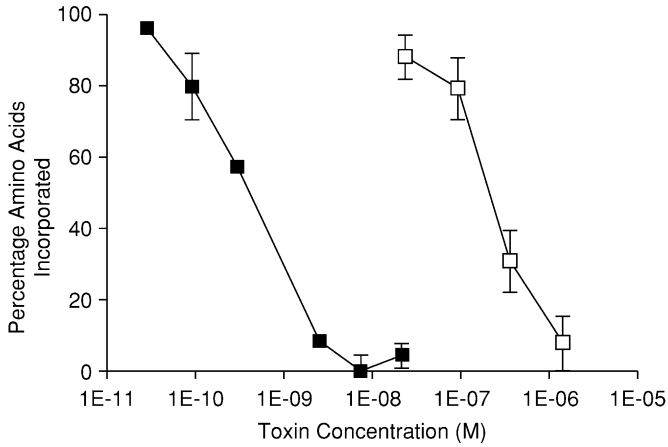

FIGURE 4 Inhibition of cellular protein synthesis by HuM195–rGel and rGel. Incorporation of ^{3}H-leucine was assessed in HL-60 cells treated with various doses of HuM195–rGel (closed squares) and recombinant gelonin (open squares). The inhibition of cellular protein synthesis was approximately 1000-fold more efficient with HuM195–rGel than with equivalent molar concentrations of the free gelonin toxin.

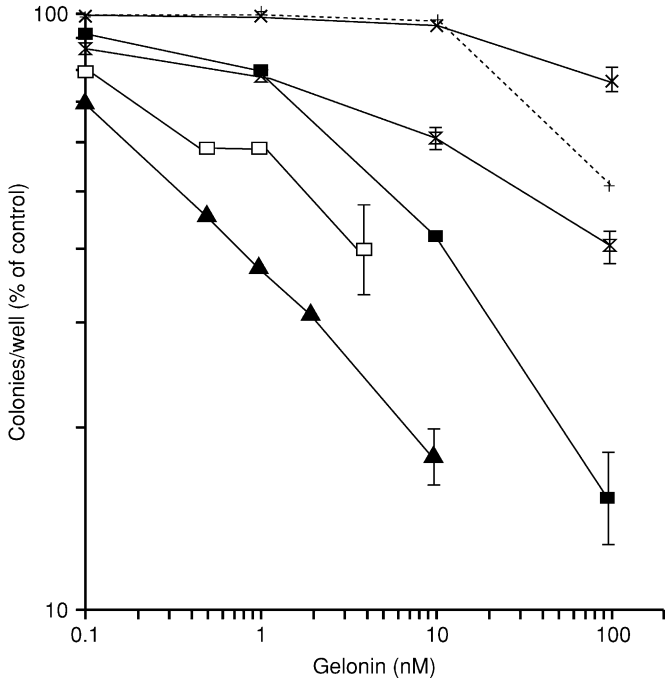

FIGURE 5 Dose–response curves of HuM195–rGel on patient isolates. Tumor cells were isolated from six different patients and were treated *ex vivo* with various doses of HuM195–rGel.

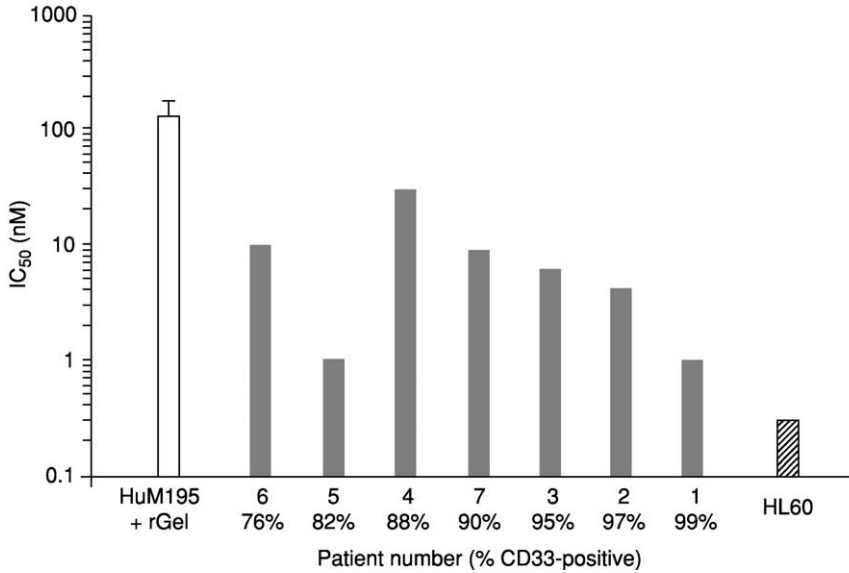

FIGURE 6 Cytotoxicity of HuM195–rGel on patient isolates-correlates with CD33 expression. The IC_{50} values for the HuM195–rGel immunotoxin on various patient isolates were assessed compared with the relative expression of CD33 on tumor cells. Increasing cellular CD33 expression appeared to directly correlate with increasing sensitivity to the cytotoxic effects of the immunotoxin.

syndrome, which has been observed with administration of RTA-based immunotoxins (Baluna and Vitetta, 1999; Lindstrom *et al*., 1997) and appears to be the dose-limiting toxicity associated with these agents. Our Phase I study has demonstrated evidence of biological effects in several patients, such as reduction in peripheral blast cells and reduction of leukemic blast cells in the bone marrow. As far as we are aware, this is the first reported clinical study of a gelonin-based immunotoxin. Although these early findings are encouraging, this trial is still ongoing, and the results described should be considered preliminary findings.

C. CD38

One of the central problems in the development of targeted therapeutic agents is the heterogenous expression of the target antigen within the tumor. This could potentially lead to ineffectiveness of the immunotoxin after multiple courses of therapy because of rapid outgrowth of tumor cells that express reduced antigen density. The CD38 antigen is a cell-surface glycoprotein (46 kDa) whose expression is generally restricted to lineage-committed lymphoid, erythroid, and myeloid precursor cells in the bone

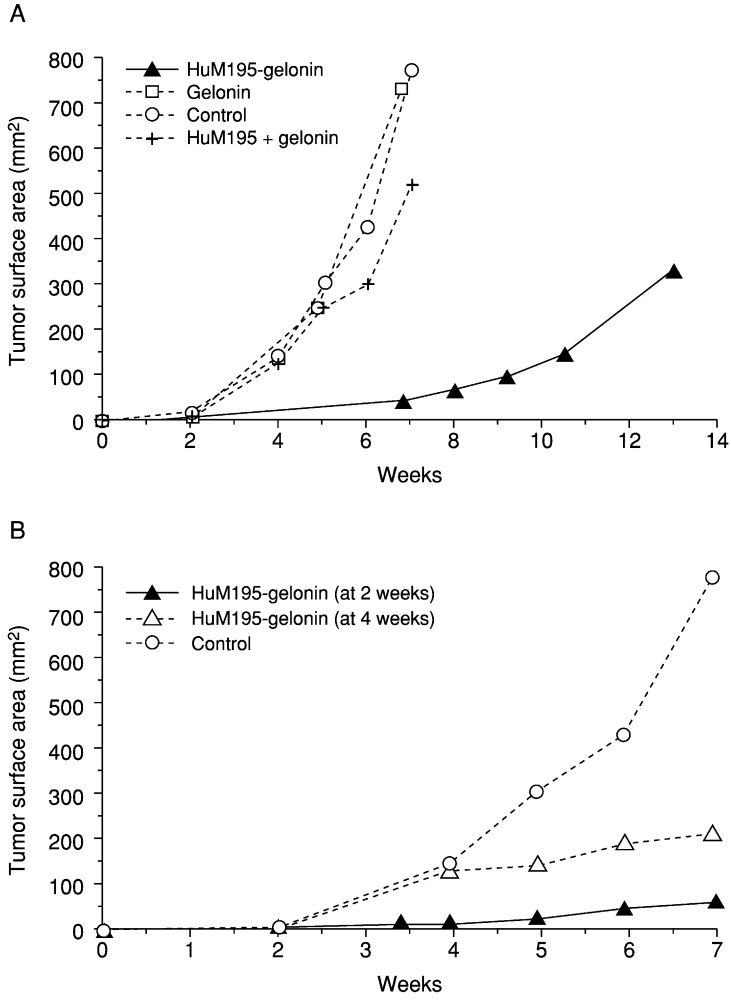

FIGURE 7 Effect of HuM195–rGel on HL-60 tumor Growth *in vivo*. Treatment of mice bearing HL–60 subcutaneous tumors with one dose of HuM195–rGel (IV) was demonstrated to effectively suppress tumor outgrowth for approximately 6 weeks after administration. In addition, treatment of mice bearing well-established tumors (Panel B) also produced dramatic suppression of tumor growth compared with that of controls.

marrow. In the lymphoid cell lineage, CD38 expression continues through the early stages of T- and B-cell development. Mature resting lymphocytes express undetectable levels of CD38, but the expression is highly upregulated during activation and differentiation of B cells into plasma cells. Transformed counterparts of normal hematopoietic cells, such as myeloid leukemia, lymphoma, and myeloma, express high levels of the CD38 antigen.

TABLE I Tumor Size 7 Weeks After HuM195–rGel Treatment

				HuM195–rGel		
Groups	*Control*	*rGel*	*HuM195 + rGel*[b]	*At 10 days*	*At 14 days*[a]	*At 28 days*[a]
Mouse 1	420	648	342	No tumor	No tumor	96
Mouse 2	525	696	400	No tumor	No tumor	160
Mouse 3	900	760	550	49	81	180
Mouse 4	1225	803	784	96	117	380
Mouse 5	Death at 6 weeks	N/A[c]	Death at 6 weeks	N/A	N/A	N/A
Mean ± SD	768 ± 398	727 ± 68	519 ± 197	36 ± 46	50 ± 59	204 ± 123

[a] These mice received six injections instead of three.
[b] Injected together, but not conjugated to each other.
[c] N/A: These groups had four mice only.

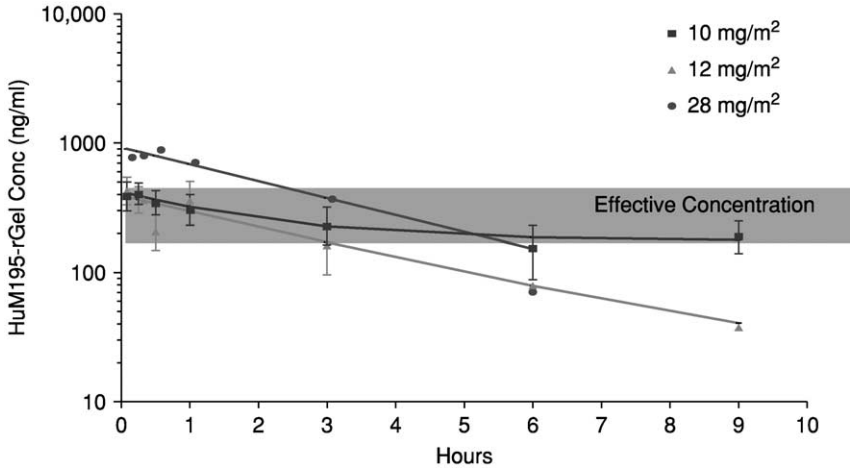

FIGURE 8 Pharmacokinetics of HuM195–rGel. The plasma clearance of intact HuM195–rGel was assessed in patients after IV administration. These data represent three patients at each dose level and triplicate analysis of each sample.

We have previously reported that retinoids in general and retinoic acid (RA) in particular induce high levels of CD38 expression in several myeloid and acute promyelocytic leukemia (APL) cell lines. We reasoned that RA pretreatment and subsequent upregulation of CD38 could provide augmented sensitivity to immunotoxins and might additionally preclude the development of cellular resistance to therapeutics targeting CD38.

We generated an anti-CD38 immunotoxin composed of the murine anti-CD38 antibody IB4 chemically conjugated to rGel toxin (Mehta *et al.*, 2004). The IB4–rGel chemical conjugate alone was capable of killing HL60 cells in culture. Coadministration of as little as 1 nM RA was effective in inducing substantial cytotoxicity (>90%) of leukemia cell clones. More importantly, leukemic blast cells isolated from myeloid leukemia patients also responded to the combination of immunotoxin and RA treatment *ex vivo*. Finally, the augmented cytotoxic effects of RA and immunotoxin were also observed on a multidrug-resistant variant of the HL60 cell line, suggesting that patients heavily pretreated with chemotherapeutic agents might be excellent candidates for such a combined therapeutic approach.

D. IL-2 Receptor

Studies by Waldmann (1987a,b) have demonstrated that resting T cells do not express interleukin-2 (IL-2) receptors, but receptors are rapidly expressed on T cells following interaction of the antigen-specific T-cell receptor complex with appropriately processed and presented antigens. In addition, IL-2 receptors are upregulated on many T-cell leukemias. DAB486IL-2 is a recombinant fusion toxin in which the native receptor-binding domain of diphtheria toxin has been replaced with human IL-2. It selectively binds and intoxicates only cells that bear the high-affinity receptor for IL-2 (Kiyokawa *et al.*, 1989; Strom *et al.*, 1993; Woodworth and Nichols, 1993). In the first clinical study of a genetically engineered ligand fusion-toxin, 18 patients with chemotherapy-resistant IL-2 receptor expressing hematologic malignancies were treated with escalating doses of DAB486IL-2 (LeMaistre *et al.*, 1993). The maximal tolerated dose of a daily intravenous bolus of DAB486IL-2 was determined to be 0.1 mg/kg/day for 10 doses. The dose-limiting toxicities were asymptomatic, reversible elevations of hepatic transaminases without changes in other tests of liver function. Other mild reversible side effects noted were rash, nausea, elevated creatinine, chest tightness, and fever. Pharmacokinetic analysis of this unique agent showed a monophasic clearance of 5.8 ± 0.7 min with peak levels of 3549 ± 1041 mg/ml at the 0.1 mg/kg dose. Approximately 50% of patients were found to develop an antibody response to DT or DAB486IL-2; however, the presence of such antibodies did not preclude patients from experiencing an antitumor response, as four of the six patients with antitumor effect had detectable antibody titers. Although this was a Phase I trial designed to define the safety of DAB486IL-2, remissions were observed in three patients lasting from 5 to more than 18 months. The ability to achieve significant tumor reductions in this group of heavily treated patients is encouraging and suggests that additional trials are warranted in hematologic malignancies. Follow-up studies were performed with a fusion construct containing a more shortened version of the toxin and designated

DAB(389)IL2 (Ontak) (Frankel *et al.*, 2003b; Kreitman, 2003). These studies demonstrated that this agent has biologic activity in the absence of significant toxicity. As a result of these studies, Ontak is approved for clinical use in advanced-stage cutaneous T-cell lymphoma.

E. GMCSF/DT

The receptor for granulocyte-macrophage colony-stimulating factor (GMCSF) is upregulated on the majority of AML blast cells and is poorly expressed on early normal hematopoietic stem cells. This makes GMCSF and its receptors excellent potential targets for therapy of AML. For this reason, a variety of growth-factor/toxin constructs composed of GMCSF and either truncated DT (Bendel *et al.*, 1997; Chan *et al.*, 1995; Frankel *et al.*, 1997; Hall *et al.*, 1998) or recombinant ricin (Burbage *et al.*, 1997) have been generated and tested. This agent is extremely potent, with IC_{50} values in the picomolar range (Hogge *et al.*, 1998). Preclinical studies demonstrate that serum from patients contain preformed concentrations of anti-DT antibodies, albeit at levels that should not preclude therapy with this agent (Hall *et al.*, 2001, 2002). Recently completed Phase I studies by Frankel *et al.* (2002) have demonstrated that this agent can be safely administered at doses up to 4 μg/kg/day, but hepatic toxicity was observed at higher doses. Complete and partial remissions were observed in 3 of 31 patients who were all treated at doses above the maximum tolerated dose. These data suggest that this targeted therapeutic agent has potential therapeutic utility if the therapeutic index of this protein can be improved.

References

Alexander, R. L., Kucera, G. L., Klein, B., and Frankel, A. E. (2000). *In vitro* interleukin-3 binding to leukemia cells predicts cytotoxicity of a diphtheria toxin/IL-3 fusion protein. *Bioconj. Chem.* **11,** 564–568.

Amlot, P. L., Stone, M. J., Cunningham, D., Fay, J., Newman, J., Collins, R., May, R., McCarthy, M., Richardson, J., and Ghetie, V. (1993). A phase I study of an anti-CD22-deglycosylated ricin A chain immunotoxin in the treatment of B-cell lymphomas resistant to conventional therapy. *Blood* **82,** 2624–2633.

Atkinson, S. F., Bettinger, T., Seymour, L. W., Behr, J. P., and Ward, C. M. (2001). Conjugation of folate via gelonin carbohydrate residues retains ribosomal-inactivating properties of the toxin and permits targeting to folate receptor positive cells. *J. Biol. Chem.* **276,** 27930–27935.

Baluna, R., and Vitetta, E. S. (1999). An *in vivo* model to study immunotoxin-induced vascular leak in human tissue. *J. Immunother.* **22,** 41–47.

Bendel, A. E., Shao, Y., Davies, S. M., Warman, B., Yang, C. H., Waddick, K. G., Uckun, F. M., and Perentesis, J. P. (1997). A recombinant fusion toxin targeted to the granulocyte-macrophage colony-stimulating factor receptor. *Leuk. Lymph.* **25,** 257–270.

Bérgamaschi, G., Perfetti, V., Tonon, L., Novella, A., Lucotti, C., Danova, M., Glennie, M., Merlini, G., and Cazzola, M. (1996). Saporin, a ribosome-inactivating protein used to prepare immunotoxins, induces cell death via apoptosis. *Br. J. Haematol.* **93,** 789–794.

Bernstein, I. D. (2002). CD33 as a target for selective ablation of acute myeloid leukemia. *Clin. Lymph.* **2**(Suppl. 1), S9–S11.

Bolognesi, A., Polito, L., Tazzari, P. L., Lemoli, R. M., Lubelli, C., Fogli, M., Boon, L., de-Boer, M., and Stirpe, F. (2000). *In vitro* anti-tumour activity of anti-CD80 and anti-CD86 immunotoxins containing type 1 ribosome-inactivating proteins. *Br. J. Haematol.* **110,** 351–361.

Bonardi, M. A., French, R. R., Amlot, P., Gromo, G., Modena, D., and Glennie, M. J. (1993). Delivery of saporin to human B-cell lymphoma using bispecific antibody: Targeting via CD22 but not CD19, CD37, or immunoglobulin results in efficient killing. *Cancer Res.* **53,** 3015–3021.

Bregni, M., Siena, S., Formosa, A., Lappi, D. A., Martineau, D., Malavasi, F., Dorken, B., Bonadonna, G., and Gianni, A. M. (1989). B-cell restricted saporin immunotoxins: Activity against B-cell lines and chronic lymphocytic leukemia cells. *Blood* **73,** 753–762.

Burbage, C., Tagge, E. P., Harris, B., Hall, P., Fu, T., Willingham, M. C., and Frankel, A. E. (1997). Ricin fusion toxin targeted to the human granulocyte-macrophage colony-stimulating factor receptor is selectively toxic to acute myeloid leukemia cells. *Leuk. Res.* **21,** 681–690.

Caron, P. C., Dumont, L., and Scheinberg, D. A. (1998). Supersaturating infusional humanized anti-CD33 monoclonal antibody HuM195 in myelogenous leukemia. *Clin. Cancer Res.* **4,** 1421–1428.

Chan, C. H., Blazar, B. R., Eide, C. R., Kreitman, R. J., and Vallera, D. A. (1995). A murine cytokine fusion toxin specifically targeting the murine granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor on normal committed bone marrow progenitor cells and GM-CSF-dependent tumor cells. *Blood* **86,** 2732–2740.

Chu, J. Y., Yang, A. D., Wang, B. M., Hu, Z., Zhu, X. M., Zhang, H. J., Qu, J. H., Luo, L. Y., Guo, R., and Shi, L. R. (1990). Monoclonal anti-human T cell antibody and PAP-s conjugate: Preparation and selective cytotoxic properties on leukemic cell. *J. Tongji Med. Univ* **10,** 15–18.

Conry, R. M., Khazaeli, M. B., Saleh, M. N., Ghetie, V., Vitetta, E. S., Liu, T., and LoBuglio, A. F. (1995). Phase I trial of an anti-CD19 deglycosylated ricin A chain immunotoxin in non-Hodgkin's lymphoma: Effect of an intensive schedule of administration. *J. Immunother. Emphasis. Tumor Immunol.* **18,** 231–241.

Dedon, P. C., Salzberg, A. A., and Xu, J. (1993). Exclusive production of bistranded DNA damage by calicheamicin. *Biochemistry* **32,** 3617–3622.

Delprino, L., Giacomotti, M., Dosio, F., Brusa, P., Ceruti, M., Grosa, G., and Cattel, L. (1993). Toxin-targeted design for anticancer therapy. II. Preparation and biological comparison of different chemically linked gelonin-antibody conjugates. *J. Pharm. Sci.* **82,** 699–704.

Douer, D. (2002). New advances in the treatment of acute promyelocytic leukemia. *Int. J. Hematol.* **76**(Suppl. 2), 179–187.

Drak, J., Iwasawa, N., Danishefsky, S., and Crothers, D. M. (1991). The carbohydrate domain of calicheamicin gamma I1 determines its sequence specificity for DNA cleavage. *Proc. Natl. Acad. Sci. USA* **88,** 7464–7468.

Engel, P., Wagner, N., Miller, A. S., and Tedder, T. F. (1995). Identification of the ligand-binding domains of CD22, a member of the immunoglobulin superfamily that uniquely binds a sialic acid-dependent ligand. *J. Exp. Med.* **181,** 1581–1586.

Engert, A., Burrows, F., Jung, W., Tazzari, P. L., Stein, H., Pfreundschuh, M., Diehl, V., and Thorpe, P. (1990). Evaluation of ricin A chain-containing immunotoxins directed against the CD30 antigen as potential reagents for the treatment of Hodgkin's disease. *Cancer Res.* **50,** 84–88.

Engert, A., Gottstein, C., Bohlen, H., Winkler, U., Schon, G., Manske, O., Schnell, R., Diehl, V., and Thorpe, P. (1995). Cocktails of ricin A-chain immunotoxins against different antigens on Hodgkin and Sternberg-Reed cells have superior anti-tumor effects against H-RS cells *in vitro* and solid Hodgkin tumors in mice. *Int. J. Cancer* **63,** 304–309.

Falasca, A., Gasperi-Campani, A., Abbondanza, A., Barbieri, L., and Stirpe, F. (1982). Properties of the ribosome-inactivating proteins gelonin, *Momordica charantia* inhibitor, and dianthins. *Biochem. J.* **207,** 505–509.

Feldman, E., Kalaycio, M., Weiner, G., Frankel, S., Schulman, P., Schwartzberg, L., Jurcic, J., Velez-Garcia, E., Seiter, K., Scheinberg, D., Levitt, D., and Wedel, N. (2003). Treatment of relapsed or refractory acute myeloid leukemia with humanized anti-CD33 monoclonal antibody HuM195. *Leukemia* **17,** 314–318.

Feuring-Buske, M., Frankel, A., Gerhard, B., and Hogge, D. (2000). Variable cytotoxicity of diphtheria toxin 388-granulocyte-macrophage colony-stimulating factor fusion protein for acute myelogenous leukemia stem cells. *Exp. Hematol.* **28,** 1390–1400.

Flavell, D. J., Boehm, D. A., Noss, A., Warnes, S. L., and Flavell, S. U. (2001). Therapy of human T-cell acute lymphoblastic leukaemia with a combination of anti-CD7 and anti-CD38-SAPORIN immunotoxins is significantly better than therapy with each individual immunotoxin. *Br. J. Cancer* **84,** 571–578.

Frankel, A. E., Powell, B. L., Hall, P. D., Cawe, L. D., and Kreitman, R. J. (2002). Phase I trial of a novel diptheria toxin/granulocyte macrophage colony-stimulating factor fusion protein (DT388GMCSF) for refractory or relapsed acute myeloid leukemia. *Clin. Cancer Res.* **8,** 1004–1013.

Frankel, A. E., Fleming, D. R., Hall, P. D., Powell, B. L., Black, J. H., Leftwich, C., and Gartenhaus, R. (2003a). A phase II study of DT fusion protein denileukin diftitox in patients with fludarabine-refractory chronic lymphocytic leukemia. *Clin. Cancer Res.* **9,** 3555–3561.

Frankel, A. E., Fleming, D. R., Powell, B. L., and Gartenhaus, R. (2003b). DAB(389)IL2 (ONTAK((R))) fusion protein therapy of chronic lymphocytic leukaemia. *Expert Opin. Biol. Ther.* **3,** 179–186.

Frankel, A. E., Hall, P. D., Burbage, C., Vesely, J., Willingham, M., Bhalla, K., and Kreitman, R. J. (1997). Modulation of the apoptotic response of human myeloid leukemia cells to a diphtheria toxin granulocyte-macrophage colony-stimulating factor fusion protein. *Blood* **90,** 3654–3661.

French, R. R., Penney, C. A., Browning, A. C., Stirpe, F., George, A. J., and Glennie, M. J. (1995). Delivery of the ribosome-inactivating protein, gelonin, to lymphoma cells via CD22 and CD38 using bispecific antibodies. *Br. J. Cancer* **71,** 986–994.

Ghetie, M. A., May, R. D., Till, M., Uhr, J. W., Ghetie, V., Knowles, P. P., Relf, M., Brown, A., Wallace, P. M., and Janossy, G. (1988). Evaluation of ricin A chain-containing immunotoxins directed against CD19 and CD22 antigens on normal and malignant human B-cells as potential reagents for *in vivo* therapy. *Cancer Res.* **48,** 2610–2617.

Ghetie, M. A., Tucker, K., Richardson, J., Uhr, J. W., and Vitetta, E. S. (1992). The antitumor activity of an anti-CD22 immunotoxin in SCID mice with disseminated Daudi lymphoma is enhanced by either an anti-CD19 antibody or an anti-CD19 immunotoxin. *Blood* **80,** 2315–2320.

Gosselaar, P. H., van Dijk, A. J., de Gast, G. C., Polito, L., Bolognesi, A., Vooijs, W. C., Verheul, A. F., Krouwer, H. G., and Marx, J. J. (2002). Transferrin toxin but not transferrin receptor immunotoxin is influenced by free transferrin and iron saturation. *Eur. J. Clin. Invest.* **32**(Suppl. 1), 61–69.

Griffin, J. D., Linch, D., Sabbath, K., Larcom, P., and Schlossman, S. F. (1984). A monoclonal antibody reactive with normal and leukemic human myeloid progenitor cells. *Leuk. Res.* **8,** 521–534.

Gunther, R., Chelstrom, L. M., Finnegan, D., Tuel-Ahlgren, L., Irvin, J. D., Myers, D. E., and Uckun, F. M. (1993a). In vivo anti-leukemic efficacy of anti-CD7-pokeweed antiviral protein immunotoxin against human T-lineage acute lymphoblastic leukemia/lymphoma in mice with severe combined immunodeficiency. *Leukemia* **7,** 298–309.

Hall, P. D., Kreitman, R. J., Willingham, M. C., and Frankel, A. E. (1998). Toxicology and pharmacokinetics of DT388-GM-CSF, a fusion toxin consisting of a truncated diphtheria toxin (DT388) linked to human granulocyte-macrophage colony-stimulating factor (GM-CSF) in C57BL/6 mice. *Toxicol. Appl. Pharmacol.* **150,** 91–97.

Hall, P. D., Razzouk, B. I., Willoughby, T. E., McLean, T. W., and Frankel, A. E. (2002). The majority of children and adolescents with acute myeloid leukemia have detectable anti-DT388-GMCSF IgG concentrations, but at concentrations that should not preclude *in vivo* activity. *J. Pediatr. Hematol. Oncol.* **24,** 521–526.

Hall, P. D., Virella, G., Willoughby, T., Atchley, D. H., Kreitman, R. J., and Frankel, A. E. (2001). Antibody response to DT-GM, a novel fusion toxin consisting of a truncated diphtheria toxin (DT) linked to human granulocyte-macrophage colony-stimulating factor (GM), during a phase I trial of patients with relapsed or refractory acute myeloid leukemia. *Clin. Immunol.* **100,** 191–197.

Harris, P., Reed, E., King, D. W., and Suciu-Foca, N. (1991). *In vitro* studies of the effect of MAb NDA 4 linked to toxin on the proliferation of a human EBV-transformed lymphoblastoid B cell line and of gibbon MLA leukemia cell line. *Cell Immunol.* **134,** 85–95.

Hogge, D. E., Willman, C. L., Kreitman, R. J., Berger, M., Hall, P. D., Kopecky, K. J., McLain, C., Tagge, E. P., Eaves, C. J., and Frankel, A. E. (1998). Malignant progenitors from patients with acute myelogenous leukemia are sensitive to a diphtheria toxin-granulocyte-macrophage colony-stimulating factor fusion protein. *Blood* **92,** 589–595.

Huang, Y. W., Burrows, F. J., and Vitetta, E. S. (1993). Cytotoxicity of a novel anti-ICAM-1 immunotoxin on human myeloma cell lines. *Hybridoma* **12,** 661–675.

Huhn, M., Sasse, S., Tur, M. K., Matthey, B., Schinkothe, T., Rybak, S. M., Barth, S., and Engert, A. (2001). Human angiogenin fused to human CD30 ligand (Ang-CD30L) exhibits specific cytotoxicity against CD30-positive lymphoma. *Cancer Res.* **61,** 8737–8742.

Hursey, M., Newton, D. L., Hansen, H., Ruby, D., Goldenberg, D. M., and Rybak, S. M. (2002). Specifically targeting the CD22 receptor of human B-cell lymphomas with RNA damaging agents: a new generation of therapeutics. *Leuk. Lymph.* **43,** 953–959.

Husain, S. R., Kreitman, R. J., Pastan, I., and Puri, R. K. (1999). Interleukin-4 receptor-directed cytotoxin therapy of AIDS-associated Kaposi's sarcoma tumors in xenograft model. *Nat. Med.* **5,** 817–822.

Ishiguro, K., Ho, P. T., and Sartorelli, A. C. (1992). Characterization of the defect in a variant of HL-60 promyelocytic leukemia cells with reduced transferrin receptor expression. *Somat. Cell Mol. Genet.* **18,** 45–63.

Joshi, B. H., Leland, P., Silber, J., Kreitman, R. J., Pastan, I., Berger, M., and Puri, R. K. (2002). IL-4 receptors on human medulloblastoma tumours serve as a sensitive target for a circular permuted IL-4-*Pseudomonas* exotoxin fusion protein. *Br. J. Cancer* **86,** 285–291.

Jurcic, J. G., DeBlasio, T., Dumont, L., Yao, T. J., and Scheinberg, D. A. (2000). Molecular remission induction with retinoic acid and anti-CD33 monoclonal antibody HuM195 in acute promyelocytic leukemia. *Clin. Cancer Res.* **6,** 372–380.

Kiyokawa, T., Shirono, K., Hattori, T., Nishimura, H., Yamaguchi, K., Nichols, J. C., Strom, T. B., Murphy, J. R., and Takatsuki, K. (1989). Cytotoxicity of interleukin 2-toxin toward lymphocytes from patients with adult T-cell leukemia. *Cancer Res.* **49,** 4042–4046.

Kreitman, R. J. (2001). Toxin-labeled monoclonal antibodies. *Curr. Pharm. Biotechnol.* **2,** 313–325.

Kreitman, R. J. (2003). Recombinant toxins for the treatment of cancer. *Curr. Opin. Mol. Ther.* **5,** 44–51.

Kreitman, R. J., Margulies, I., Stetler-Stevenson, M., Wang, Q. C., FitzGerald, D. J., and Pastan, I. (2000). Cytotoxic activity of disulfide-stabilized recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) toward fresh malignant cells from patients with B-cell leukemias. *Clin. Cancer Res.* **6,** 1476–1487.

Kreitman, R. J., and Pastan, I. (2003). Immunobiological treatments of hairy-cell leukaemia. *Best Pract. Res. Clin. Haematol.* **16,** 117–133.

Kreitman, R. J., Wang, Q. C., FitzGerald, D. J., and Pastan, I. (1999). Complete regression of human B-cell lymphoma xenografts in mice treated with recombinant anti-CD22 immunotoxin RFB4(dsFv)-PE38 at doses tolerated by cynomolgus monkeys. *Int. J. Cancer* **81,** 148–155.

Kreitman, R. J., Wilson, W. H., Bergeron, K., Raggio, M., Stetler-Stevenson, M., FitzGerald, D. J., and Pastan, I. (2001). Efficacy of the anti-CD22 recombinant immunotoxin BL22 in chemotherapy-resistant hairy-cell leukemia. *N. Engl. J. Med.* **345,** 241–247.

Kristensen, J. S., and Hokland, P. (1991). Monoclonal antibodies in myeloid diseases: Prognostic use in acute myeloid leukaemia. *Leuk. Res.* **15,** 693–700.

LeMaistre, C. F., Craig, F. E., Meneghetti, C., McMullin, B., Parker, K., Reuben, J., Boldt, D. H., Rosenblum, M., and Woodworth, T. (1993). Phase I trial of a 90-minute infusion of the fusion toxin DAB486IL-2 in hematological cancers. *Cancer Res.* **53,** 3930–3934.

Lindstrom, A. L., Erlandsen, S. L., Kersey, J. H., and Pennell, C. A. (1997). An *in vitro* model for toxin-mediated vascular leak syndrome: Ricin toxin A chain increases the permeability of human endothelial cell monolayers. *Blood* **90,** 2323–2334.

Liu, T. C., Tan, G. B., Wong, C. L., and Han, P. (1991). Immunophenotypic characterisation of monocytoid differentiation markers in acute non-lymphoblastic leukaemias. *Ann. Acad. Med. Singapore* **20,** 353–355.

Lorimer, I. A., Wikstrand, C. J., Batra, S. K., Bigner, D. D., and Pastan, I. (1995). Immunotoxins that target an oncogenic mutant epidermal growth factor receptor expressed in human tumors. *Clin. Cancer Res.* **1,** 859–864.

Mansfield, E., Amlot, P., Pastan, I., and FitzGerald, D. J. (1997a). Recombinant RFB4 immunotoxins exhibit potent cytotoxic activity for CD22-bearing cells and tumors. *Blood* **90,** 2020–2026.

Mansfield, E., Chiron, M. F., Amlot, P., Pastan, I., and FitzGerald, D. J. (1997b). Recombinant RFB4 single-chain immunotoxin that is cytotoxic towards CD22-positive cells. *Biochem. Soc. Trans.* **25,** 709–714.

Mansfield, E., Pastan, I., and FitzGerald, D. J. (1996). Characterization of RFB4-*Pseudomonas* exotoxin A immunotoxins targeted to CD22 on B-cell malignancies. *Bioconj. Chem.* **7,** 557–563.

Marcil, J., Ravindranath, N., and Sairam, M. R. (1993). Cytotoxic activity of lutropin-gelonin conjugate in mouse Leydig tumor cells: Potentiation of the hormonotoxin activity by different drugs. *Mol. Cell Endocrinol.* **92,** 83–90.

Mason, D. Y., Comans-Bitter, W. M., Cordell, J. L., Verhoeven, M. A., and van Dongen, J. J. (1990). Antibody L26 recognizes an intracellular epitope on the B-cell-associated CD20 antigen. *Am. J. Pathol.* **136,** 1215–1222.

May, R. D., Vitetta, E. S., Moldenhauer, G., and Dorken, B. (1986). Selective killing of normal and neoplastic human B cells with anti-CD19- and anti-CD22-ricin A chain immunotoxins. *Cancer Drug Deliv.* **3,** 261–272.

McGrath, M. S., Rosenblum, M. G., Philips, M. R., and Scheinberg, D. A. (2003). Immunotoxin resistance in multidrug resistant cells. *Cancer Res.* **63,** 72–79.

McGraw, K. J., Rosenblum, M. G., Cheung, L., and Scheinberg, D. A. (1994). Characterization of murine and humanized anti-CD33, gelonin immunotoxins reactive against myeloid leukemias. *Cancer Immunol. Immunother.* **39,** 367–374.

Mehta, K., Ocanas, L., Malavasi, F., Marks, J. W., and Rosenblum, M. G. (2004). Retinoic acid-induced CD38 antigen as a target for immunotoxin-mediated killing of leukemia cells. *Mol. Cancer Ther.* **3,** 345–352.

Messinger, Y., Yanishevski, Y., Avramis, V. I., Ek, O., Chelstrom, L. M., Gunther, R., Myers, D. E., Irvin, J. D., Evans, W., and Uckun, F. M. (1996). Treatment of human B-cell precursor leukemia in SCID mice using a combination of the investigational biotherapeutic agent B43-PAP with cytosine arabinoside. *Clin. Cancer Res.* **2,** 1533–1542.

Myers, D. E., and Uckun, F. M. (1995). An anti-CD72 immunotoxin against therapy-refractory B-lineage acute lymphoblastic leukemia. *Leuk. Lymphoma* **18,** 119–122.

Nabhan, C., and Tallman, M. S. (2002). Early phase I/II trials with gemtuzumab ozogamicin (Mylotarg®) in acute myeloid leukemia. *Clin. Lymph.* **2**(Suppl. 1), S19–S23.

Nichols, J., Foss, F., Kuzel, T. M., LeMaistre, C. F., Platanias, L., Ratain, M. J., Rook, A., Saleh, M., and Schwartz, G. (1997). Interleukin-2 fusion protein: An investigational therapy for interleukin-2 receptor expressing malignancies. *Eur. J. Cancer* **33**(Suppl. 1), S34–S36.

O'Hare, M., Brown, A. N., Hussain, K., Gebhardt, A., Watson, G., Roberts, L. M., Vitetta, E. S., Thorpe, P. E., and Lord, J. M. (1990). Cytotoxicity of a recombinant ricin-A-chain fusion protein containing a proteolytically-cleavable spacer sequence. *FEBS Lett.* **273,** 200–204.

Oldham, R. K., Lewis, M., Orr, D. W., Avner, B., Liao, S. K., Ogden, J. R., Avner, B., and Birch, R. (1988). Adriamycin custom-tailored immunoconjugates in the treatment of human malignancies. *Mol. Biother.* **1,** 103–113.

Pagliaro, L. C., Liu, B., Munker, R., Andreeff, M., Freireich, E. J., Scheinberg, D. A., and Rosenblum, M. G. (1998). Humanized M195 monoclonal antibody conjugated to recombinant gelonin: An anti-CD33 immunotoxin with antileukemic activity. *Clin. Cancer Res.* **4,** 1971–1976.

Pastan, I. (2003). Immunotoxins containing *Pseudomonas* exotoxin A: A short history. *Cancer Immunol. Immunother.* **52,** 338–341.

Psarras, K., Ueda, M., Tanabe, M., Kitajima, M., Aiso, S., Komatsu, S., and Seno, M. (2000). Targeting activated lymphocytes with an entirely human immunotoxin analogue: Human pancreatic RNase1-human IL-2 fusion. *Cytokine* **12,** 786–790.

Robertson, M. J., Soiffer, R. J., Freedman, A. S., Rabinowe, S. L., Anderson, K. C., Ervin, T. J., Murray, C., Dear, K., Griffin, J. D., and Nadler, L. M. (1992). Human bone marrow depleted of CD33-positive cells mediates delayed but durable reconstitution of hematopoiesis: Clinical trial of MY9 monoclonal antibody-purged autografts for the treatment of acute myeloid leukemia. *Blood* **79,** 2229–2236.

Roboz, G. J., Knovich, M. A., Bayer, R. L., Schuster, M. W., Seiter, K., Powell, B. L., Woodruff, R. D., Silver, R. T., Frankel, A. E., and Feldman, E. J. (2002). Efficacy and safety of gemtuzumab ozogamicin in patients with poor-prognosis acute myeloid leukemia. *Leuk. Lymph.* **43,** 1951–1955.

Rosenblum, M. G., Cheung, L., Kim, S. K., Mujoo, K., Donato, N. J., and Murray, J. L. (1996). Cellular resistance to the antimelanoma immunotoxin ZME-gelonin and strategies to target resistant cells. *Cancer Immunol. Immunother.* **42,** 115–121.

Rosenblum, M. G., Kohr, W. A., Beattie, K. L., Beattie, W. G., Marks, W., Toman, P. D., and Cheung, L. (1995). Amino acid sequence analysis, gene construction, cloning, and expression of gelonin, a toxin derived from *Gelonium multiflorum*. *J. Interferon Cytokine Res.* **15,** 547–555.

Rosenblum, M. G., Marks, J. W., and Cheung, L. H. (1999). Comparative cytotoxicity and pharmacokinetics of antimelanoma immunotoxins containing either natural or recombinant gelonin. *Cancer Chemother. Pharmacol.* **44,** 343–348.

Rozemuller, H., Chowdhury, P. S., Pastan, I., and Kreitman, R. J. (2001). Isolation of new anti-CD30 scFvs from DNA-immunized mice by phage display and biologic activity of

recombinant immunotoxins produced by fusion with truncated *Pseudomonas* exotoxin. *Int. J. Cancer* **92,** 861–870.

Sausville, E. A., Headlee, D., Stetler-Stevenson, M., Jaffe, E. S., Solomon, D., Figg, W. D., Herdt, J., Kopp, W. C., Rager, H., and Steinberg, S. M. (1995). Continuous infusion of the anti-CD22 immunotoxin IgG-RFB4-SMPT-dgA in patients with B-cell lymphoma: A phase I study. *Blood* **85,** 3457–3465.

Scheinberg, D. A., Tanimoto, M., McKenzie, S., Strife, A., Old, L. J., and Clarkson, B. D. (1989). Monoclonal antibody M195: a diagnostic marker for acute myelogenous leukemia. *Leukemia* **3,** 440–445.

Shen, G. L., Li, J. L., Ghetie, M. A., Ghetie, V., May, R. D., Till, M., Brown, A. N., Relf, M., Knowles, P., and Uhr, J. W. (1988). Evaluation of four CD22 antibodies as ricin A chain-containing immunotoxins for the *in vivo* therapy of human B-cell leukemias and lymphomas. *Int. J. Cancer* **42,** 792–797.

Shin, Y. K., Choi, Y. L., Choi, E. Y., Kim, M. K., Kook, M. C., Chung, J., Choi, Y. K., Kim, H. S., Song, H. G., and Park, S. H. (2003). Targeted cytotoxic effect of anti-JL1 immunotoxin against a human leukemic cell line and its clinical implications. *Cancer Immunol. Immunother.* **52,** 506–512.

Shinohara, H., Morita, S., Kawai, M., Miyamoto, A., Sonoda, T., Pastan, I., and Tanigawa, N. (2002). Expression of HER2 in human gastric cancer cells directly correlates with antitumor activity of a recombinant disulfide-stabilized anti-HER2 immunotoxin. *J. Surg. Res.* **102,** 169–177.

Siena, S., Bregni, M., Formosa, A., Brando, B., Marenco, P., Lappi, D. A., Bonadonna, G., and Gianni, A. M. (1989). Immunotoxin-mediated inhibition of chronic lymphocytic leukemia cell proliferation in humans. *Cancer Res.* **49,** 3328–3332.

Simmons, D., and Seed, B. (1988). Isolation of a cDNA encoding CD33, a differentiation antigen of myeloid progenitor cells. *J. Immunol.* **141,** 2797–2800.

Stadtmauer, E. A. (2002). Gemtuzumab ozogamicin in the treatment of acute myeloid leukemia. *Curr. Oncol. Rep.* **4,** 375–380.

Stirpe, F., Olsnes, S., and Pihl, A. (1980). Gelonin, a new inhibitor of protein synthesis, nontoxic to intact cells. Isolation, characterization, and preparation of cytotoxic complexes with concanavalin A. *J. Biol. Chem.* **255,** 6947–6953.

Strom, T. B., Kelley, V. R., Murphy, J. R., Nichols, J., and Woodworth, T. G. (1993). Interleukin-2 receptor-directed therapies: Antibody- or cytokine-based targeting molecules. *Annu. Rev. Med.* **44,** 343–353.

Sweeney, E. B., Foss, F. M., Murphy, J. R., and vanderSpek, J. C. (1998). Interleukin 7 (IL-7) receptor-specific cell killing by DAB389 IL-7: A novel agent for the elimination of IL-7 receptor positive cells. *Bioconj. Chem.* **9,** 201–207.

Talpaz, M., Hagop, K., Freireich, E., Lopez, V., Zhang, W., Cortes-Franco, J., Scheinberg, D., and Rosenblum, M. G. (2003). Phase I clinical trial of the anti-CD-33 immunotoxin HuM195/rGel. Proceedings of the AACR. Cadmus Professional Communications, Linthicum, MD, Abstract # 5362, 44, 1228.

Tanimoto, M., Scheinberg, D. A., Cordon-Cardo, C., Huie, D., Clarkson, B. D., and Old, L. J. (1989). Restricted expression of an early myeloid and monocytic cell surface antigen defined by monoclonal antibody M195. *Leukemia* **3,** 339–348.

Tazzari, P. L., Zhang, S., Chen, Q., Sforzini, S., Bolognesi, A., Stirpe, F., Xie, H., Moretta, A., and Ferrini, S. (1993). Targeting of saporin to CD25-positive normal and neoplastic lymphocytes by an anti-saporin/anti-CD25 bispecific monoclonal antibody: *In vitro* evaluation. *Br. J. Cancer* **67,** 1248–1253.

Terenzi, A., Bolognesi, A., Pasqualucci, L., Flenghi, L., Pileri, S., Stein, H., Kadin, M., Bigerna, B., Polito, L., Tazzari, P. L., Martelli, M. F., Stirpe, F., and Falini, B. (1996). Anti-CD30 (BER=H2) immunotoxins containing the type-1 ribosome-inactivating proteins momordin and PAP-S (pokeweed antiviral protein from seeds) display powerful antitumour

activity against CD30+ tumour cells *in vitro* and in SCID mice. *Br. J. Haematol.* **92,** 872–879.

Thorburn, J., Frankel, A. E., and Thorburn, A. (2003). Apoptosis by leukemia cell-targeted diphtheria toxin occurs via receptor-independent activation of Fas-associated death domain protein. *Clin. Cancer Res.* **9,** 861–865.

Thorpe, P. E., Brown, A. N., Ross, W. C., Cumber, A. J., Detre, S. I., Edwards, D. C., Davies, A. J., and Stirpe, F. (1981). Cytotoxicity acquired by conjugation of an anti-Thy1.1 monoclonal antibody and the ribosome-inactivating protein, gelonin. *Eur. J. Biochem.* **116,** 447–454.

Toba, K., Hanawa, H., Fuse, I., Sakaue, M., Watanabe, K., Uesugi, Y., Higuchi, W., Takahashi, M., and Aizawa, Y. (2002). Difference in CD22 molecules in human B cells and basophils. *Exp. Hematol.* **30,** 205–211.

Trail, P. A., King, H. D., and Dubowchik, G. M. (2003). Monoclonal antibody drug immunoconjugates for targeted treatment of cancer. *Cancer Immunol. Immunother.* **52,** 328–337.

Viele, C. S. (2002). Gemtuzumab ozogamicin. *Clin. J. Oncol. Nurs.* **6,** 298–299, 304.

Vitetta, E. S., Stone, M., Amlot, P., Fay, J., May, R., Till, M., Newman, J., Clark, P., Collins, R., and Cunningham, D. (1991). Phase I immunotoxin trial in patients with B-cell lymphoma. *Cancer Res.* **51,** 4052–4058.

Voutsadakis, I. A. (2002). Gemtuzumab ozogamicin (CMA-676, Mylotarg) for the treatment of CD33+ acute myeloid leukemia. *Anticancer Drugs* **13,** 685–692.

Waldmann, T. A. (1987a). The interleukin-2 receptor on normal and malignant lymphocytes. *Adv. Exp. Med. Biol.* **213,** 129–137.

Waldmann, T. A. (1987b). The role of the multichain IL-2 receptor complex in the control of normal and malignant T-cell proliferation. *Environ. Health Perspect.* **75,** 11–15.

Woodworth, T. G., and Nichols, J. C. (1993). Recombinant fusion toxins—a new class of targeted biologic therapeutics. *Cancer Treat. Res.* **68,** 145–160.

Xu, Y., Xu, Q., Rosenblum, M. G., and Scheinberg, D. A. (1996). Antileukemic activity of recombinant humanized M195-gelonin immunotoxin in nude mice. *Leukemia* **10,** 321–326.

Yazdi, P. T., and Murphy, R. M. (1994). Quantitative analysis of protein synthesis inhibition by transferrin-toxin conjugates. *Cancer Res.* **54,** 6387–6394.

Zein, N., Sinha, A. M., McGahren, W. J., and Ellestad, G. A. (1988). Calicheamicin gamma 1I: An antitumor antibiotic that cleaves double-stranded DNA site specifically. *Science* **240,** 1198–1201.

Zola, H., Neoh, S. H., Potter, A., Melo, J. V., De Oliveria, M. S., and Catovsky, D. (1987). Markers of differentiated B cell leukaemia: CD22 antibodies and FMC7 react with different molecules. *Dis. Markers.* **5,** 227–235.

George J. Weiner and Brian K. Link

Holden Comprehensive Cancer Center
Department of Internal Medicine
University of Iowa
Iowa City, Iowa 52242

Antibody Therapy of Lymphoma

I. Chapter Overview

Studies in the early 1980s with anti-idiotype monoclonal antibodies (mAbs) provided the clinical proof of concept that mAbs could be used to treat lymphoma. It was not until the mid-1990s with the development of the chimeric anti-CD20 mAb rituximab that clinical therapy of lymphoma with mAbs became practical. Early studies with rituximab demonstrated significant, if transient, clinical responses in approximately half the patients treated. Modification of the initial weekly four-dose treatment regimen resulted in modest improvement in response rates and duration of response, although the clinical significance of these changes remains unclear. Antilymphoma mAbs that recognize other target antigens have been evaluated clinically. Although each demonstrates unique and interesting characteristics, the value added beyond rituximab remains undefined at the present time. A number of mechanisms of action, including signaling-induced apoptosis,

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

antibody-dependent cellular cytotoxicity, and complement-mediated cytotoxicity, have been identified that could contribute to the observed antilymphoma effects of mAbs. Growing evidence suggests that multiple interacting mechanisms are likely involved. Radioimmunotherapy with iodine-131 (^{131}I)- and yttrium-90 (^{90}Y)-labeled anti-CD20, and combinations of mAbs and chemotherapy, might offer advantages over monotherapy with mAbs alone. The studies comparing mAb monotherapy, radioimmunotherapy, and combination therapy are still relatively young, and further follow-up and research are needed before the true clinical value of these approaches is known. In addition, research that allows for the rational design of the next generation of mAb-based regimens is needed before we can take full advantage of the revolution in management of lymphoma that has resulted from mAb-based therapy.

II. History

A. Dawn of Antibody Therapy

It has been over 100 years since Paul Ehrlich coined the term *antibody* and suggested that these highly specific proteins could serve as "magic bullets" that would allow for targeted therapy without toxicity (Ehrlich, 1906). In the first half of the twentieth century, immunotherapy of a number of infectious diseases led to major breakthroughs that had an immense impact on public health. However, successful cancer immunotherapy in general, and antibody-based therapy in particular, was much more elusive. Attempts to treat cancer with polyclonal antisera from animals that had been immunized with human cancer cells or tumor extracts were largely unsuccessful. The first attempts at radioimmunotherapy were also performed with polyclonal antisera and showed limited success (Order, 1976). Overall, serum sickness was common, clinical responses were uncommon, and production of consistent lots of the therapeutic agent was impossible. These major obstacles limited the enthusiasm for cancer immunotherapy. To many, immunotherapy of cancer was considered a "failed hypothesis."

B. Introduction of Monoclonal Antibodies

There was a renewed claim of the "magic bullet" for cancer, and a reawakening of interest in cancer immunotherapy, in the mid-1970s following the development of the hybridoma technique by Kohler and Milstein (1975). Over the next several years, multiple mAbs of defined specificity and class were made by using this technique and found to react with subsets of

both normal lymphocytes and lymphoma cells. Although not strictly tumor specific, these mAbs were lineage specific and, in some cases, capable of inducing measurable changes in benign and malignant lymphocytes. This resulted in a revolution in our understanding of immunology and also demonstrated that lymphocytes were more sensitive than other cell populations to changes induced by mAbs. Given these findings, lymphomas were justifiably seen as prime candidates for mAb-based cancer therapy.

C. Clinical Proof of Concept

Early clinical evaluation demonstrated that murine mAbs could be administered relatively safely to patients and had *in vivo* immunologic effects (Nadler *et al.*, 1980, 1981). However, factors such as the presence of circulating antigen and limited information on how to select the proper target limited efficacy. The idiotype (Id) expressed by B lymphocytes as part of the surface immunoglobulin is highly specific and known to play a major role as the B-cell receptor in the behavior of the B-cell. The Id therefore seemed to be an attractive—if not necessarily practical—target for mAb-based therapy. In a series of landmark studies in the 1980s, Levy and colleagues produced tailormade murine anti-Id mAbs directed toward the surface immunoglobulin expressed by follicular lymphomas (Meeker *et al.*, 1985a,b). Remarkably, an overall response rate (ORR) of approximately 50% was noted in patients treated in these early trials. Three major factors limited more widespread use of this therapy: the need to produce tailormade mAbs for essentially every individual patient, the emergence of Id-variant malignant cells that lacked the target antigen, and the murine nature of the mAbs that resulted in a relatively short half-life and, in some cases, development of the human antimouse antibody (HAMA). Nevertheless, the observed clinical responses led those who had claimed that immunotherapy was a failed hypothesis to reconsider. It also encouraged a new generation of investigators who viewed these studies as a valid proof of concept to begin working toward addressing these limitations.

The past 15 years have seen dramatic advances in our understanding of immunology and molecular biology, and these in turn have led to practical advances that have allowed us to overcome some of the problems identified by Levy and colleagues. We can at present produce human-like mAbs by replacing the murine Fc with human Fc, which results in so-called chimeric human–mouse mAbs. We can also graft the complementarity-determining regions (CDRs) that are responsible for the specificity of a murine mAb into a construct containing both a human Fc and human variable region framework. This results in a so-called CDR-grafted humanized mAb that is 95% human in sequence but maintains the specificity of the parent murine mAb.

III. Chimeric Anti-CD20—A Clinical Breakthrough

A. Early Studies of Chimeric Rituximab

The first clinically approved approach to mAb-based therapy of lymphoma involved the anti-CD20 chimeric mAb known as rituximab (Rituxan®, Mabthera®). In the initial Phase I trial with this mAb, Maloney and coworkers selected patients with previously treated B-cell lymphomas. Four weekly infusions of rituximab as monotherapy were administered. The treatment was well tolerated, with transient and relatively minor infusion-related toxicity. No maximal tolerated dose was identified. Approximately half the patients treated on this Phase I trial enjoyed a clinical response, with the majority being partial responses (PRs). Median duration of observed response was just more than 8 months. No immune response to the infused mAb was noted (Maloney *et al*., 1997a,b).

McLaughlin *et al*. (1998) confirmed these observations in a Phase II trial of 166 patients with low-grade or follicular lymphoma. Four weekly infusions of rituximab at a dose of 375 mg/m^2 resulted in a 48% ORR and 6% complete responses (CRs), using strict response evaluation techniques and definitions. The median duration of response was 11.6 months. The ORR was only 13% in the 30 subjects with small lymphocytic lymphoma, but 60% in the 132 patients with follicular lymphoma. As in the Phase I trial, infusions were associated with a constellation of largely grade I and II symptoms, including fever, chills, rash, bronchospasm, and hypotension, which were easily managed. There was a low (<5%) rate of grade III/IV hematologic toxicities. Depletion of B cells from the peripheral blood lasted 6–9 months, but was not associated with significant hypogammaglobulinemia or identifiable infections. The chimeric mAb was not immunogenic, with human antichimeric antibody detected in only one patient. Rituximab had a serum half-life of 205 h following the fourth infusion, with generally higher concentrations in responding patients and in patients with lower tumor burdens. These results were a true breakthrough in the development of mAbs as clinically useful agents for the treatment of lymphoma.

B. Refining Rituximab Treatment Schedule

These early studies demonstrated that rituximab was clearly of clinical value, but they were not designed to define an optimal dosing strategy. Additional studies were designed to assess whether other schedules might be more effective. Given that rituximab steady-state serum levels are not achieved with four weekly infusions, and that sustained rituximab serum levels correlate with response (Berinstein *et al*., 1998), it was hypothesized that failure to respond to rituximab might be related in some cases to inadequate exposure to rituximab. Strategies such as weekly dosing beyond

4 weeks and repeated series of four weekly doses (either at the time of relapsed disease or as a maintenance strategy) were developed (Hainsworth, 2002). Piro *et al*. (1999) studied eight weekly doses of rituximab (375 mg/m^2) in 37 relapsed or refractory low-grade or follicular lymphoma patients. An ORR of 57% was observed in this single-arm Phase II study, with a median duration of response estimated at 13.4+ months. Although this appears to be a higher response rate and a longer duration of response than those seen in the McLaughlin trial, this difference may be due to differing methods of response determination, potential patient selection, and other hazards that come with comparing serial Phase II studies. At this point, it is unclear whether longer therapy is better therapy. Ongoing randomized Phase III studies are exploring this question.

In a related approach, studies were performed to assess the efficacy of repeated courses of therapy separated by a number of months as a form of maintenance therapy. In a Phase II study by Hainsworth (2003), subjects who had not progressed after an initial 4-week course of rituximab received additional 4-week courses of rituximab at 6-month intervals for up to 2 years. Actuarial progression-free survival was 67% at 2 years. In a randomized study by the Swiss Group for Clinical Cancer Research, 151 follicular lymphoma patients were treated with an initial 4-week course of rituximab and then randomized to observation vs. maintenance with a single 375 mg/m^2 rituximab dose every 8 weeks. At 3 years' follow-up, median event-free survival in the maintenance arm was 23 months compared to 12 months in the observation arm (Ghielmini, 2002). This study, although promising, did not address the question of whether maintenance therapy is a superior long-term approach compared to rituximab retreatment at relapse. Two cooperative group studies by the National Cancer Institute (NCI) are addressing the role of maintenance rituximab in large randomized trials. Although the safety of repeated cycles has been established and the Hainsworth and Swiss data are intriguing, adoption of maintenance rituximab therapy as a standard treatment for lymphoma will need to wait until the results of the randomized trials are available.

C. Treatment of Relapse

Davis and coworkers studied repeated therapy with rituximab in 57 subjects who had previously responded to rituximab but had relapsed. All subjects continued to express CD20. A second course of rituximab was not associated with new toxicities and was nonimmunogenic (Davis *et al*., 2000). The response rate to this second course was 40%. Although the response rate was low, both the duration of response and time to progression (15.7+ and 17.3+ months) were longer when compared with the first course of rituximab for these patients (9.8 and 12.4 months). Whether this is a disappointing result because of the low response rate or a promising result

because of the longer duration of responses can be debated. Nevertheless, the longer duration of the responses is not commonly seen with other forms of cancer therapy and raises questions on the mechanism of action of rituximab (see Section V).

D. Upfront Treatment

The studies outlined previously involved subjects who were refractory to standard therapy. The timing and choice of initial therapy for low-grade lymphomas remain highly controversial as becomes apparent quickly at any gathering of lymphoma experts. Given this uncertainty, it was reasonable in the eyes of many to explore the activity of this new, effective, largely nontoxic treatment modality in previously untreated low-grade lymphoma patients. To date, two studies have evaluated rituximab as a single-agent frontline therapy in patients with low-grade B-cell lymphomas. Colombat *et al*. (2001) found an 80% ORR to rituximab as a single agent following treatment with the standard 4-week schedule with 41% CR/CRu and 39% PR. Mature follow-up for this study is not yet available. Hainsworth and coworkers reported a Phase II trial of previously untreated patients treated upfront with the same regimen. Patients in this trial with ongoing response or stable disease at 6-month intervals received subsequent courses of rituximab for up to 2 years as outlined previously. The eventual ORR was 65% (27% CR). One- and two-year progression-free survival rates for all patients were 69% and 67%, respectively, with minimum follow-up of 15 months (Hainsworth, 2002, 2003). The high response rate observed in both of these studies could be a function of the favorable pretreatment characteristics of the patients and does not allow for conclusions to be reached regarding potential increased mAb activity in chemotherapy-naïve patients. However, these results support evaluation of upfront therapy with rituximab in randomized trials.

IV. Alternative Molecular Targets

CD20 was selected as a target because it is lineage specific and is expressed in high density by a variety of B-cell malignancies, including most follicular lymphomas. The functional role of CD20 is still unclear. Although the clinical results outlined previously suggest that anti-CD20 is indeed an excellent target for mAb therapy, there is no reason to assume *a priori* that CD20 is the best or only target worth evaluating. mAbs that target other antigens are also of interest and are currently under development.

A. CD22

Epratuzumab (AMG-412, hLL2) is a CDR-grafted, humanized mAb that binds to CD22 (Leung *et al*., 1995). The CD22 antigen is a B-cell-restricted molecule expressed in more than 80% of B-cell malignancies. CD22 is known to play a role in B-cell activation and interaction with T cells. Although it is not normally shed from the surface of antigen-bearing cells, CD22 is rapidly internalized when bound by mAb and can be re-expressed on the cell membrane after modulation. In a Phase I/II dose-escalation study, epratuzumab was safely administered in four weekly doses to subjects with CD22-expressing B-cell malignancies (including lymphoma, acute lymphocytic leukemia, and Waldenstrom's macroglobulinemia) (Leonard *et al*., 2003). More than 100 patients with low-grade and aggressive B-cell lymphoma have been treated in Phase II studies of epratuzumab. Aggregate response rates (within select dose ranges) were approximately 40% in patients with follicular lymphomas and 30% in patients with relapsed diffuse large cell lymphoma (Leonard *et al*., 2000). A larger Phase II study using epratuzumab 360 mg/m^2/week in patients with follicular lymphoma refractory to rituximab is currently underway. In a Phase II study of combined therapy with epratuzumab plus rituximab in patients with relapsed lymphoma, early reports describe 8 of 16 objective responses in patients with indolent histology (Leonard *et al*., 2002). These data demonstrate that epratuzumab has clear antilymphoma activity. It is too early to know whether it will be a clinically valuable agent.

B. CD52

Alemtuzumab (Campath-1H) is a chimeric humanized antibody that binds to a glycoprotein (CDw52) expressed by benign and malignant T cells, B cells, and monocytes (Salisbury *et al*., 1994). Recent clinical trials with alemtuzumab have largely focused on chronic lymphocytic leukemia (CLL) because of early observations that the greatest efficacy was observed in the blood and marrow compartments. In a multicenter Phase II European trial, 42% of 29 patients with relapsed or refractory CLL responded to alemtuzumab delivered as thrice-weekly intravenous infusions (Osterborg *et al*., 1997). Subsequent trials of alemtuzumab using a similar thrice-weekly schedule in patients with nodal lymphomas demonstrated low ORR, with only 6 of 42 patients achieving PR (Khorana *et al*., 2001; Lundin *et al*., 1998). Nine episodes of bacterial septicemia were noted along with three deaths from infection, thus limiting enthusiasm for further evaluation of alemtuzumab in lymphomas.

C. HLA-DR

Class II human leukocyte antigen differs in important ways from other target antigens. Binding of class II molecules such as HLA-DR by mAbs can induce rapid apoptosis mediated by a pathway that appears to be different from that mediated by other anti-B-cell mAbs (Bains *et al*., 2003). In addition, mAbs directed against HLA-DR have been shown to be more effective than anti-CD20 mAbs at mediating antibody-dependent cellular cytotoxicity (ADCC) when effector cells are stimulated with the granulocyte colony-stimulating factor (G-CSF) to express high levels of Fcγ receptor I (CD64) (Valerius *et al*., 1997). On the other hand, class II expression is not limited to B cells. Targeting of HLA-DR or other class II molecules could lead to unintentional damage to a variety of benign tissues. The humanized mAb apolizumab (Remitogen®, Hu1D10), directed against a polymorphic determinant of HLA-DR expressed on normal and malignant B cells, is capable of inducing ADCC, complement-mediated lysis, and direct apoptosis of lymphoma cell lines and fresh human B-cell tumors (Kostelny *et al*., 2001). In a Phase I dose-escalation study, patients with relapsed B-cell lymphoma were treated weekly for four doses with apolizumab. In contrast to anti-CD20 and anti-CD22, toxicity was seen at higher dose levels (5 mg/kg). Pharmacokinetics demonstrated marked intersubject variability in clearance of apolizumab, with little evidence for accumulation of the mAb even at higher doses, suggesting a significant antigen sink. Despite these problems, four PRs were documented among eight patients with follicular lymphoma treated at a variety of doses of apolizumab. Three of these patients had previously been refractory to therapy with rituximab. Interestingly, and distinct from the pattern of responses typically seen with rituximab, the median time to response was 106 days. Three of the four responders remained progression free at 13, 17, and 21 months (Link *et al*., 2001a). Day 100 serum from a responding patient contained autologous antilymphoma IgG, suggesting that the patient had developed an active humoral antilymphoma immune response (Link *et al*., 2001b). Taken together, these studies suggest that clinical response that results following the targeting of class II could be due to a unique antitumor mechanism of action (induction of an active immune response), whereas nonspecific targeting results in enhanced toxicity. Additional studies are needed to assess whether the observed toxicity is prohibitive, to confirm a unique mechanism of action, and to determine how this apparent difference might be clinically useful.

V. Mechanisms of Action

The clinical results outlined previously related to use of mAbs as a single agent to treat lymphoma have been extremely exciting and have led to major changes in our current approaches to clinical management. However, both

scientific and practical questions remain. Although there is strong evidence that multiple mechanisms of action can contribute to the efficacy of mAb therapy, we are only now beginning to understand the clinical relevance of these mechanisms. Understanding potential mechanisms of action and how they might interact will be critical to the rational design of the next generation of mAb-based treatments, including use of radiolabeled mAbs and combination regimens. We therefore first address what we know—and do not know—about mechanisms of mAb action and then return to more practical questions concerning clinical use of mAbs in lymphoma.

A. Challenges in Understanding Mechanisms of Action

A number of factors have complicated our ability to understand mAb mechanisms of action. *In vitro* studies have provided valuable hints, but have significant limitations as well. *In vitro* assays of transmembrane signaling or cytotoxicity generally are limited to a few hours or, at most, a few days. In contrast, clinical response of lymphoma to mAb therapy often takes months. With primary tumor samples, *in vitro* analysis is particularly difficult because of rapid death of cells *in vitro* and other artifacts resulting from the manipulation of the tissue and placing it in culture. Because of these problems, most *in vitro* assays exploring mAb mechanisms of action involve use of cell lines, not primary tumor samples. This brings in additional problems. Lymphoma cell lines proliferate rapidly and have been selected to remain viable in the absence of external signals. This is not the case in clinical lymphoma, in which proliferation is often slow, and cross-talk between lymphoma cells and benign cells, and other environmental factors such as cytokines, chemokines, and vascularity, has a major impact on the behavior of the lymphoma. Animal models can be quite useful, but also have their limitations. For practical reasons, animal models are usually based on rapidly growing tumors that develop from cell lines that can also grow *in vitro*. Therefore, many of the limitations outlined previously related to the differences between cell lines and primary tumors also impact on *in vivo* animal models. There are other obvious differences between humans and rodents. Clinical correlative studies have proven to be particularly helpful because they clearly address the patient population in question. However, these results need to be interpreted with caution because correlation and causation are not the same.

Despite these limitations, the combination of *in vitro* studies, animal modeling, and clinical correlation is helping us understand the relative importance of various mechanisms of action. Key among them are signaling-induced apoptosis, ADCC, and complement-mediated cytotoxicity (CMC). In reality, a combination of these mechanisms, plus other complex mechanisms we are only now beginning to understand, is likely responsible for the observed clinical effects.

B. Signaling-Induced Apoptosis

In some but not all cell lines, direct signaling can lead to apoptosis. With anti-CD20, various intermediate changes, including activation of scr-family tyrosine kinases, have been identified that lead to activation of caspase 3 (Hofmeister *et al*., 2000; Shan *et al*., 2000). The epitope targeted by mAbs, and the ability to cross-link, has a significant impact on signaling. There is heterogeneity in the ability of anti-CD20 mAbs to cross-link CD20 and to induce movement of CD20 into membrane lipid rafts. This, in turn, might contribute to the strength of the signal and the resulting impact on growth or death (Cragg *et al*., 2003; Deans *et al*., 2002). Signaling mediated by other anti-B-cell mAbs can be mediated by a different pathway than that seen with anti-CD20. For example, treatment of cells with the anti-HLA-DR mAb Hu1D10 results in rapid disruption of the inner mitochondrial transmembrane potential and selective release of apoptosis-inducing factor (AIF) from the mitochondria (Bains *et al*., 2003).

Complex factors, including antigen density, association with membrane rafts, cross-linking mediated by Fc receptors, cell density, and interaction with other environmental factors such as other soluble and surface-bound ligands, also likely impact on the strength and the downstream effects of mAb-mediated signaling *in vivo*. This complicates our ability to determine whether *in vitro* observations of signaling have clinical relevance. An interesting set of studies by Byrd and coworkers provides the strongest evidence to date that signaling might indeed play a role in observed clinical responses. These authors harvested CLL cells from the blood of patients treated with rituximab and found *in vivo* activation of caspase-9, caspase-3, and poly(ADP-ribose) polymerase (PARP). Patients having caspase-3 activation and PARP cleavage *in vivo* had a significant lowering of the cell count as compared with those without caspase activation (Byrd *et al*., 2002). The mechanism of rapid clearance of circulating leukemic cells might be different from the mechanisms responsible for the slower clearance of malignant nodal masses; nevertheless, these data provide evidence that signaling, and likely apoptosis, do occur to some degree in patients in response to mAbs.

C. Antibody-Dependent Cellular Cytotoxicity

ADCC has long been felt to be responsible for much of the antitumor activity of mAbs. *In vitro*, mAbs can target lysis by a variety of effector cell populations, including NK cells, monocytes/macrophages, and activated granulocytes. In animal models, depletion of select effector cell populations, including NK cells and granulocytes, decreases the efficacy of mAb therapy (van Ojik *et al*., 2003). Clynes *et al*. (2000) performed an elegant series of

studies exploring mAb therapy in mice that lacked specific Fcγ receptors (FcγR). The antitumor effect of mAb was enhanced in mice lacking Fcγ RII2B (the so-called inhibitory FcγR), whereas the same mAb had essentially no antitumor activity in mice lacking the FcγR subunit responsible for sending the activation signal to the immune effector cell. Perhaps the strongest evidence to date that ADCC is a central mechanism of action for antitumor mAb therapy comes from clinical correlative analysis of low-affinity FcγRIII CD16 genetic polymorphisms in patients treated with rituximab. Patients homozygous for the higher-affinity allele of CD16 demonstrated significantly higher clinical response rates to rituximab than did patients heterozygous or homozygous for the lower-affinity allele (Cartron *et al*., 2002). This observation has now been confirmed by other groups and in a different population of patients. The emerging evidence that FcγR is important in mediating the antitumor effects of mAbs certainly points to ADCC as being a major mechanism of action. On the other hand, FcγR could contribute to cross-linking of mAbs on the tumor cell surface or to inducing effector cells to produce cytokines that have antitumor activity. Thus, the convincing evidence that FcγR is important suggests, but does not prove, that ADCC is a key mechanism of action.

D. Complement-Mediated Cytotoxicity

Among the first effector functions identified for antibodies in general was their ability to fix complement. For many years, investigators believed that this mechanism was active in the mAb lysis of prokaryotes, but not important for mAbs targeted to autologous eukaryotic cells that express proteins that inhibit complement activation. There is now growing, but conflicting, evidence that complement indeed might play an important role in the antitumor effect of mAbs. *In vitro*, expression of the inhibitory proteins CD55, and to a lesser extent CD59, on the surface of the target cell correlates with anti-CD20-directed complement-mediated lysis of cell lines and primary samples (Golay *et al*., 2000; Harjunpaa *et al*., 2000). This lysis is mediated not by caspase activation but by reactive oxygen species (Bellosillo *et al*., 2001). However, the level of inhibitory molecule expression on the surface of lymphoma samples does not predict for clinical response to rituximab (Weng and Levy, 2001). Recent studies suggest that complement might be important in some animal models. Di Gaetano and colleagues evaluated the efficacy of anti-CD20 mAbs in a model that used a clone of the murine EL4 lymphoma cell line that expresses human CD20. In this model, anti-CD20 mAb had antitumor effects in wild-type mice but not in syngeneic knockout mice lacking C1q, the first component of the classical complement pathway (Di Gaetano *et al*., 2003). This suggests that an intact complement pathway is necessary for antitumor activity of mAbs.

Importantly, this does not necessarily imply that complement directly induces cytotoxicity. The ability of anti-CD20 mAbs to fix complement has been shown to be associated with the movement of CD20 to lipid rafts on the cell surface (Cragg *et al.*, 2003), which in turn could impact cell signaling and mAb-induced apoptosis. Fixation of complement also results in release of opsinins, which could contribute to ADCC. At present, the role of complement in the antitumor activity of antilymphoma mAbs, both in mediating lysis directly and in interaction with other proposed mechanisms of mAb antitumor activity, remains unclear and deserves further evaluation.

E. Other Complex Mechanisms

Direct signaling-induced apoptosis, ADCC, and CMC are the potential mechanisms of action that have received the most attention, in large part because they can be studied *in vitro*. Other mechanisms that are more complex might also be important in the observed clinical responses to antilymphoma mAbs. Signals that result following binding of mAb to lymphoma cells might induce phenotypic change in the lymphoma cell without inducing apoptosis. This theoretically could result in enhanced immunogenicity and development of an enhanced active antilymphoma immune response. Uptake of tumor antigen by professional antigen-presenting cells could also be enhanced by mAbs, after the mAbs induce lysis by other mechanisms. mAbs bound to target cells can induce activation of effector cells by cross-linking FcγR. The resulting production of cytokines and chemokines by the activated effector cells could induce a broad range of regional changes within the malignant tissue, including changes in chemotaxis of other cell populations, vascular permeability, blood flow, or angiogenesis. These mechanisms will be difficult to assess and measure, but evaluation of their role in the observed clinical efficacy of mAb therapy could have a significant impact on the design of the next generation of mAb-based treatments. Most likely, multiple interactive mechanisms of action are involved in the observed clinical responses to mAb in lymphoma, and continued exploration of their relative role in the antilymphoma activity of mAb will be critical for the rational design of the next generation of regimens.

Use of radioimmunoconjugates that combine the direct antilymphoma effects of mAbs with radiation effects, or combining mAbs with chemotherapy, would be expected to take advantage of the signaling effects of mAbs and enhance target cell apoptosis, whereas combining mAbs with various immunologically active molecules would enhance immune-mediated antitumor activity. Clinical evaluation of radioimmunotherapy, mAbs combined with chemotherapy, and mAbs combined with immunotherapy are ongoing and are showing promise.

VI. Radioimmunotherapy

Multiple animal models have demonstrated that mAbs can be used to deliver toxic moieties to tumors. A variety of immunotoxins have been evaluated in patients with lymphoma. This work is reviewed elsewhere in this volume and is not discussed further here. Given the variety of mAbs available and the relative sensitivity of lymphomas to radiation, development of radioimmunoconjugates for the treatment of lymphoma is a logical approach. The deceptively simple concept of radioimmunotherapy is actually quite complex and requires contributions from multiple scientific disciplines to deal with issues such as mAb construct, choice of chelator, selection of optimal radionuclide, optimization of pharmacokinetics, dosimetry, and use of cold mAb predosing. Each of these can have a significant impact on the efficacy and toxicity of therapy. Despite these multiple variables, clinical data from the past 5 years have confirmed the promise of this approach and allowed us to begin to define the role of radiolabeled mAbs as a treatment for lymphoma (Dillman, 2002; Illidge and Johnson, 2000). Initial clinical studies exploring radioimmunotherapy of lymphoma involved the use of ^{131}I, which has an extensive history of use in clinical medicine. Advantages of this isotope include emitting energy with a focused 0.8-mm path length that theoretically minimizes toxicity to surrounding tissues. Concurrent emissions are useful for dosimetry. A variety of other isotopes are being used at present. A more detailed discussion of the advantages and disadvantages of such isotopes for the radioimmunotherapy of hematologic malignancies is presented elsewhere in this volume and is not discussed in detail here.

A. Tositumomab

Initial pilot studies of the radioimmunotherapy of lymphoma by Kaminski *et al.* (1992) involved the use of ^{131}I-labeled anti-CD37. Subsequent studies demonstrated that ^{131}I-labeled anti-CD20 mAbs, now known as tositumomab (Bexxar®), was a more promising agent. The initial Phase I dose-escalation trial by this group with ^{131}I anti-CD20 was designed to remain below severely myelosuppressive doses. A total of 34–161 mCi (designed to achieve whole body doses of 25–85 cGy) was administered. Calculated mean maximal radiation dose to the tumors was 9.25 Gy. After a single therapeutic dose, 22 patients (79%) had objective responses, with two-thirds of those responses being complete. All patients with low-grade lymphomas responded (77% CR), with a median duration of response of more than 16 months. As expected, myelosuppression was dose limiting at a whole body radiation dose of 75 cGy. Nonhematologic toxicity was mild, and 6 of the 34 enrolled patients developed HAMAs (Kaminski *et al.*, 1996). These observations were confirmed in several multicenter Phase II studies

such as the one reported by Vose and coworkers in which 46 subjects (37 with low-grade histologies and 9 with transformed lymphoma) were treated. In this patient population with several adverse prognostic features, ORR was 65% (81% of those with a low-grade histology), but median duration of the responses was only 6.5 months (Vose *et al.*, 2000b).

Press and colleagues used the same agents but administered higher doses of radiation with the expectation that autologous marrow would be administered after therapy because of the risk of marrow ablation. In a Phase I/II experience, 29 patients with relapsed lymphoma (mostly low grade) received ^{131}I coupled to murine anti-CD20 or anti-CD37. An 86% ORR and a 79% CR rate were observed at doses of 280–785 mCi with calculated radiation doses of 22–92 Gy to the tumors (median 38 Gy). Stringent selection criteria were used such that most subjects with larger tumor burdens or splenomegaly were ineligible for therapy. The therapy was associated with significant myelosuppression, but was well tolerated otherwise. In a median follow-up of 42 months, 14 of 29 subjects remained progression free (Liu *et al.*, 1998).

B. Ibritumomab Tiuxetan

Witzig and coworkers reported a series of trials evaluating ibritumomab tiuxetan (Zevlin®), a murine anti-CD20 mAb conjugated with the radioisotope ^{90}Y. ^{90}Y is a pure β-emitting isotope with a 5-mm mean path length, theoretically allowing for improved penetrance in bulky tumors. The lack of γ emission complicates the detection of biodistribution, requiring a dosimetry strategy using an indium-111 labeled surrogate. However, this isotope allows patients to be treated with theoretically less irradiation to distant organs and fewer public health concerns. A Phase I/II trial with ^{90}Y ibritumomab tiuxetan included 51 patients with confirmed relapsed or refractory low-grade or follicular B-cell lymphoma, or relapsed intermediate-grade and mantle cell lymphoma (Witzig *et al.*, 1999). The maximum tolerated dose was 0.4 mCi/kg for patients with a platelet count higher than 150,000 and 0.3 mCi/kg for patients with a platelet count of 100,000–149,000. The ORR was 67%, with 26% CR and 41% PR. The ORR was 82% in patients with low-grade non-Hodgkin's lymphoma and 43% in patients with intermediate-grade histologies. Estimated median time to progression was 12.9+ months, with median duration of response being 11.7+ months. One patient developed a HAMA and human antichimeric antibody (HACA) 2 months after treatment. This study demonstrated that ^{90}Y-labeled anti-CD20 mAbs could safely be administered in the outpatient setting at a dose demonstrating clinical activity without the need for autologous progenitor cell support (Witzig *et al.*, 2003). A randomized controlled trial comparing the ^{90}Y ibritumomab tiuxetan regimen to rituximab alone was

conducted in 143 patients with relapsed or refractory low-grade, follicular, or CD20+-transformed lymphoma. The ORR was 80% in the ^{90}Y ibritumomab tiuxetan arm and 56% in the rituximab arm ($p = 0.002$), with 30% and 16% CR rates, respectively ($p = 0.04$). Estimated median duration of response for all patients was 14.2 months in the ibritumomab tiuxetan arm and 12.1 months in the rituximab arm (Witzig *et al.*, 2002).

It is not yet possible to directly compare the clinical value of ^{131}I tositumomab to that of ^{90}Y ibritumomab tiuxetan. Administration of both agents is complex, but not prohibitively so, and both are well tolerated. Both ^{131}I tositumomab and ^{90}Y ibritumomab tiuxetan appear to result in response rates higher than those seen with rituximab as a single agent. On the other hand, evidence to date suggests that duration of response with the radioimmunoconjugates is similar to that seen with rituximab. One obvious potential indication for these drugs is in patients refractory to both standard chemotherapy and rituximab. Studies of radioimmunotherapy upfront are relatively young and not yet mature enough for us to make any formal conclusions concerning use of radioimmunotherapy as initial therapy.

C. Other Radioimmunoconjugates

Radiolabled mAb-targeting molecules other than CD20 are under development as well. ^{131}I- and ^{90}Y-labeled epratuzumab have been employed in both low-dose and high-dose (with stem cell support) regimens, with clear evidence of antilymphoma activity and acceptable toxicity (Vose *et al.*, 2000a). Lym-1, a murine IgG2a mAb targeted against HLA-DR, has been conjugated to ^{131}I, ^{90}Y, and copper-67 (^{67}Cu) and tested in patients with lymphoma. Toxicity was acceptable, and some antilymphoma activity was seen with each of these isotopes (DeNardo *et al.*, 1997, 1999). The relative advantage or disadvantage of these radioimmunoconjugates over CD20-based therapy remains to be determined.

VII. mAbs Combined with Chemotherapy

Many investigators have explored the impact of rituximab plus chemotherapy *in vitro* and found some evidence for synergy (Wilson, 2000). These results, and the clinical experience of single-agent rituximab that demonstrated the safety and efficacy of rituximab, have led to further studies looking at rituximab combined with cytotoxic chemotherapy in both indolent and aggressive non-Hodgkin's lymphomas.

A. CHOP–Rituximab in Low-Grade Lymphoma

An often-cited early experience combining rituximab and chemotherapy is a Phase II study by Czuczman *et al.* (1999) evaluating 40 patients with newly diagnosed or relapsed or refractory low-grade or follicular lymphoma treated with rituximab and CHOP chemotherapy. ORR in this heterogeneous population of patients was 95%, with 55% CR. Median progression-free survival was not reached after a median observation time of 65 months (Imrie *et al.*, 2002). This study demonstrated that mAb therapy can be safely added to an aggressive chemotherapy regimen. The data on durable responses are intriguing and justify prospective Phase III studies to further explore the clinical utility of such a combination; however, it is also important not to overinterpret these results given the hazards of premature conclusions from small pilot Phase II studies.

B. CHOP–Rituximab in Aggressive Lymphoma

The combination of CHOP and rituximab has also been examined in a series of trials involving patients with previously untreated aggressive non-Hodgkin's lymphoma. In a Phase II study, 33 patients with previously untreated intermediate-grade B-cell lymphoma were treated with six cycles of the combination of rituximab and standard-dose CHOP (Vose *et al.*, 2001). The study population was mostly patients with diffuse large B-cell lymphoma, but seven patients had follicular large-cell lymphoma. Overall, subjects in this trial were a good prognosis group; 69% had low or low-intermediate risk categorization by the International Prognostic Index. In this study, as in the Czuczman study, there was no appreciable increase in serious toxicity over that expected for CHOP alone and CHOP normalized dose intensity was maintained at 94%. The ORR was 94%, with 61% CR and 33% PR. Twenty-nine of 31 patients who achieved PR or CR were in continued remission, with a median observation time of 26 months from entry into remission. As with the previous pilot studies, caution needs to be taken in reaching conclusions from a Phase II trial in good-prognosis patients. Nevertheless, these intriguing data justify larger Phase III randomized trials.

Two well-designed and well-executed Phase III studies have now completed accrual, and early data are available. In a French multicenter (GELA) study of 399 patients between the ages 60 and 80 with untreated diffuse large-cell lymphoma, subjects were randomized to eight cycles of CHOP vs. eight cycles of CHOP plus rituximab (Coiffier *et al.*, 2002). In contrast to the Phase II study outlined previously, the majority of subjects in this Phase III trial had poor prognosis as per the age-adjusted International Prognostic Index. No convincing difference in clinical toxicity was identified between the two regimens. CR or CRu at the end of treatment for CHOP–rituximab was 75% and for CHOP was 63% ($p = 0.005$). Progression during treatment was 9% and 22%, and death without progression was 6% and 6%,

respectively. With median follow-up of 24 months, events (progression, relapse, or death) were observed in 43% of patients treated with CHOP–rituximab and 61% of patients treated with CHOP ($p < 0.001$). The 2-year overall survival was 70% in the CHOP–rituximab arm and 57% in the CHOP arm ($p = 0.007$). An important North American Intergroup study comparing CHOP–rituximab to CHOP has completed accrual of 631 patients older than age 60 with previously untreated diffuse large-cell lymphoma. Preliminary results from this important study were broadly confirmatory, and rituximab plus CHOP is now accepted as superior to CHOP in this population of patients (Habermann *et al*., 2003).

C. Fludarabine and Rituximab

The efficacy of fludarabine alone or in combination with other agents has been well documented in the treatment of low-grade B-cell non-Hodgkin's lymphomas. *In vitro* studies suggest some synergy between fludarabine and rituximab (Di Gaetano *et al*., 2001). There is a concern regarding the cumulative immunologic deficits that could result from the depletion of T-cell function by fludarabine and B-cell function by rituximab. Investigators at the M. D. Anderson Cancer Center randomized 78 patients to receive either fludarabine, mitoxantrone, and dexamethasone (FND) with concurrent rituximab followed by interferon maintenance or FND alone followed by interferon maintenance and delayed rituximab (McLaughlin *et al*., 2000). An analysis of toxicity in the two arms demonstrated a slightly higher incidence of grade 4 granulocytopenia in the arm with concurrent FND plus rituximab; however, at the initial analysis, no difference in the rate of infection was seen between the two arms. Nonhematologic toxicity in both arms was described as modest. Molecular remission (as determined by the bcl-2 PCR assay) was higher at 6 months when rituximab was given concurrently with FND. Czuczman *et al*. (2002) also presented data on the safety of rituximab combined with fludarabine in a heterogeneous population of patients with low-grade lymphomas. Prolonged cytopenias were observed with the initial dosing regimen chosen, leading to adjustments, including 40% reduction in fludarabine dosing and discontinuation of prophylactic trimethoprim/sulfmethsoxasole. Transient treatment delays were still observed in 10 of 30 subsequent patients treated. Of the 40 subjects, 6 developed herpes simplex/zoster infections. Clinical response rates in both studies were predictably high. It is too early to reach a consensus on the relative risks and benefits of combining nucleoside analogs and rituximab.

D. Other Combinations

The combinations of rituximab and chemotherapy outlined previously are only the tip of the iceberg. Multiple chemotherapy regimens are currently being combined with mAbs in Phase I, II, and III trials. We will learn

more about the potential of these various combinations as treatment for various lymphomas as these trials mature over the next several years. Given the potency of radioimmunotherapy and the success of chemoimmunotherapy, investigators have begun the exploration of chemotherapy combined with radioimmunotherapy. The Southwest Oncology Group reported on 90 patients with previously untreated advanced-stage follicular lymphoma treated with CHOP followed 4–8 weeks later by ^{131}I tositumomab. Treatment was well tolerated, and the overall response rate to the regimen was 90%, with 67% CRs and a 2-year progression-free survival estimate of 81% (Press *et al.*, 2003). These results are promising, but follow-up is needed before we know the long-term impact of this aggressive, upfront therapy.

VIII. mAbs Combined with Immunotherapy

As outlined previously, ADCC appears to contribute to the antilymphoma activity of mAbs. Agents that can activate the effector cells that mediate ADCC should be able to improve the efficacy of therapy. *In vitro* and in animal models, agents such as IFNα, IL2, IL-12, and CpG oligodeoxynucleotides that activate effector cell populations also enhance antitumor activity of mAbs (Basham *et al.*, 1987; Berinstein *et al.*, 1988; Ozaki *et al.*, 1999; Wooldridge *et al.*, 1997). Clinical trials exploring the efficacy of mAbs, plus immunostimulatory agents are not as advanced as those exploring mAbs plus chemotherapy; nevertheless, this approach continues to hold promise.

A. Interferon

The combination of IFNα and anti-Id mAb was explored by Levy and colleagues in the early 1980s in patients with follicular lymphoma. Of the 12 subjects in this small trial, 9 responded to the combination (Brown *et al.*, 1989). This approach was revisited by a number of groups when rituximab was found to be active as a single agent. Davis and coworkers treated 38 patients with the combination of rituximab and IFN-α2a. The ORR was 45%, with a median response duration and the median time to progression in responders of 22.3 and 25.2 months, respectively (Davis *et al.*, 1998). Results from the study of Sacchi *et al.* (2001) in 64 subjects were similar, with an ORR of 70% and a median duration of response of 19 months. Kimby and colleagues performed a study based on a complex schema in which the addition of IFN-α2a appeared to significantly increase responsiveness to a second course of rituximab in patients with a partial response or minor response to an initial course of rituximab monotherapy (Kimby, 2002). Taken together, these studies hint at increased activity with the

addition of interferon, although it remains to be determined whether any increased activity is worth the increased toxicity.

B. IL-2

Ongoing studies are exploring synergy between rituximab and IL-2. Friedberg and colleagues treated 20 patients with rituximab and IL-2 administered subcutaneously at a dose of 1.2 mg/m^2 daily for 52 days. The treatment was well tolerated, and consistent increases in CD8(+) and CD56(+) cell numbers were noted. The study was too small to assess efficacy (Friedberg *et al*., 2002). This approach is at present being evaluated in larger trials.

C. IL-12

Ansell and coworkers have been exploring the combination of rituximab and IL-12. At higher doses of IL-12, constitutional symptoms and liver enzyme elevations were dose limiting. An ORR of 69% was noted, with 8 of 11 CRs seen at IL-12 doses of 300 ng/kg or higher. This therapy also upregulated γ interferon and IP-10 expression and increased NK cell lytic activity (Ansell *et al*., 2002).

D. Immunostimulatory CpG ODN

Immunostimulatory CpG ODN can activate monocytes and NK cells and induce production of both type 1 interferon and IL-12 (Jahrsdorfer and Weiner, 2003). In animal models, the combination of mAbs plus CpG ODN is highly effective (Wooldridge *et al*., 1997). CpG ODN can also induce upregulation of CD20 by primary malignant B-cells (Jahrsdorfer *et al*., 2001). Clinical evaluation of the combination of rituximab and immunostimulatory CpG ODN has recently begun.

IX. Conclusion

We no longer hear clinicians speak of a failed hypothesis when discussing the clinical value of mAbs in the treatment of lymphoma. Without question, mAb-based therapy is now an important part of lymphoma management. However, we are not yet ready to claim that we have found Ehrlich's "magic bullet" for lymphoma. There is much about the biology and clinical utility of mAb-based therapy that we do not yet know. Ongoing laboratory research and rationally designed clinical trials based on our growing understanding of the biology and clinical effects of mAb-based therapy are needed to allow us to expand on this exciting beginning of a new era in lymphoma management.

References

Ansell, S. M., Witzig, T. E., Kurtin, P. J., Sloan, J. A., Jelinek, D. F., Howell, K. G., Markovic, S. N., Habermann, T. M., Klee, G. G., Atherton, P. J., and Erlichman, C. (2002). Phase 1 study of interleukin-12 in combination with rituximab in patients with B-cell non-Hodgkin lymphoma. *Blood* **99,** 67–74.

Bains, S. K., Mone, A., Yun Tso, J., Lucas, D., Byrd, J. C., Weiner, G. J., and Green, J. M. (2003). Mitochondria control of cell death induced by anti-HLA-DR antibodies. *Leukemia* **17,** 1357–1365.

Basham, T. Y., Kaminski, M. S., Kitamura, K., Levy, R., and Merigan, T. C. (1987). Synergistic antitumor effect of interferon and anti-idiotype monoclonal antibody in murine lymphoma. *J. Immunol.* **137,** 3019–3024.

Bellosillo, B., Villamor, N., Lopez-Guillermo, A., Marce, S., Esteve, J., Campo, E., Colomer, D., and Montserrat, E. (2001). Complement-mediated cell death induced by rituximab in B-cell lymphoproliferative disorders is mediated in vitro by a caspase-independent mechanism involving the generation of reactive oxygen species. *Blood* **98,** 2771–2777.

Berinstein, N., Starnes, C. O., and Levy, R. (1988). Specific enhancement of the therapeutic effect of anti-idiotype antibodies on a murine B cell lymphoma by IL-2. *J. Immunol.* **140,** 2839–2845.

Berinstein, N. L., Grillo-Lopez, A. J., White, C. A., Bence-Bruckler, I., Maloney, D., Czuczman, M., Green, D., Rosenberg, J., McLaughlin, P., and Shen, D. (1998). Association of serum rituximab (IDEC-C2B8) concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma. *Ann. Oncol.* **9,** 995–1001.

Brown, S. L., Miller, R. A., Horning, S. J., Czerwinski, D., Hart, S. M., McElderry, R., Basham, T., Warnke, R. A., Merigan, T. C., and Levy, R. (1989). Treatment of B-cell lymphomas with anti-idiotype antibodies alone and in combination with alpha interferon. *Blood* **73,** 651–661.

Byrd, J. C., Kitada, S., Flinn, I. W., Aron, J. L., Pearson, M., Lucas, D., and Reed, J. C. (2002). The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: Evidence of caspase activation and apoptosis induction. *Blood* **99,** 1038–1043.

Cartron, G., Dacheux, L., Salles, G., Solal-Celigny, P., Bardos, P., Colombat, P., and Watier, H. (2002). Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. *Blood* **99,** 754–758.

Clynes, R. A., Towers, T. L., Presta, L. G., and Ravetch, J. V. (2000). Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. *Nat. Med.* **6,** 443–446.

Coiffier, B., Lepage, E., Briere, J., Herbrecht, R., Tilly, H., Bouabdallah, R., Morel, P., Van Den Neste, E., Salles, G., Gaulard, P., Reyes, F., Lederlin, P., and Gisselbrecht, C. (2002). CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N. Engl. J. Med.* **346,** 235–242.

Colombat, P., Salles, G., Brousse, N., Eftekhari, P., Soubeyran, P., Delwail, V., Deconinck, E., Haioun, C., Foussard, C., Sebban, C., Stamatoullas, A., Milpied, N., Boue, F., Taillan, B., Lederlin, P., Najman, A., Thieblemont, C., Montestruc, F., Mathieu-Boue, A., Benzohra, A., and Solal-Celigny, P. (2001). Rituximab (anti-CD20 monoclonal antibody) as single first-line therapy for patients with follicular lymphoma with a low tumor burden: clinical and molecular evaluation. *Blood* **97,** 101–106.

Cragg, M. S., Morgan, S. M., Chan, H. T., Morgan, B. P., Filatov, A. V., Johnson, P. W., French, R. R., and Glennie, M. J. (2003). Complement-mediated lysis by anti-CD20 mAb correlates with segregation into lipid rafts. *Blood* **101,** 1045–1052.

Czuczman, M. S., Fallon, A., Mohr, A., Stewart, C., Bernstein, Z. P., McCarthy, P., Skipper, M., Brown, K., Miller, K., Wentling, D., Klippenstein, D., Loud, P., Rock, M. K., Benyunes, M., Grillo-Lopez, A. J., and Bernstein, S. H. (2002). Rituximab in combination with CHOP or fludarabine in low-grade lymphoma. *Semin. Oncol.* **29,** 36–40.

Czuczman, M. S., Grillo-Lopez, A. J., White, C. A., Saleh, M., Gordon, L., LoBuglio, A. F., Jonas, C., Klippenstein, D., Dallaire, B., and Varns, C. (1999). Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. *J. Clin. Oncol.* **17,** 268–276.

Davis, T. A., Grillo-Lopez, A. J., White, C. A., McLaughlin, P., Czuczman, M. S., Link, B. K., Maloney, D. G., Weaver, R. L., Rosenberg, J., and Levy, R. (2000). Rituximab anti-CD20 monoclonal antibody therapy in non-Hodgkin's lymphoma: Safety and efficacy of re-treatment. *J. Clin. Oncol.* **18,** 3135–3143.

Davis, T. A., Maloney, D. G., Czerwinski, D. K., Liles, T. M., and Levy, R. (1998). Anti-idiotype antibodies can induce long-term complete remissions in non-Hodgkin's lymphoma without eradicating the malignant clone. *Blood* **92,** 1184–1190.

Deans, J. P., Li, H., and Polyak, M. J. (2002). CD20-mediated apoptosis: Signalling through lipid rafts. *Immunology* **107,** 176–182.

DeNardo, G. L., Kukis, D. L., Shen, S., DeNardo, D. A., Meares, C. F., and DeNardo, S. J. (1999). 67Cu-versus 131I-labeled Lym-1 antibody: Comparative pharmacokinetics and dosimetry in patients with non-Hodgkin's lymphoma. *Clin. Cancer. Res.* **5,** 533–541.

DeNardo, G. L., Lamborn, K. R., Goldstein, D. S., Kroger, L. A., and Denardo, S. J. (1997). Increased survival associated with radiolabeled Lym-1 therapy for non-Hodgkins lymphoma and chronic lymphocytic leukemia. *Cancer* **80,** 2706–2711.

Di Gaetano, N., Cittera, E., Nota, R., Vecchi, A., Grieco, V., Scanziani, E., Botto, M., Introna, M., and Golay, J. (2003). Complement activation determines the therapeutic activity of rituximab in vivo. *J. Immunol.* **171,** 1581–1587.

Di Gaetano, N., Xiao, Y., Erba, E., Bassan, R., Rambaldi, A., Golay, J., and Introna, M. (2001). Synergism between fludarabine and rituximab revealed in a follicular lymphoma cell line resistant to the cytotoxic activity of either drug alone. *Br. J. Haematol.* **114,** 800–809.

Dillman, R. O. (2002). Radiolabeled anti-CD20 monoclonal antibodies for the treatment of B-cell lymphoma. *J. Clin. Oncol.* **20,** 3545–3557.

Ehrlich, P. (1906). "Collected Studies on Immunity." John Wiley & Sons, New York.

Friedberg, J. W., Neuberg, D., Gribben, J. G., Fisher, D. C., Canning, C., Koval, M., Poor, C. M., Green, L. M., Daley, J., Soiffer, R., Ritz, J., and Freedman, A. S. (2002). Combination immunotherapy with rituximab and interleukin 2 in patients with relapsed or refractory follicular non-Hodgkin's lymphoma. *Br. J. Haematol.* **117,** 828–834.

Ghielmini, M. (2002). Prolonged treatment with rituximab significantly improves event free survival and duration of response in patients with follicular lymphoma: A randomised SAKK trial. *Proc. Am. Soc. Hematol.* **100,** 161a.

Golay, J., Zaffaroni, L., Vaccari, T., Lazzari, M., Borleri, G. M., Bernasconi, S., Tedesco, F., Rambaldi, A., and Introna, M. (2000). Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab *in vitro*: CD55 and CD59 regulate complement-mediated cell lysis. *Blood* **95,** 3900–3908.

Habermann, T. M., Weller, E. A., Morrison, V. A., Cassileth, P. A., Cohn, J. B., Dakhil, S. R., Gascoyne, R. D., Woda, G., Fisher, R. I., Peterson, B. A., and Horning, S. J. (2003). Phase III trial of rituximab-CHOP vs. CHOP with a secondary randomization to maintenance rituximab or observation in patients 60 years of age and older with diffuse large B-cell lymphoma. *Blood* **102,** 6a.

Hainsworth, J. D. (2002). Rituximab as first-line and maintenance therapy for patients with indolent non-Hodgkin's lymphoma: Interim follow-up of a multicenter phase II trial. *Semin. Oncol.* **29,** 25–29.

Hainsworth, J. D. (2003). First-line and maintenance treatment with rituximab for patients with indolent non-Hodgkin's lymphoma. *Semin. Oncol.* **30,** 9–15.

Harjunpaa, A., Junnikkala, S., and Meri, S. (2000). Rituximab (anti-CD20) therapy of B-cell lymphomas: Direct complement killing is superior to cellular effector mechanisms. *Scand. J. Immunol.* **51,** 634–641.

Hofmeister, J. K., Cooney, D., and Coggeshall, K. M. (2000). Clustered CD20 induced apoptosis: src-family kinase, the proximal regulator of tyrosine phosphorylation, calcium influx, and caspase 3-dependent apoptosis. *Blood Cells Mol. Dis.* **26,** 133–143.

Illidge, T. M., and Johnson, P. W. (2000). The emerging role of radioimmunotherapy in haematological malignancies. *Br. J. Haematol.* **108,** 679–688.

Imrie, K. R., Linch, D. C., and Czuczman, M. S. (2002). Debate on the conservative and aggressive treatment options for the optimal management of indolent non-Hodgkin's lymphoma. *Anticancer Drugs* **13**(Suppl. 2), S19–S24.

Jahrsdorfer, B., Hartmann, G., Racila, E., Jackson, W., Muhlenhoff, L., Meinhardt, G., Endres, S., Link, B. K., Krieg, A. M., and Weiner, G. J. (2001). CpG DNA increases primary malignant B cell expression of costimulatory molecules and target antigens. *J. Leukoc. Biol.* **69,** 81–88.

Jahrsdorfer, B., and Weiner, G. J. (2003). Immunostimulatory CpG oligodeoxynucleotides and antibody therapy of cancer. *Semin. Oncol.* **30,** 476–482.

Kaminski, M. S., Fig, L. M., Zasadny, K. R., Koral, K. F., DelRosario, R. B., Francis, I. R., Hanson, C. A., Normolle, D. P., Mudgett, E., Liu, C. P., Moon, S., Scott, P., Miller, R. A., and Wahl, R. L. (1992). Imaging, dosimetry and radioimmunotherapy with iodine 131-labeled anti-CD37 antibody in B-cell lymphoma. *J. Clin. Oncol.* **10,** 1696–1711.

Kaminski, M. S., Zasadny, K. R., Francis, I. R., Fenner, M. C., Ross, C. W., Milik, A. W., Estes, J., Tuck, M., Regan, D., Fisher, S., Glenn, S. D., and Wahl, R. L. (1996). Iodine-131-Anti-B1 radioimmunotherapy for B-cell lymphoma. *J. Clin. Oncol.* **14,** 1974–1981.

Khorana, A., Bunn, P., McLaughlin, P., Vose, J., Stewart, C., and Czuczman, M. S. (2001). A phase II multicenter study of CAMPATH-1H antibody in previously treated patients with nonbulky non-Hodgkin's lymphoma. *Leuk. Lymph.* **41,** 77–87.

Kimby, E. (2002). Beyond immunochemotherapy: Combinations of rituximab with cytokines interferon-alpha2a and granulocyte colony stimulating factor [corrected] *Semin. Oncol.* **29,** 7–10.

Kohler, G., and Milstein, C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256,** 495.

Kostelny, S. A., Link, B. K., Tso, J. Y., Vasquez, M., Jorgensen, B. H., Wang, H., Hall, W. C., and Weiner, G. J. (2001). Humanization and characterization of the anti-HLA-DR antibody 1D10. *Int. J. Cancer* **93,** 556–565.

Leonard, J. P., Coleman, M., Schuster, M. W., Feldman, E. J., Chadburn, A., Ely, S., Hansen, H. J., Wegener, W. A., and Goldenberg, D. M. (2000). Immunotherapy of NHL with epratuzumab (anti-CD22 monoclonal antibody): Excellent tolerability with objective responses. **19**, 17a.

Leonard, J. P., Coleman, M., Matthews, J. C., Fiore, J. M., Dosik, A., Shore, T., Kapushoc, H., Macri, M., Wegener, W. A., Cesano, A., and Goldenberg, D. M. (2002). Epratuzumab (anti-CD22) and rituximab (anti-CD20) combination immunotherapy for non-Hodgkin's lymphoma: Preliminary response data. *Proc. Am. Soc. Clin. Oncol.* **21,** 266a.

Leonard, J. P., Coleman, M., Ketas, J. C., Chadburn, A., Ely, S., Furman, R. R., Wegener, W. A., Hansen, H. J., Ziccardi, H., Eschenberg, M., Gayko, U., Cesano, A., and Goldenberg, D. M. (2003). Phase I/II trial of epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma. *J. Clin. Oncol.* **21,** 3051–3059.

Leung, S. O., Goldenberg, D. M., Dion, A. S., Pellegrini, M. C., Shevitz, J., Shih, L. B., and Hansen, H. J. (1995). Construction and characterization of a humanized, internalizing, B-cell (CD22)-specific, leukemia/lymphoma antibody, LL2. *Mol. Immunol.* **32,** 1413–1427.

Link, B. K., Wang, H., Byrd, J. C., Leonard, J. P., Davis, T. A., Flinn, I., Hall, W. C., Turner, J. F., Levitt, D., and Weiner, G. J. (2001a). The humanized monoclonal antibody Hu1D10 was well tolerated and demonstrated activity in patients with relapsed B-cell lymphoma. *Proc. Am. Soc. Clin. Oncol.* **280,** 284a.

Link, B. K., Wang, H., Byrd, J. C., Leonard, J. P., Davis, T. A., Flinn, I., Hall, W. C., Turner, J. F., Bowles, J., Shannon, M., Levitt, D., and Weiner, G. J. (2001b). Prolonged clinical responses in patients with follicular lymphoma treated on a Phase I trial of the anti-HLA-DR monoclonal antibody remitogen™ (Hu1D10). *Proc. Am. Soc. Hematol.* **98,** 244b.

Liu, S. Y., Eary, J. F., Petersdorf, S. H., Martin, P. J., Maloney, D. G., Appelbaum, F. R., Matthews, D. C., Bush, S. A., Durack, L. D., Fisher, D. R., Gooley, T. A., Bernstein, I. D., and Press, O. W. (1998). Follow-up of relapsed B-cell lymphoma patients treated with iodine-131-labeled anti-CD20 antibody and autologous stem-cell rescue. *J. Clin. Oncol.* **16,** 3270–3278.

Lundin, J., Osterborg, A., Brittinger, G., Crowther, D., Dombret, H., Engert, A., Epenetos, A., Gisselbrecht, C., Huhn, D., Jaeger, U., Thomas, J., Marcus, R., Nissen, N., Poynton, C., Rankin, E., Stahel, R., Uppenkamp, M., Willemze, R., and Mellstedt, H. (1998). CAMPATH-1H monoclonal antibody in therapy for previously treated low-grade non-Hodgkin's lymphomas: A phase II multicenter study. European Study Group of CAMPATH-1H Treatment in Low-Grade Non-Hodgkin's Lymphoma. *J. Clin. Oncol.* **16,** 3257–3263.

Maloney, D. G., Grillo-Lopez, A. J., Bodkin, D. J., White, C. A., Liles, T. M., Royston, I., Varns, C., Rosenberg, J., and Levy, R. (1997a). IDEC-C2B8: Results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma [see comments] *J. Clin. Oncol.* **15,** 3266–3274.

Maloney, D. G., Grillo-Opez, A. J., White, C. A., Bodkin, D., Schilder, R. J., Neidhart, J. A., Janakiraman, N., Foon, K. A., Liles, T. M., Dallaire, B. K., Wey, K., Royston, I., Davis, T., and Levy, R. (1997b). Idec-C2b8 (rituximab) anti-Cd20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood* **90,** 2188–2195.

McLaughlin, P., Grillo-Lopez, A. J., Link, B. K., Levy, R., Czuczman, M. S., Williams, M. E., Heyman, M. R., Bence-Bruckler, I., White, C. A., Cabanillas, F., Jain, V., Ho, A. D., Lister, J., Wey, K., Shen, D., and Dallaire, B. K. (1998). Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: Half of patients respond to a four-dose treatment program. *J. Clin. Oncol.* **16,** 2825–2833.

McLaughlin, P., Hagemeister, F. B., Rodriguez, M. A., Sarris, A. H., Pate, O., Younes, A., Lee, M. S., Dang, N. H., Romaguera, J. E., Preti, A. H., McAda, N., and Cabanillas, F. (2000). Safety of fludarabine, mitoxantrone, and dexamethasone combined with rituximab in the treatment of stage IV indolent lymphoma. *Semin. Oncol.* **27,** 37–41.

Meeker, T., Lowder, J., Cleary, M. L., Stewart, S., Warnke, R., Sklar, J., and Levy, R. (1985a). Emergence of idiotype variants during treatment of B-cell lymphoma with anti-idiotype antibodies. *N. Engl. J. Med.* **312,** 1658–1665.

Meeker, T. C., Lowder, J., Maloney, D. G., Miller, R. A., Thielemans, K., Warnke, R., and Levy, R. (1985b). A clinical trial of anti-idiotype therapy for B cell malignancy. *Blood* **65,** 1349–1363.

Nadler, L. M., Ritz, J., Griffin, J. D., Todd, R. F., 3rd, Reinherz, E. L., and Schlossman, S. F. (1981). Diagnosis and treatment of human leukemias and lymphomas utilizing monoclonal antibodies. *Prog. Hematol.* **12,** 187–225.

Nadler, L. M., Stashenko, P., Hardy, R., Kaplan, W. D., Button, L. N., Kufe, D. W., Antman, K. H., and Schlossman, S. F. (1980). Serotherapy of a patient with a monoclonal antibody directed against a human lymphoma-associated antigen. *Cancer Res.* **40,** 3147–3154.

Order, S. E. (1976). The history and progress of serologic immunotherapy and radiodiagnosis. *Radiology* **118,** 219–223.

Osterborg, A., Dyer, M. J., Bunjes, D., Pangalis, G. A., Bastion, Y., Catovsky, D., and Mellstedt, H. (1997). Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia. European Study Group of CAMPATH-1H Treatment in Chronic Lymphocytic Leukemia. *J. Clin. Oncol.* **15,** 1567–1574.

Ozaki, S., Kosaka, M., Wakahara, Y., Ozaki, Y., Tsuchiya, M., Koishihara, Y., Goto, T., and Matsumoto, T. (1999). Humanized anti-HM1.24 antibody mediates myeloma cell cytotoxicity that is enhanced by cytokine stimulation of effector cells. *Blood* **93,** 3922–3930.

Piro, L. D., White, C. A., Grillo-Lopez, A. J., Janakiraman, N., Saven, A., Beck, T. M., Varns, C., Shuey, S., Czuczman, M., Lynch, J. W., Kolitz, J. E., and Jain, V. (1999). Extended rituximab (anti-CD20 monoclonal antibody) therapy for relapsed or refractory low-grade or follicular non-Hodgkin's lymphoma. *Ann. Oncol.* **10,** 655–661.

Press, O. W., Unger, J. M., Braziel, R. M., Maloney, D. G., Miller, T. P., LeBlanc, M., Gaynor, E. R., Rivkin, S. E., and Fisher, R. I. (2003). A phase 2 trial of CHOP chemotherapy followed by tositumomab/iodine I 131 tositumomab for previously untreated follicular non-Hodgkin lymphoma: Southwest Oncology Group Protocol S9911. *Blood* **102,** 1606–1612.

Sacchi, S., Federico, M., Vitolo, U., Boccomini, C., Vallisa, D., Baldini, L., Petrini, M., Rupoli, S., Di Raimondo, F., Merli, F., Liso, V., Tabilio, A., Saglio, G., Vinci, G., Brugiatelli, M., and Dastoli, G. (2001). Clinical activity and safety of combination immunotherapy with IFN-alpha 2a and rituximab in patients with relapsed low grade non-Hodgkin's lymphoma. *Haematologica* **86,** 951–958.

Salisbury, J. R., Rapson, N. T., Codd, J. D., Rogers, M. V., and Nethersell, A. B. (1994). Immunohistochemical analysis of CDw52 antigen expression in non-Hodgkin's lymphomas. *J. Clin. Pathol.* **47,** 313–317.

Shan, D., Ledbetter, J. A., and Press, O. W. (2000). Signaling events involved in anti-CD20-induced apoptosis of malignant human B cells. *Cancer Immunol. Immunother.* **48,** 673–683.

Valerius, T., Elsasser, D., Repp, R., Van de Winkel, J. G., Gramatzki, M., and Glennie, M. (1997). HLA class II antibodies recruit G-CSF activated neutrophils for treatment of B cell malignancies. *Leuk. Lymph.* **26,** 261–269.

van Ojik, H. H., Bevaart, L., Dahle, C. E., Bakker, A., Jansen, M. J. H., van Vugt, M. J., van de winkel, J. G. J., and Weiner, G. J. (2003). CpG-A and B ODN enhance the efficacy of antibody therapy by activating different effector cell populations. *Cancer Res.* **63,** 5595–5600.

Vose, J. M., Colcher, D., Gobar, L., Bierman, P. J., Augustine, S., Tempero, M., Leichner, P., Lynch, J. C., Goldenberg, D., and Armitage, J. O. (2000a). Phase I/II trial of multiple dose ^{131} iodine-MAb LL2 (CD22) in patients with recurrent non-Hodgkin's lymphoma. *Leuk. Lymph.* **38,** 91–101.

Vose, J. M., Link, B. K., Grossbard, M. L., Czuczman, M., Grillo-Lopez, A., Gilman, P., Lowe, A., Kunkel, L. A., and Fisher, R. I. (2001). Phase II study of rituximab in combination with CHOP chemotherapy in patients with previously untreated, aggressive non-Hodgkin's lymphoma. *J. Clin. Oncol.* **19,** 389–397.

Vose, J. M., Wahl, R. L., Saleh, M., Rohatiner, A. Z., Knox, S. J., Radford, J. A., Zelenetz, A. D., Tidmarsh, G. F., Stagg, R. J., and Kaminski, M. S. (2000b). Multicenter phase II study of iodine-131 tositumomab for chemotherapy-relapsed/refractory low-grade and transformed low-grade B-cell non-Hodgkin's lymphomas. *J. Clin. Oncol.* **18,** 1316–1323.

Weng, W. K., and Levy, R. (2001). Expression of complement inhibitors CD46, CD55, and CD59 on tumor cells does not predict clinical outcome after rituximab treatment in follicular non-Hodgkin lymphoma. *Blood* **98,** 1352–1357.

Wilson, W. H. (2000). Chemotherapy sensitization by rituximab: Experimental and clinical evidence. *Semin. Oncol.* **27,** 30–36.

Witzig, T. E., Gordon, L. I., Cabanillas, F., Czuczman, M. S., Emmanouilides, C., Joyce, R., Pohlman, B. L., Bartlett, N. L., Wiseman, G. A., Padre, N., Grillo-Lopez, A. J., Multani, P., and White, C. A. (2002). Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. *J. Clin. Oncol.* **20,** 2453–2463.

Witzig, T. E., White, C. A., Gordon, L. I., Wiseman, G. A., Emmanouilides, C., Murray, J. L., Lister, J., and Multani, P. S. (2003). Safety of yttrium-90 ibritumomab tiuxetan radioimmunotherapy for relapsed low-grade, follicular, or transformed non-Hodgkin's lymphoma. *J. Clin. Oncol.* **21,** 1263–1270.

Witzig, T. E., White, C. A., Wiseman, G. A., Gordon, L. I., Emmanouilides, C., Raubitschek, A., Janakiraman, N., Gutheil, J., Schilder, R. J., Spies, S., Silverman, D. H., Parker, E., and Grillio-Lopez, A. J. (1999). Phase I/II trial of IDEC-Y2B8 radioimmunotherapy for treatment of relapsed or refractory CD20(+) B-cell non-Hodgkin's lymphoma. *J. Clin. Oncol.* **17,** 3793–3803.

Wooldridge, J. E., Ballas, Z., Krieg, A. M., and Weiner, G. J. (1997). Immunostimulatory oligodeoxynucleotides containing CpG motifs enhance the efficacy of monoclonal antibody therapy of lymphoma. *Blood* **89,** 2994–2998.

Sijie Lu
Eric Wieder
Krishna Komanduri
Qing Ma
Jeffrey J. Molldrem

The University of Texas M. D. Anderson Cancer Center
Houston, Texas 77030

Vaccines in Leukemia

I. Chapter Overview

Immunity to leukemia and leukemia-associated antigens has been demonstrated in animal models and preclinical *in vitro* studies. In addition, data from allogeneic stem cell transplantation have provided direct evidence that T-lymphocyte immunity is important in inducing and maintaining remission in patients with myeloid forms of leukemia. Laboratory studies have more recently focused on the identification of the effector mechanisms and antigens targeted by T lymphocytes. Clinical trials are currently underway with protein-, peptide-, and cellular-based vaccination strategies, and results of these ongoing studies will facilitate our understanding of leukemia immunity. Vaccine-based immunotherapy can potentially offer an alternative treatment strategy for patients with an otherwise poor prognosis. In this chapter, we review the biologic basis of antileukemia immunity and highlight potential leukemia-associated target antigens. In addition, we

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

describe ongoing treatment strategies and the preliminary results from recent vaccine trials.

II. Introduction

The most compelling evidence that lymphocytes meditate an antileukemia effect comes from studies in which allogeneic donor lymphocyte infusions (DLIs) have been used to treat relapse of myeloid leukemia after allogeneic bone marrow transplantation (BMT) (Antin, 1993; Giralt and Kolb, 1996; Kolb and Holler, 1997; Kolb *et al.*, 1995, 1996). Lymphocyte infusions from the original donor used for prior BMT can induce both hematologic and cytogenetic responses in approximately 70–80% of patients with chronic myeloid leukemia (CML) in chronic phase (CP) (Kolb *et al.*, 1996). A complete cytogenetic response is usually obtained between 1 and 4 months after DLIs (van Rhee *et al.*, 1994), and approximately 80% of responders achieve reverse transcriptase-polymerase chain reaction (RT-PCR) negativity for the BCR/ABL translocation [the fusion product of the t(9;22) translocation found in CML] within a mean of 6 months (van Rhee *et al.*, 1994). Acute myeloid leukemia (AML) is also susceptible to the graft-versus-leukemia (GVL) effect, with 15–40% of patients obtaining remission with DLIs alone (Collins *et al.*, 1997). Although significant graft-versus-host disease (GVHD) occurs in 50% of patients treated with DLI and disease response occurs in 90% of CML patients, 55% of patients who do not experience GVHD also have disease response (Giralt and Kolb, 1996; Kolb and Holler, 1997). This demonstrates that GVL is separable from GVHD in some patients, and several potential antigens that drive the donor's lymphocyte response preferentially against the leukemia have been identified. There is also evidence of an autologous immune response against both CML and AML, which is directed against some of the same antigens. Interestingly, chronic GVHD is associated with more GVL activity than acute GVHD, and chronic GVHD resembles autoimmunity, suggesting that the antigens involved in this reaction might be self-antigens. Remissions after DLIs for AML are generally not as durable as those obtained in chronic-phase CML, which may reflect the rapid kinetics of tumor growth outpacing the kinetics of the developing immune response as well as a potentially less immunogenic target cell. However, if more antigens could be determined, and if large numbers of antigen-specific cytotoxic T lymphocytes (CTLs) could be elicited vis-à-vis vaccination strategies, it would allow for development of leukemia-specific therapies.

To understand the nature of vaccine-induced T-cell immunity, we first review some of the principles of antigen recognition and highlight a recent discovery that has aided our ability to study T-cell interactions. T cells recognize peptide antigens that are presented on the cell surface in

combination with major histocompatibility complex (MHC) antigens. Peptides derived from cytoplasmic proteins that are 8–11 amino acids in length bind in the groove of class I MHC molecules and are transported through the endoplasmic reticulum to the cell surface. Larger peptides, typically 12–18 amino acids in length, derived from the processing of extracellular proteins bind class II MHC molecules and are presented to T cells on the cell surface. Both peptide/MHC-I and peptide/MHC-II are recognized by the heterodimeric T-cell receptor (TCR) on CD8 or CD4 T lymphocytes, respectively, with weak affinity and rapid off rates. Points of contact between the TCR and the peptide/MHC surface include surface amino acids contributed by the two alpha-helical domains of the MHC molecule that flank the peptide antigen binding pocket, as well as amino acids from the peptide itself.

Our understanding of the nature of antigen-specific T-cell responses has been greatly improved by the discovery that antigen-specific TCR can be reversibly labeled with soluble peptide/MHC tetramers (Altman *et al.*, 1996). Peptide antigen, β_2-microglobulin, and the MHC-I heavy chain are folded together, and, through a biotinylation signal sequence at the C-terminus of the MHC-I heavy chain, are linked covalently to streptavidin in a 4:1 molecular ratio. When the streptavidin molecule is linked to a fluorescent dye such as phycoerythrin, the resulting peptide/MHC tetramers can be used to identify antigen-specific T cells by FACS analysis because of their higher binding avidity to the cognate TCR. Using tetramers, it has been determined that up to 45% of all peripheral circulating T cells might be specific for a single dominant antigen at the height of an immune response to Epstein-Barr virus (EBV) infection (Callan *et al.*, 1998) and similar dominance can be seen during other viral infections (Komanduri *et al.*, 1998, 2001). Tetramers have also been used to study immune responses to tumor antigens (Lee *et al.*, 1999), and they have also aided in their discovery (Molldrem *et al.*, 2000).

III. Potential Target Antigens

Various methods have been used to determine the nature of the target antigens involved in leukemia immunity. For instance, tissue-restricted minor histocompatibility antigens (mHAs) that are derived from proteins expressed only in hematopoietic tissue have been shown to be the targets of alloreactive T cells (den Haan *et al.*, 1995, 1998; Dolstra *et al.*, 1997; Murata *et al.*, 2003; Warren *et al.*, 1998). These mHAs often result from polymorphic differences between donor and recipient in the coding regions of peptide antigens that bind within the groove of MHC molecules and are recognized by donor T cells. Recently, however, a newly described mHA was found to result from differential expression in donor and recipient due to a gene deletion (Murata *et al.*, 2003). Heterologous T-cell clones that

demonstrate alloreactivity toward mHAs have been established from patients with severe GVHD following BMT with an HLA-matched donor (Faber *et al.*, 1995a,b, 1996; van der Harst *et al.*, 1994). Some of these mHA-specific CTL clones react only with hematopoietic-derived cells, suggesting tissue specificity (Faber *et al.*, 1996) and therefore potentially shared antigens on leukemia. In one study, GVHD correlated closely with differences in the minor antigen HA-1 in HLA-identical sibling transplants (Goulmy *et al.*, 1996). Expression of two human mHAs, identified as HA-1 and HA-2, is confined to hematopoietic tissues, and HA-2 was identified as a peptide derived from the non-filament-forming class I myosin family by using mHA-reactive CTL clones to screen peptide fractions eluted from MHC class I molecules (den Haan *et al.*, 1995). Although this methodology has successfully defined the first CTL alloantigens, it is labor intensive and it is unclear whether CTLs specific for any minor antigens identified thus far convey only leukemia-specific immunity without concomitant GVHD. Immunization of leukemia patients after allogeneic stem cell transplant (vaccination by proxy) with mHA might promote GVL and reduce GVHD if appropriate hematopoietic-restricted mHA could be targeted (such as HA-1 or HA-2). In a recent report of three CML patients who received DLIs after relapse, however, GVHD occurred in each patient concomitant to a rise in HA-1- or HA-2-specific CTLs and cytogenetic remission, albeit grade 2 or less (Marijt *et al.*, 2003). Perhaps more importantly, a practical limit of immunotherapy targeting these mHAs is that only 10% of individuals would be expected to have the relevant HA-1 alternative allele, and $<1\%$ would have the HA-2 alternative allele, which makes donor availability quite limiting.

An alternative immunologic method to determine leukemia-specific CTL epitopes has been applied to determine whether BCR/ABL fusion region peptides could be used to elicit CML-specific T-cell responses. Using this method, peptides are synthesized based on an educated guess strategy about which proteins are potential target antigens for a selective antileukemia CTL response. The proteins are then examined for short peptides that fit the binding motif of the most common HLA alleles. These peptides are then synthesized, HLA binding is confirmed, and peptide-specific CTL responses are elicited *in vitro*. Because BCR/ABL is present in nearly all Philadelphia chromosome-positive (Ph+) CML patients, it is thought to represent a potentially unique leukemia antigen. The ABL coding sequences upstream (5′) of exon II on chromosome 9 are translocated to chromosome 22 and fused in-frame with the BCR gene downstream (3′) of exon III, resulting in the most common chimeric mRNA transcript (b3a2), which is translated into a chimeric protein ($p210^{BCR/ABL}$). Translation of b3a2 mRNA results in the coding of a unique amino acid (lysine) within the fusion region. Some HLA-A2, HLA-A3, HLA-A22, and HLA-B8-restricted overlapping peptides inclusive of this lysine could bind to their respective HLA alleles and could

be used to elicit T-cell proliferative responses when the peptide was either pulsed onto HLA-matched normal antigen presenting cells or onto HLA-B8-positive CML cells (Bocchia *et al*., 1995, 1996; Dermime *et al*., 1995). However, when the b3a2 peptides were used to elicit b3a2-specific T-lymphocyte lines *in vitro*, the resulting T cells could not specifically lyse fresh CML cells that had not previously been pulsed with the peptide (Bocchia *et al*., 1996). This could be due to a low affinity of the peptide-specific CTLs or the peptide might not be processed or presented on CML cells. More recently, however, b3a2-specific CTLs were identified in the peripheral blood of chronic-phase CML patients, using soluble b3a2 peptide/MHC tetramers (Clark *et al*., 2001). Although the tetramer-positive CTLs from the patients were not examined for their ability to kill autologous CML target cells, b3a2-specific CTL elicited *in vitro* from healthy donors were able to kill CML cells. This suggests that BCR/ABL fusion peptides might also be targets of CTL immunity.

To adapt what has been learned about immunity against solid tumor antigens to the study of myeloid leukemia antigens, we studied myeloid-restricted proteins that are highly expressed in the leukemias relative to normal hematopoietic progenitors. Myeloid leukemias express a number of differentiation antigens associated with granule formation. An example of an aberrantly expressed tumor antigen in human leukemia is proteinase 3 (Pr3), a 26-kDa neutral serine protease that is stored in primary azurophil granules and is maximally expressed at the promyelocyte stage of myeloid differentiation (Chen *et al*., 1994; Muller-Berat, *et al*., 1994; Sturrock *et al*., 1992). Pr3 and two other azurophil granule proteins, neutrophil elastase and azurocidin, are coordinately regulated and the transcription factors PU.1 and C/EBPα, which are responsible for normal myeloid differentiation from stem cells to monocytes or granulocytes, are important in mediating their expression (Zhang *et al*., 2002). These transcription factors have been implicated in leukemogenesis (Behre *et al*., 1999), and Pr3 itself might also be important in maintaining a leukemia phenotype because Pr3 antisense oligonucleotides halt cell division and induce maturation of the HL-60 promyelocytic leukemia cell line (Bories *et al*., 1989). A more recent study has shown that neutrophil elastase, a serine protease that shares 54% sequence homology with Pr3, is required in a murine model of acute promyelocytic leukemia for PML-RARα-induced transformation (Lane and Ley, 2003), which supports the role of these serine proteases in the genesis of leukemia.

We have also studied another myeloid-restricted protein, myeloperoxidase (MPO), a heme protein synthesized during very early myeloid differentiation that constitutes the major component of neutrophil azurophilic granules. Produced as a single-chain precursor, MPO is subsequently cleaved into a light chain and a heavy chain. The mature MPO enzyme is composed of two light chains and two heavy chains (Borregaard and Cowland, 1997) that produce hypohalous acids central to the microbicidal activity of

neutrophils. Importantly, MPO and Pr3 are both overexpressed in a variety of myeloid leukemia cells, including 75% of CML patients, approximately 50% of AML patients, and approximately 30% of myelodysplastic syndrome patients (Dengler *et al.*, 1995).

What might be critical for our ability to identify T-cell antigens in these proteins is the observation that Pr3 is the target of autoimmune attack in Wegener's granulomatosis (Franssen *et al.*, 1996) and MPO is a target antigen in patients with small-vessel vasculitis (Borregaard and Cowland, 1997; Brouwer *et al.*, 1994; Franssen *et al.*, 2000). There is evidence for both T-cell and humoral immunity in patients with these diseases. Wegener's granulomatosis is associated with production of *cytoplasmic* antineutrophil cytoplasmic antibodies (cANCAs) with specificity for Pr3 (Williams *et al.*, 1994), whereas microscopic polyangiitis and Churg–Strauss syndrome are associated with the production of *perinuclear* ANCAs (pANCAs) with specificity for MPO (Jennette *et al.*, 2001; Savige *et al.*, 1999). T cells taken from affected individuals proliferate in response to crude extracts from neutrophil granules and to the purified proteins (Ballieux *et al.*, 1995; Brouwer *et al.*, 1994). These findings suggest that T-cell responses against these proteins might be relatively easy to elicit *in vitro*, using a deductive strategy to identify HLA-restricted peptide epitopes. Based on this hypothesis, we identified PR1, an HLA-A2.1-restricted nonamer derived from Pr3, as a leukemia-associated antigen (Molldrem *et al.*, 1996, 1997, 1999, 2000) by first searching the length of the protein using the HLA-A2.1-binding motif, the most prevalent HLA allele. Peptides predicted to have high-affinity binding to HLA-A2.1 were synthesized, confirmed to bind, and then used to elicit peptide-specific CTLs *in vitro* from healthy donor lymphocytes.

We have found that PR1 can be used to elicit CTL from HLA-A2.1+ normal donors *in vitro* and that T-cell immunity to PR1 is present in healthy donors and in many patients with CML who are in remission. These PR1-specific CTLs show preferential cytotoxicity toward allogeneic HLA-A2.1+ myeloid leukemia cells over HLA-identical normal donor marrow (Molldrem *et al.*, 1996). In addition, PR1-specific CTLs inhibit colony-forming unit granulocyte-macrophage (CFU-GM) from the marrow of CML patients but not CFU-GM from normal HLA-matched donors (Molldrem *et al.*, 1997), suggesting that leukemia progenitors are also targeted.

Using PR1/HLA-A2 tetramers to detect CTL specific for PR1 (PR1-CTL), we found a significant correlation with cytogenetic remission after treatment with interferon-α and the presence of PR1-CTL (Molldrem *et al.*, 2000). Somewhat surprisingly, PR1-CTL was also identified in the peripheral blood of some allogeneic transplant recipients who achieved molecular remission and who had converted to 100% donor chimerism. PR1/HLA-A2 tetramer-sorted allogeneic CTLs from patients in remission were able to kill CML cells but not normal bone marrow cells in 4-hr cytotoxicity assays,

thus demonstrating that the PR1 self-antigen is also recognized by allogeneic CTLs (Molldrem *et al*., 2000). These studies have established PR1 as a human leukemia-associated antigen and that PR1-specific CTLs contribute to the elimination of CML (Molldrem *et al*., 2000).

Recently, we found that another peptide, referred to as MY4, a nine-amino-acid peptide derived from MPO that binds to HLA-A2.1, can be used to elicit CTLs from HLA-A2.1+ normal donors *in vitro* (Braunschweig *et al*., 2000). MY4-specific CTLs show preferential cytotoxicity toward allogeneic HLA-A2.1+ myeloid leukemia cells over HLA-identical normal donor marrow (Braunschweig *et al*., 2000). MY4-specific CTLs also inhibit CFU-GM from the marrow of CML patients, but not CFU-GM from normal HLA-matched donors. Like PR1, MY4 is therefore a peptide antigen that can elicit leukemia-specific CTLs.

Several other HLA-restricted epitopes have been identified as potentially relevant leukemia-associated antigens. The Wilms' tumor antigen-1 (WT-1) has emerged as a very potent immunogen containing multiple unique HLA-restricted epitopes (Azuma *et al*., 2002; Bellantuono *et al*., 2002; Gao *et al*., 2000; Oka *et al*., 2000; Scheibenbogen *et al*., 2002), and it might also be a marker of minimal residual disease, because it is aberrantly expressed in both myeloid and lymphoid acute leukemia (Bergmann *et al*., 1997a,b; Brieger *et al*., 1995). Various surface molecules on leukemia cells, such as CD45, present on all hematopoietic cells, and CD33 and CD19 on myeloid and lymphoid cells, respectively, have also been studied by deductive means to uncover potentially immunogenic epitopes (Amrolia *et al*., 2002; Chen *et al*., 1995; Raptis *et al*., 1998). Although some HLA-restricted epitopes have been identified, it is unclear whether any of these are leukemia-associated antigens. The method of serologic screening of cDNA expression libraries with autologous serum (SEREX) has also been used to identify MAGE-1 and to confirm WT-1 as potential leukemia-associated antigens, although there might be some controversy on whether the MAGE proteins are expressed in leukemia blasts (Chambost *et al*., 2001).

In addition to these tissue-restricted epitopes in myeloid leukemias, other potential antigens that might be useful as target antigens in vaccine therapies include the idiotypes associated with lymphoid malignancies, such as immunoglobulin idiotypes (Hsu *et al*., 1996, 1997; Kim *et al*., 2003; Ruffini *et al*., 2002) and the CDR3 variable region associated with the TCR (Berger *et al*., 1998, 2001). Furthermore, antigens that are aberrantly expressed in most tumors, such as telomerase (Vonderheide *et al*., 1999) and CYP1B1 (Nagai *et al*., 2002; Xie *et al*., 2002), contain epitopes that are recognized by CTLs *in vitro*, which preferentially kill tumor cells but not normal cells. Other potential targets include antigens from virus-induced hematologic malignancies, such as the EBV antigens (Heslop *et al*., 1994a,b, 1996; Sing *et al*., 1997), which are highly immunogenic. EBV-specific CTLs administered as adoptive cellular immunotherapy can induce complete

remission in patients with EBV-related lymphoproliferative disorders after transplant, making this system ideal for understanding the nature of highly effective antigen-specific immune responses.

A cellular-based approach to induce leukemia-specific immunity has been studied by using leukemia cells or cytokine-modified leukemia as vaccines. Several groups have shown that cytokines that are used to elicit dendritic cells (DCs) *in vitro*, such as GM-CSF and IL-4, and cytokines used to mature the DCs, such as TFN-α or IL-1, can also be used to alter the phenotype of acute and chronic myeloid leukemia cells (Choudhury *et al.*, 1997; Smit *et al.*, 1997; Wang *et al.*, 1998; Westers *et al.*, 2003; Woiciechowsky *et al.*, 2001). These altered leukemia cells have been used as antigen-presenting cells (APCs) to elicit leukemia-specific CTL responses *in vitro*, which has provided a basis for clinical trials using these leukemia-derived APCs. However, other investigators have noted important deficits in these leukemia-derived APCs, which might adversely affect their ability to induce potent immune responses *in vivo* (Dong *et al.*, 2003; Lindner *et al.*, 2003; Rezvany *et al.*, 2001). Preliminary results of a recent clinical trial suggest that this might be important because antigen-specific immune responses were noted in the absence of any clinical responses after leukemia-derived APC vaccination of CML patients (Takahashi *et al.*, 2003).

An alternative strategy employs the use of gene-modified bystander cells or leukemia cells that secrete cytokines such as GM-CSF. A murine model has shown the effectiveness of this approach after autologous BMT is used to achieve a minimal disease state (Borrello *et al.*, 2000), and clinical trials are currently underway to use GM-CSF gene-modified leukemia cell lines as a cellular vaccine to induce leukemia-specific T-cell immunity. This approach might enhance cross-presentation of antigens derived from the modified leukemia cell lines (Huang *et al.*, 1996), inducing a broader repertoire of leukemia-specific T cells.

IV. Clinical Vaccine Trials with Leukemia-Associated Antigens

Besides peptides derived from the idiotypes of lymphoid malignancies, peptides derived from the BCR/ABL fusion transcript have undergone perhaps the most extensive clinical testing. The results of a previous Phase I trial in CML patients showed that although a combination of fusion region-derived peptides was safe when administered subcutaneously and immune responses could also be measured by ELISPOT after vaccination, meaningful clinical responses were not observed. More recently, the same group at the Memorial Sloan-Kettering Cancer Center reported on 14 patients in a Phase II study who were given five injections of six peptides over 10 weeks. A decrease in the percentage of Ph+ cells was noted in four patients in previous

hematologic remission; three were also receiving interferon, and one was receiving imatinib mesylate (Panilla *et al.*, 2003). Transient PCR negativity was also noted in a few additional patients, although these patients had received prior allogeneic transplant and donor lymphocyte infusions.

Because the heat shock protein 70 (HSP70) is associated with antigenic peptides and is involved in chaperoning these peptides in the MHC-1 antigen processing pathway, autologous cellular extracts containing HSP70–peptide complexes have been studied as a vaccine in chronic-phase CML patients. At the University of Connecticut, HSP70–peptide complexes purified from leukapheresis products were administered to CML patients who had not yet achieved a major cytogenetic response after 6 months of imatinib mesylate treatment. Of the first five patients who completed all eight weekly subcutaneous injections, major cytogenetic responses were noted in all five, and only mild cutaneous reactions were seen (Li *et al.*, 2003). Importantly, ELISPOT responses to the vaccine preparation were also noted in some patients.

Although results from both the HSP70 and the BCR/ABL vaccine studies are important because they demonstrate that the vaccines can induce immune responses in CML patients and clinical responses are possible, true cause and effect has not been established because patients in both studies concomitantly received other therapies. It is therefore not possible to determine with certainty whether the vaccines contributed to the cytogenetic or molecular responses. For instance, major cytogenetic remissions after imatinib treatment continue to be observed in more than 30% of patients beyond 6 months of therapy, and small fluctuations in the percentage of Ph+ cells might be seen throughout treatment.

Clinical studies are also being conducted in Germany and Japan, using WT-1 peptides specific for the HLA-A2 and-A24 alleles as vaccines. A case report detailing responses in two patients with MDS and secondary AML were recently reported by investigators in Osaka, Japan (Oka *et al.*, 2003). These two patients received a single intradermal HLA-A24-restricted WT-1 peptide vaccination, and in one patient WT-1-specific CTL increased within 48 h from 1–6% of all CD8+ T lymphocytes by tetramer analysis. Steroids were required in both patients to treat severe cytopenia that developed after vaccination, and sepsis occurred in one patient. Nevertheless, blasts initially decreased in both the patients, suggesting that WT-1 might induce antileukemia immunity.

In addition, peptides derived from the hTERT telomerase protein, which is widely overexpressed in leukemia, hematopoietic progenitors, and most solid tumors, have also been seen in Phase I/II trials at the University of Pennsylvania and elsewhere. Preliminary results from many of these studies might be available by 2005. Interestingly, in a recent study, T cells that recognized hTERT-derived peptides *in vitro* showed promiscuous recognition of the same peptides in the context of different HLA alleles, consistent with results of experiments using the PR1 peptide (Schroers *et al.*, 2003).

This suggests that hTERT peptide-based vaccines might be used to induce immunity across different HLA alleles, opening the possibility to treat more patients with single peptides.

The PR1 peptide is also undergoing Phase I/II study, and the single peptide epitope is combined with incomplete Freund's adjuvant and GM-CSF and administered every 3 weeks for three vaccinations. Patients with AML, CML, and MDS are eligible, and the first 15 patients are fully evaluable. To judge whether a clinical response was because of the vaccine, eligible patients were required to have progression, relapse, or second or more than second CR (AML patients only) prior to vaccination. Immune responses, measured using PR1/HLA-A2 tetramers, were noted in eight of the patients and clinical responses in five of those patients. Notably, the TCR avidity of the vaccine-induced PR1-specific CTLs was higher in the clinical responders than in the nonresponders, and durable molecular remissions were noted in two refractory AML patients followed from 11 months to 3 years.

V. Conclusion

We are beginning to learn more about the nature of the antigens targeted by T cells that mediate autologous antileukemia immunity and those that are targets of the allogeneic GVL effect. Some self-antigens might also be the targets of alloreactive CTL, as we have shown for PR1. As more antigens are identified, logical immunotherapy strategies such as vaccines or adoptive cellular therapies can be tested in patients. Obstacles to this approach remain, however. We must identify which of the hematopoietic tissue-restricted peptides are recognized by T cells and improve our understanding of the nature of peripheral T-cell tolerance in order to break immune tolerance to certain peptide determinants without causing potentially destructive autoimmunity. In the future, allogeneic stem cell transplantation is likely to evolve as a platform for delivering antigen-specific adoptive cellular therapies and for posttransplant vaccination strategies in which donor CTLs are elicited in the recipient. Both autologous and allogeneic transplant might reset T-cell homeostasis and allow a more complete T-cell repertoire to emerge postgrafting that could be further expanded selectively against tumor antigens by vaccination posttransplant, as in a vaccination by proxy therapy in the case of allogeneic transplantation.

References

Altman, J. D., Moss, P. A. H., Goulder, P. J. R., Barouch, D. H., McHeyzer-Williams, M. G., Bell, J. I., McMichael, A. J., and Davis, M. M. (1996). Phenotypic analysis of antigen-specific T lymphocytes. *Science* **274**, 94–96.

Amrolia, P. J., Reid, S. D., Gao, L., Schultheis, B., Dotti, G., Brenner, M. K., Melo, J. V., Goldman, J. M., and Stauss, H. J. (2003). Allorestricted cytotoxic T cells specific for human CD45 show potent antileukemic activity. *Blood* **101,** 1007–1014.

Antin, J. H. (1993). Graft-versus-leukemia: No longer an epiphenomenon. *Blood* **82,** 2273–2277.

Azuma, T., Makita, M., Ninomiya, K., Fujita, S., Harada, M., and Yasukawa, M. (2002). Identification of a novel WT1-derived peptide which induces human leucocyte antigen-A24-restricted anti-leukaemia cytotoxic T lymphocytes. *Br. J. Haematol.* **116,** 601–603.

Ballieux, B. E., van der Burg, S. H., Hagen, E. C., van der Woude, F. J., Melief, C. J., and Daha, M. R. (1995). Cell-mediated autoimmunity in patients with Wegener's granulomatosis (WG). *Clin. Exp. Immunol.* **100,** 186–193.

Behre, G., Zhang, P., Zhang, D. E., and Tenen, D. G. (1999). Analysis of the modulation of transcriptional activity in myelopoiesis and leukemogenesis. *Methods* **17,** 231–237.

Bellantuono, I., Gao, L., Parry, S., Marley, S., Dazzi, F., Apperley, J., Goldman, J. M., and Stauss, H. J. (2002). Two distinct HLA-A0201-presented epitopes of the Wilms tumor antigen 1 can function as targets for leukemia-reactive CTL. *Blood* **100,** 3835–3837.

Berger, C. L., Longley, B. J., Imaeda, S., Christensen, I., Heald, P., and Edelson, R. L. (1998). Tumor-specific peptides in cutaneous T-cell lymphoma: association with class I major histocompatibility complex and possible derivation from the clonotypic T-cell receptor. *Int. J. Cancer* **76,** 304–311.

Berger, C. L., Longley, J., Hanlon, D., Girardi, M., and Edelson, R. (2001). The clonotypic T cell receptor is a source of tumor-associated antigens in cutaneous T cell lymphoma. *Ann. N. Y. Acad. Sci.* **941,** 106–122.

Bergmann, L., Maurer, U., and Weidmann, E. (1997). Wilms tumor gene expression in acute myeloid leukemias. *Leuk Lymphoma* **25,** 435–443.

Bergmann, L., Miething, C., Maurer, U., Brieger, J., Karakas, T., Weidmann, E., and Hoelzer, D. (1997). High levels of Wilms' tumor gene (wt1) mRNA in acute myeloid leukemias are associated with a worse long-term outcome. *Blood* **90,** 1217–1225.

Bocchia, M., Wentworth, P. A., Southwood, S., Sidney, J., McGraw, K., Scheinberg, D. A., and Sette, A. (1995). Specific binding of leukemia oncogene fusion protein peptides to HLA class I molecules. *Blood* **85,** 2680–2684.

Bocchia, M., Korontsvit, T., Xu, Q., Mackinnon, S., Yang, S. Y., Sette, A., and Scheinberg, D. A. (1996). Specific human cellular immunity to bcr-abl oncogene-derived peptides. *Blood* **87,** 3587–3592.

Bories, D., Raynal, M. C., Solomon, D. H., Darzynkiewicz, Z., and Cayre, Y. E. (1989). Down-regulation of a serine protease, myeloblastin, causes gwoth arrest and differentiation of promyelocytic leukemia cells. *Cell* **59,** 959.

Borregaard, N., and Cowland, J. B. (1997). Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* **89,** 3503–3521.

Borrello, I., Sotomayor, E. M., Rattis, F. M., Cooke, S. K., Gu, L., and Levitsky, H. I. (2000). Sustaining the graft-versus-tumor effect through posttransplant immunization with granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing tumor vaccines. *Blood* **95,** 3011–3019.

Braunschweig, I., Wang, C., and Molldrem, J. (2000). Cytotoxic T lymphocytes (CTL) specific for myeloperoxidase-derived HLA-A2-restricted peptides specifically lyse AML and CML cells. *Blood* **96,** 3291.

Brieger, J., Weidmann, E., Maurer, U., Hoelzer, D., Mitrou, P. S., and Bergmann, L. (1995). The Wilms' tumor gene is frequently expressed in acute myeloblastic leukemias and may provide a marker for residual blast cells detectable by PCR. *Ann. Oncol.* **6,** 811–816.

Brouwer, E., Stegeman, C. A., Huitema, M. G., Limburg, P. C., and Kallenberg, C. G. (1994). T cell reactivity to proteinase 3 and myeloperoxidase in patients with Wegener's granulomatosis (WG). *Clin. Exp. Immunol.* **98,** 448–453.

Callan, M. F., Tan, L., Annels, N., Ogg, G. S., Wilson, J. D., O'Callaghan, C. A., Steven, N., McMichael, A. J., and Rickinson, A. B. (1998). Direct visualization of antigen-specific CD8+ T cells during the primary immune response to Epstein-Barr virus *In vivo*. *J. Exp. Med.* **187,** 1395–1402.

Chambost, H., van Baren, N., Brasseur, F., and Olive, D. (2001). MAGE-A genes are not expressed in human leukemias. *Leukemia* **15,** 1769–1771.

Chen, T., Meier, R., Ziemiecki, A., Fey, M. F., and Tobler, A. (1994). Myeloblastin/proteinase 3 belongs to the set of negatively regulated primary response genes expressed during in vitro myeloid differentiation. *Biochem. Biophys. Res. Commun.* **200,** 1130–1135.

Chen, W., Chatta, K., Rubin, W., Liggitt, D. H., Kusunoki, Y., Martin, P., and Cheever, M. A. (1995). Polymporphic segments of CD45 can serve as targets for GVHD and GVL responses. *Blood* **86**(Suppl. 1), 157a.

Choudhury, A., Gajewski, J. L., Liang, J. C., Popat, U., Claxton, D. F., Kliche, K. O., Andreeff, M., and Champlin, R. E. (1997). Use of leukemic dendritic cells for the generation of antileukemic cellular cytotoxicity against Philadelphia chromosome-positive chronic myelogenous leukemia. *Blood* **89,** 1133–1142.

Clark, R. E., Dodi, I. A., Hill, S. C., Lill, J. R., Aubert, G., Macintyre, A. R., Rojas, J., Bourdon, A., Bonner, P. L., Wang, L. *et al.* (2001). Direct evidence that leukemic cells present HLA-associated immunogenic peptides derived from the BCR-ABL b3a2 fusion protein. *Blood* **98,** 2887–2893.

Collins, R. H., Jr., Shpilberg, O., Drobyski, W. R., Porter, D. L., Giralt, S., Champlin, R., Goodman, S. A., Wolff, S. N., Hu, W., Verfaillie, C. *et al.* (1997). Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J. Clin. Oncol.* **15,** 433–444.

den Haan, J. M. M., Sherman, N. E., Blokland, E., Huczko, E., Koning, F., Drijfhout, J. W., Skipper, J., Shabanowitz, J., Hunt, D. F., Engelhard, V. H. *et al.* (1995). Identification of a graft versus host disease-associated human minor histocompatibility antigen. *Science* **268,** 1476–1480.

den Haan, J. M. M., Meadows, L. M., Wang, W., Pool, J., Blokland, E., Bishop, T., Reinhardus, C., Shabanowitz, J., Offringa, R., Hunt, D. F. *et al.* (1998). The minor histocompatibility antigen HA-1: A diallelic gene with a single amino acid polymorphism. *Science* **279,** 1054–1057.

Dengler, R., Munstermann, U., al-Batran, S., Hausner, I., Faderl, S., Nerl, C., and Emmerich, B. (1995). Immunocytochemical and flow cytometric detection of proteinase 3 (myeloblastin) in normal and leukaemic myeloid cells. *Br. J. Haematol.* **89,** 250–257.

Dermime, S., Molldrem, J., Parker, K. C., Jiang, Y. Z., Mavroudis, D., Hensel, N., Couriel, D., Mahoney, M., Coligan, J. E., and Barrett, A. J. (1995). Human CD8+ T lymphocytes recognize the fusion region of bcr/abl hybrid protein present in chronic myelogenous leukemia. *Blood* **86**(Suppl. 1), 158a.

Dolstra, H., Fredrix, H., Preijers, F., Goulmy, E., Figdor, C. G., de Witte, T. M., and van de Wiel-van Kemenade, E. (1997). Recognition of a B cell leukemia-associated minor histocompatibility antigen by CTL. *J. Immunol.* **158,** 560–565.

Dong, R., Cwynarski, K., Entwistle, A., Marelli-Berg, F., Dazzi, F., Simpson, E., Goldman, J. M., Melo, J. V., Lechler, R. I., Bellantuono, I. *et al.* (2003). Dendritic cells from CML patients have altered actin organization, reduced antigen processing, and impaired migration. *Blood* **101,** 3560–3567.

Faber, L. M., van der Hoeven, J., Goulmy, E., Hooftman-den Otter, A. L., van Luxemburg-Heijs, S. A., Willemze, R., and Falkenburg, J. H. (1995). Recognition of clonogenic leukemic cells, remission bone marrow and HLA-identical donor bone marrow by CD8+ or CD4+ minor histocompatibility antigen-specific cytotoxic T lymphocytes. *J. Clin. Invest.* **96,** 877–883.

Faber, L. M., van Luxemburg-Heijs, S. A., Veenhof, W. F., Willemze, R., and Falkenburg, J. H. (1995). Generation of CD4+ cytotoxic T-lymphocyte clones from a patient with severe graft-versus-host disease after allogeneic bone marrow transplantation: Implications for graft-versus-leukemia reactivity. *Blood* **86,** 2821–2828.

Faber, L. M., van Luxemburg-Heijs, S. A., Rijnbeek, M., Willemze, R., and Falkenburg, J. H. (1996). Minor histocompatibility antigen-specific, leukemia-reactive cytotoxic T cell clones can be generated *in vitro* without *in vivo* priming using chronic myeloid leukemia cells as stimulators in the presence of alpha-interferon. *Biol. Blood Marrow Transplant* **2,** 31–36.

Franssen, C. F., Cohen Tervaert, J. W., Stegeman, C. A., and Kallenberg, C. G. (1996). c-ANCA as a marker of Wegener's disease. *Lancet* **347,** 116; discussion 118.

Franssen, C. F., Stegeman, C. A., Kallenberg, C. G., Gans, R. O., De Jong, P. E., Hoorntje, S. J., and Tervaert, J. W. (2000). Antiproteinase 3- and antimyeloperoxidase-associated vasculitis. *Kidney Int.* **57,** 2195–2206.

Gao, L., Bellantuono, I., Elsasser, A., Marley, S. B., Gordon, M. Y., Goldman, J. M., and Stauss, H. J. (2000). Selective elimination of leukemic CD34(+) progenitor cells by cytotoxic T lymphocytes specific for WT1. *Blood* **95,** 2198–2203.

Giralt, S. A., and Kolb, H. J. (1996). Donor lymphocyte infusions. *Curr. Opin. Oncol.* **8,** 96–102.

Goulmy, E., Schipper, R., Pool, J., Blokland, E., Falkenburg, J. H., Vossen, J., Grathwohl, A., Vogelsang, G. B., van Houwelingen, H. C., and van Rood, J. J. (1996). Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. *N. Engl. J. Med.* **334,** 281–285.

Heslop, H. E., Brenner, M. K., and Rooney, C. M. (1994). Donor T cells to treat EBV-associated lymphoma. *N. Engl. J. Med.* **331,** 679–680.

Heslop, H. E., Brenner, M. K., Rooney, C., Krance, R. A., Roberts, W. M., Rochester, R., Smith, C. A., Turner, V., Sixbey, J., Moen, R. *et al.* (1994). Administration of neomycin-resistance-gene-marked EBV-specific cytotoxic T lymphocytes to recipients of mismatched-related or phenotypically similar unrelated donor marrow grafts. *Hum. Gene. Ther.* **5,** 381–397.

Heslop, H. E., Ng, C. Y., Li, C., Smith, C. A., Loftin, S. K., Krance, R. A., Brenner, M. K., and Rooney, C. M. (1996). Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. *Nat. Med.* **2,** 551–555.

Hsu, F. J., Benike, C., Fagnoni, F., Liles, T. M., Czerwinski, D., Taidi, B., Engleman, E. G., and Levy, R. (1996). Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nat. Med.* **2,** 52–58.

Hsu, F. J., Caspar, C. B., Czerwinski, D., Kwak, L. W., Liles, T. M., Syrengelas, A., Taidi-Laskowski, B., and Levy, R. (1997). Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma–long-term results of a clinical trial. *Blood* **89,** 3129–3135.

Huang, A. Y., Bruce, A. T., Pardoll, D. M., and Levitsky, H. I. (1996). In vivo cross-priming of MHC class I-restricted antigens requires the TAP transporter. *Immunity* **4,** 349–355.

Jennette, J. C., Thomas, D. B., and Falk, R. J. (2001). Microscopic polyangiitis (microscopic polyarteritis). *Semin. Diagn. Pathol.* **18,** 3–13.

Kim, S. B., Baskar, S., and Kwak, L. W. (2003). *In vitro* priming of myeloma antigen-specific allogeneic donor T cells with idiotype pulsed dendritic cells. *Leuk Lymphoma* **44,** 1201–1208.

Kolb, H. J., Schattenberg, A., Goldman, J. M., Hertenstein, B., Jacobsen, N., Arcese, W., Ljungman, P., Ferrant, A., Verdonck, L., Niederwieser, D. *et al.* (1995). Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. European

Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. *Blood* **86,** 2041–2050.

Kolb, H. J., Mittermuller, J., Holler, E., Thalmeier, K., and Bartram, C. R. (1996). Graft-versus-host reaction spares normal stem cells in chronic myelogenous leukemia. *Bone Marrow Transplant* **17,** 449–452.

Kolb, H. J., and Holler, E. (1997). Adoptive immunotherapy with donor lymphocyte transfusions. *Curr. Opin. Oncol.* **9,** 139–145.

Komanduri, K. V., Viswanathan, M. N., Wieder, E. D., Schmidt, D. K., Bredt, B. M., Jacobson, M. A., and McCune, J. M. (1998). Restoration of cytomegalovirus-specific CD4+ T-lymphocyte responses after ganciclovir and highly active antiretroviral therapy in individuals infected with HIV-1. *Nat. Med.* **4,** 953–956.

Komanduri, K. V., Donahoe, S. M., Moretto, W. J., Schmidt, D. K., Gillespie, G., Ogg, G. S., Roederer, M., Nixon, D. F., and McCune, J. M. (2001). Direct measurement of CD4+ and CD8+ T-cell responses to CMV in HIV-1-infected subjects. *Virology* **279,** 459–470.

Lane, A. A., and Ley, T. J. (2003). Neutrophil elastase cleaves PML-RARa and is important for the development of acute promyelocytic leukemia in mice. *Cell* **115,** 305–318.

Lee, P. P., Yee, C., Savage, P. A., Fong, L., Brockstedt, D., Weber, J. S., Johnson, D., Swetter, S., Thompson, J., Greenberg, P. D. *et al.* (1999). Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. *Nat. Med.* **5,** 677–685.

Li, Z., Qiao, Y., Laska, E., Julko, J., Bona, R., Gaffney, J., Hegde, U., Moyo, P., and Srivastava, P. (2003). "Combination of imatinib mesylate with autologous leukocyte-derived heat shock protein 70 vaccine for chronic myelogenous leukemia." American Society of Clinical Oncology. p. 664. San Francisco, CA.

Lindner, I., Kharfan-Dabaja, M. A., Ayala, E., Kolonias, D., Carlson, L. M., Beazer-Barclay, Y., Scherf, U., Hnatyszyn, J. H., and Lee, K. P. (2003). Induced dendritic cell differentiation of chronic myeloid leukemia blasts is associated with down-regulation of BCR-ABL. *J. Immunol.* **171,** 1780–1791.

Marijt, W. A., Heemskerk, M. H., Kloosterboer, F. M., Goulmy, E., Kester, M. G., van der Hoorn, M. A., van Luxemburg-Heys, S. A., Hoogeboom, M., Mutis, T., Drijfhout, J. W. *et al.* (2003). Hematopoiesis-restricted minor histocompatibility antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. *Proc. Natl. Acad. Sci. USA* **100,** 2742–2747.

Molldrem, J., Dermime, S., Parker, K., Jiang, Y. Z., Mavroudis, D., Hensel, N., Fukushima, P., and Barrett, A. J. (1996). Targeted T-cell therapy for human leukemia: Cytotoxic T lymphocytes specific for a peptide derived from proteinase 3 preferentially lyse human myeloid leukemia cells. *Blood* **88,** 2450–2457.

Molldrem, J. J., Clave, E., Jiang, Y. Z., Mavroudis, D., Raptis, A., Hensel, N., Agarwala, V., and Barrett, A. J. (1997). Cytotoxic T lymphocytes specific for a nonpolymorphic proteinase 3 peptide preferentially inhibit chronic myeloid leukemia colony-forming units. *Blood* **90,** 2529–2534.

Molldrem, J. J., Lee, P. P., Wang, C., Champlin, R. E., and Davis, M. M. (1999). A PR1-human leukocyte antigen-A2 tetramer can be used to isolate low-frequency cytotoxic T lymphocytes from healthy donors that selectively lyse chronic myelogenous leukemia. *Cancer Res.* **59,** 2675–2681.

Molldrem, J. J., Lee, P. P., Wang, C., Felio, K., Kantarjian, H. M., Champlin, R. E., and Davis, M. M. (2000). Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. *Nat. Med.* **6,** 1018–1023.

Muller-Berat, N., Minowada, J., Tsuji-Takayama, K., Drexler, H., Lanotte, M., Wieslander, J., and Wiik, A. (1994). The phylogeny of proteinase 3/myeloblastin, the autoantigen in Wegener's granulomatosis, and myeloperoxidase as shown by immunohistochemical studies on human leukemic cell lines. *Clin. Immunol. Immunopathol.* **70,** 51–59.

Murata, M., Warren, E. H., and Riddell, S. R. (2003). A human minor histocompatibility antigen resulting from differential expression due to a gene deletion. *J. Exp. Med.* **197,** 1279–1289.

Nagai, F., Hiyoshi, Y., Sugimachi, K., and Tamura, H. O. (2002). Cytochrome P450 (CYP) expression in human myeloblastic and lymphoid cell lines. *Biol. Pharm. Bull.* **25,** 383–385.

Oka, Y., Elisseeva, O. A., Tsuboi, A., Ogawa, H., Tamaki, H., Li, H., Oji, Y., Kim, E. H., Soma, T., Asada, M. *et al.* (2000). Human cytotoxic T-lymphocyte responses specific for peptides of the wild-type Wilms' tumor gene (WT1) product. *Immunogenetics* **51,** 99–107.

Oka, Y., Tsuboi, A., Murakami, M., Hirai, M., Tominaga, N., Nakajima, H., Elisseeva, O. A., Masuda, T., Nakano, A., Kawakami, M. *et al.* (2003). Wilms tumor gene peptide-based immunotherapy for patients with overt leukemia from myelodysplastic syndrome (MDS) or MDS with myelofibrosis. *Int. J. Hematol.* **78,** 56–61.

Panilla, J., Cathcart, K., Korontsvit, T., Schwartz, J. D., Zakheleva, E., Papadopoulos, E., and Scheinberg, D. A. (2003). "A phase II trial of patients with CML using a multivalent BCR-ABL oncogene product fusion peptide vaccine." American Society of Clinical Oncology. p. 674. San Francisco, CA.

Raptis, A., Clave, E., Mavroudis, D., Molldrem, J., Van Rhee, F., and Barrett, A. J. (1998). Polymorphism in CD33 and CD34 genes: A source of minor histocompatibility antigens on haemopoietic progenitor cells? *Br. J. Haematol.* **102,** 1354–1358.

Rezvany, M. R., Jeddi-Tehrani, M., Biberfeld, P., Soderlund, J., Mellstedt, H., Osterborg, A., and Rabbani, H. (2001). Dendritic cells in patients with non-progressive B-chronic lymphocytic leukaemia have a normal functional capability but abnormal cytokine pattern. *Br. J. Haematol.* **115,** 263–271.

Ruffini, P. A., Neelapu, S. S., Kwak, L. W., and Biragyn, A. (2002). Idiotypic vaccination for B-cell malignancies as a model for therapeutic cancer vaccines: From prototype protein to second generation vaccines. *Haematologica* **87,** 989–1001.

Savige, J., Gillis, D., Benson, E., Davies, D., Esnault, V., Falk, R. J., Hagen, E. C., Jayne, D., Jennette, J. C., Paspaliaris, B. *et al.* (1999). International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA). *Am. J. Clin. Pathol.* **111,** 507–513.

Scheibenbogen, C., Letsch, A., Thiel, E., Schmittel, A., Mailaender, V., Baerwolf, S., Nagorsen, D., and Keilholz, U. (2002). CD8 T-cell responses to Wilms tumor gene product WT1 and proteinase 3 in patients with acute myeloid leukemia. *Blood* **100,** 2132–2137.

Schroers, R., Shen, L., Rollins, L., Rooney, C. M., Slawin, K., Sonderstrup, G., Huang, X. F., and Chen, S. Y. (2003). Human Telomerase Reverse Transcriptase-Specific T-Helper Responses Induced by Promiscuous Major Histocompatibility Complex Class II-Restricted Epitopes. *Clin. Cancer Res.* **9,** 4743–4755.

Sing, A. P., Ambinder, R. F., Hong, D. J., Jensen, M., Batten, W., Petersdorf, E., and Greenberg, P. D. (1997). Isolation of Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes that lyse Reed-Sternberg cells: implications for immune-mediated therapy of EBV+ Hodgkin's disease. *Blood* **89,** 1978–1986.

Smit, W. M., Rijnbeek, M., van Bergen, C. A., de Paus, R. A., Vervenne, H. A., van de Keur, M., Willemze, R., and Falkenburg, J. H. (1997). Generation of dendritic cells expressing bcr-abl from CD34-positive chronic myeloid leukemia precursor cells. *Hum. Immunol.* **53,** 216–223.

Sturrock, A. B., Franklin, K. F., Rao, G., Marshall, B. C., Rebentisch, M. B., Lemons, R. S., and Hoidal, J. R. (1992). Structure, chromosomal assignment, and expression of the gene for proteinase 3. *J. Biol. Chem.* **267**(29), 21193–21199.

Takahashi, T., Tanaka, Y., Nieda, M., Azuma, T., Chiba, S., Juji, T., Shibata, Y., and Hirai, H. (2003). Dendritic cell vaccination for patients with chronic myelogenous leukemia. *Leuk. Res.* **27,** 795–802.

van der Harst, D., Goulmy, E., Falkenburg, J. H., Kooij-Winkelaar, Y. M., van Luxemburg-Heijs, S. A., Goselink, H. M., and Brand, A. (1994). Recognition of minor histocompatibility antigens on lymphocytic and myeloid leukemic cells by cytotoxic T-cell clones. *Blood* **83,** 1060–1066.

van Rhee, F., Lin, F., Cullis, J. O., and Goldman, J. (1994). Relapse of chronic myeloid leukemia after allogeneic bone marrow transplant: The case for giving donor lymphocyte transfusions before the onset of hematological relapse. *Blood* **83,** 3377–3383.

Vonderheide, R. H., Hahn, W. C., Schultze, J. L., and Nadler, L. M. (1999). The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. *Immunity* **10,** 673–679.

Wang, J., Saffold, S., Cao, X., Krauss, J., and Chen, W. (1998). Eliciting T cell immunity against poorly immunogenic tumors by immunization with dendritic cell-tumor fusion vaccines. *J. Immunol.* **161,** 5516–5524.

Warren, E. H., Greenberg, P. D., and Riddell, S. R. (1998). Cytotoxic T-lymphocyte-defined human minor histocompatibility antigens with a restricted tissue distribution. *Blood* **91,** 2197–2207.

Westers, T. M., Stam, A. G., Scheper, R. J., Regelink, J. C., Nieuwint, A. W., Schuurhuis, G. J., van de Loosdrecht, A. A., and Ossenkoppele, G. J. (2003). Rapid generation of antigen-presenting cells from leukaemic blasts in acute myeloid leukaemia. *Cancer Immunol. Immunother.* **52,** 17–27.

Williams, R. C., Staud, R., Malone, C. C., Payabyab, J., Byres, L., and Underwood, D. (1994). Epitopes on proteinase 3 recognized by antibodies from patients with Wegener's granulomatosis. *J. Immunol.* **152,** 4722–4732.

Woiciechowsky, A., Regn, S., Kolb, H. J., and Roskrow, M. (2001). Leukemic dendritic cells generated in the presence of FLT3 ligand have the capacity to stimulate an autologous leukemia-specific cytotoxic T cell response from patients with acute myeloid leukemia. *Leukemia* **15,** 246–255.

Xie, H. J., Lundgren, S., Broberg, U., Finnstrom, N., Rane, A., and Hassan, M. (2002). Effect of cyclophosphamide on gene expression of cytochromes p450 and beta-actin in the HL-60 cell line. *Eur. J. Pharmacol.* **449,** 197–205.

Zhang, P., Nelson, E., Radomska, H. S., Iwasaki-Arai, J., Akashi, K., Friedman, A. D., and Tenen, D. G. (2002). Induction of granulocytic differentiation by 2 pathways. *Blood* **99,** 4406–4412.

John M. Timmerman

Division of Hematology/Oncology
University of California, Los Angeles
Center for Health Sciences 42-121
Los Angeles, California 90095-1678

Therapeutic Idiotype Vaccines for Non-Hodgkin's Lymphoma

I. Chapter Overview

The immune system of the lymphoma-bearing host can be manipulated to recognize the tumor-specific immunoglobulin (idiotype) sequences expressed by the tumor. Observations from Phase I/II clinical studies of therapeutic idiotype vaccination in patients with follicular lymphoma have included (1) induction of anti-idiotype antibody and T-cell responses that correlate with improved survival, (2) clearance of residual tumor cells from the peripheral blood to achieve molecular remission, and (3) durable objective tumor regressions, particularly using idiotype-loaded dendritic cells. These studies have provided rationale for two large randomized, controlled trials of idiotype vaccination following initial cytoreductive chemotherapy in follicular lymphoma. As the results of these Phase III trials are eagerly awaited, a host of new second-generation idiotype vaccines are undergoing

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

development, aimed at improving both the potency and practicality of this individualized approach to lymphoma therapy.

II. Introduction

Although passive monoclonal antibody treatments have proven to be potent antilymphoma agents, they represent only part of the potential for immunotherapeutic strategies against lymphoid cancers. Elicitation of an active host immune response against tumor can offer several advantages over passively administered antibodies. An adaptive immune response can display remarkable specificity for tumor-associated or tumor-specific antigens, which in theory should spare normal tissues from damage. An active host antitumor response can also include polyclonal antibody (B-cell) and T-cell responses, each of which encompasses immunologic memory to carry our ongoing surveillance against tumor cells. Together, these features should serve to decrease the chance of tumor escape from recognition and destruction. Despite their usual resistance to cure with conventional cytotoxic agents, B-cell lymphomas, particularly those of the follicular subtype, appear to be among the most immune responsive of all human cancers. This is manifested in their capacity for spontaneous regression (Horning and Rosenberg, 1984; Krikorian *et al*., 1980), their occasional responsiveness to nonspecific immune activators such as bacillus Calmette-Guerin (Anonymous), interleukin-2 (Snozl, 1995), and IL-12 (Younes *et al*., 2002), and high rates of response to monoclonal antibody therapies (Davis *et al*., 1998; McLaughlin *et al*., 1998; Miller *et al*., 1982) and tumor-specific vaccines (Bendandi *et al*., 1999; Hsu *et al*., 1997; Kwak *et al*., 1992; Timmerman *et al*., 2002a). Thus, tumor–host immune system interactions have the potential to profoundly influence lymphoma growth, and B-cell lymphoma should be considered an important testing ground for new immunotherapeutic approaches to cancer treatment.

This chapter focuses on therapeutic vaccines targeting lymphoma idiotype, since these are the best studied of all lymphoma vaccines. Other lymphoma immunotherapies such as systemic cytokines, tumor cell-based vaccines, and monoclonal antibodies have been recently reviewed (Timmerman, 2003) or are covered elsewhere in this volume.

III. Development of the Idiotype Vaccine Approach

A. Immunologic Basis for Idiotype Vaccines

B-cell lymphomas usually express clonal immunoglobulin whose variable regions comprise a unique set of immunogenic determinants known as the idiotype (Id) (Timmerman and Levy, 2000a). Each normal B cell expresses an immunoglobulin with unique variable region sequences that

are used to bind antigen. These protein sequences are formed by the stochastic recombination of gene segments during B-cell development, yielding one of many millions of possible combinations. When a B cell undergoes malignant transformation, these unique sequences are maintained by the tumor cell clone, and can thus serve as a truly tumor-specific antigen. The cost for this level of specificity when targeting tumor idiotype is high; however, a custom-made reagent must be created for each patient.

At the present time, idiotype is the best-characterized tumor antigen available as a target for the active immunotherapy of lymphomas. Human studies were first made possible by the finding that large quantities of the patient-specific tumor immunoglobulin (idiotype) could be obtained by the technique of rescue hybridization, in which a patient's lymphoma cells are fused with myeloma cells yielding stable hybrid cell lines secreting the tumor-specific idiotype protein (Fig. 1) (Levy and Dilley, 1978). Idiotype was first validated as a susceptible target antigen for human B-cell lymphoma in studies employing custom-made murine anti-idiotype monoclonal antibodies for the treatment of follicular lymphoma (Miller *et al.*, 1982). Ronald Levy and colleagues at Stanford University treated 45 patients with 52

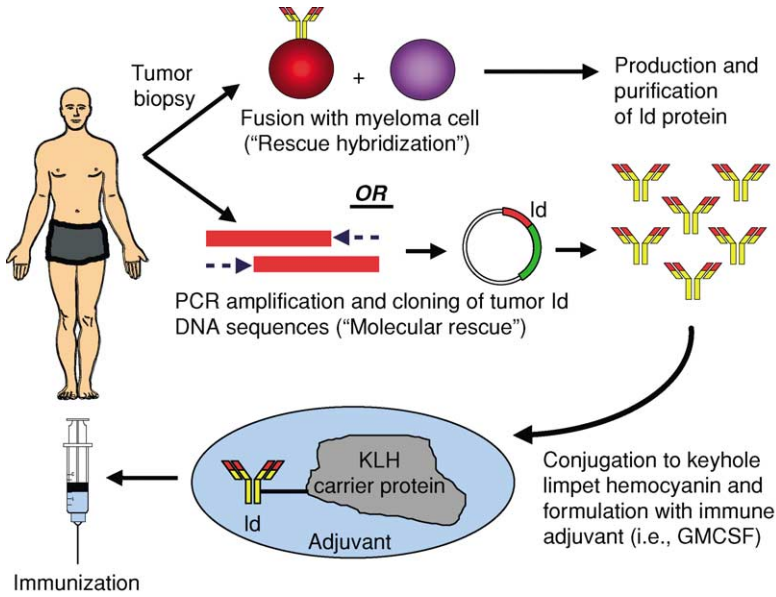

FIGURE 1 Schema for production of patient-specific idiotype (Id) vaccines. Idiotype vaccines are custom-made from each patient's own tumor cells either by fusing to an immortal myeloma cell (rescue hybrid method) or by genetic engineering techniques (molecular rescue method). In each case, an unlimited supply of the vaccine can be made. The Id protein is then chemically linked to the foreign protein keyhole limpet hemocyanin (KLH), combined with an immune stimulant (adjuvant), and injected under the skin.

courses of these patient-specific antibodies, and objective tumor regressions were seen in 66% of cases (Davis *et al*., 1998). In eight cases, these regressions were complete, with some patients being apparently cured, having remained tumor-free for many years without additional treatments. However, despite these impressive results, this custom-made antibody approach ultimately proved too cumbersome for large-scale application and has since been replaced by an active immunotherapeutic strategy that uses tumor-derived idiotype protein as a patient-specific tumor antigen (Timmerman and Levy, 2000a).

Injection of idiotype protein into a host along with an immunologic adjuvant has the potential to elicit three levels of attack against lymphoma cells bearing idiotypic determinants. These include the induction of a polyclonal anti-idiotype antibody response (Campbell *et al*., 1987; George *et al*., 1987; Kwak *et al*., 1992), and recruitment of CD4+ (Campbell *et al*., 1987; Hsu *et al*., 1997; Kwak *et al*., 1992; Lauritzsen *et al*., 1994; Lundin *et al*., 2003) and CD8+ (Abe *et al*., 1996; Cao *et al*., 1994; Chakrabarti and Ghosh, 1992a,b; Osterroth *et al*., 2000; Trojan *et al*., 2000; Wen *et al*., 2001) T cells that recognize idiotype-derived peptides on the cell surface bound to class II and class I major histocompatibility complex (MHC) molecules, respectively. It should be appreciated that an individual patient, based on his or her MHC haplotype and functional T- and B-cell repertoire, could mount all three types of responses, or the response could be limited to two, one, or none of the above. However, as described later, it appears that at least one of these responses is evoked in most vaccinated subjects.

B. Early Trials of Idiotype Vaccination for Follicular Lymphoma

Three key clinical trials in patients with follicular lymphoma have served to establish the proof of principle for therapeutic idiotype vaccination. In the first study performed at Stanford University, patients were immunized with their tumor idiotype protein chemically conjugated (using glutaraldehyde) to the highly immunogenic carrier protein keyhole limpet hemocyanin (Id-KLH) (Hsu *et al*., 1997; Kwak *et al*., 1992). This foreign protein, from the sea mollusk *Megathura crenulata*, served to render the weakly immunogenic self-derived idiotype more recognizable by the human immune system. This is because of KLH-specific CD4+ T helper cells producing cytokines and other factors that provide help to idiotype-specific B cells and CD8+ cytolytic T cells. The Id-KLH complex was emulsified in an oil-in-water type immunologic adjuvant before subcutaneous (SC) injection. In this initial trial, 41 patients were treated, including 32 in their first chemotherapy-induced remission and 9 in subsequent remissions. Side effects were minimal and confined largely to local injection site reactions attributable to the adjuvant. Of the 41 patients, 20 (49%) developed idiotype-specific antibody or T-cell

proliferative responses following vaccination. Antibody responses were detected more often than cellular proliferative responses (85% vs. 35%, respectively). Anti-idiotype immune responses were more frequent in patients in complete remission at the time of vaccination (75%) than in those with residual tumor (25%). Of 20 patients (10%) with residual tumor at the time of vaccination, 2 experienced complete regression following vaccination; both had idiotype-specific immune responses. Among the 32 first-remission patients vaccinated, roughly half (14 of 32) mounted anti-idiotype immune responses (antibody or T-cell proliferation) to the vaccine. In long-term follow-up, the development of an anti-idiotype immune response was highly correlated with improved disease-free ($p < 0.0001$) and overall ($p = 0.04$) survival (Hsu *et al.*, 1997). These results suggested a therapeutic effect of the vaccine, but cause and effect could not be proven in this nonrandomized study. Nonetheless, these observations prompted the search for new methods of improving both the frequency and potency of anti-idiotype immune responses in lymphoma patients.

C. Idiotype-Pulsed Dendritic Cell Vaccines

Stanford investigators next chose dendritic cells as a means of improving the potency of idiotype vaccines. The central role played by dendritic cells in the initiation of immune responses makes them an attractive addition to cancer vaccines (Banchereau *et al.*, 2000). These cells can be isolated directly from the peripheral blood in small numbers or generated *in vitro* from monocytes or CD34+ hematopoietic progenitors, using cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-4, and tumor necrosis factor-α. When briefly cocultured (i.e., pulsed) with tumor-derived peptides or proteins, or transduced with tumor antigen-encoding viruses or RNA and administered as a cellular vaccine, dendritic cells have been shown to promote antitumor immunity in a number of murine cancer models (Timmerman and Levy, 1999). These studies have provided a strong rationale for the clinical use of dendritic cells in therapeutic vaccination strategies against human cancers.

Hsu and colleagues were the first to report that dendritic cells pulsed with a tumor antigen (idiotype) could have clinically significant antitumor effects (Hsu *et al.*, 1996; Timmerman *et al.*, 2002a). In this study, autologous peripheral blood dendritic cells were isolated from patients with follicular lymphoma by leukapheresis and density gradient centrifugation, pulsed with idiotype protein, and administered intravenously. Four infusions of relatively small numbers of cells (approximately 5–10 million) were given, with each infusion followed 2 weeks later by booster injections of soluble idiotype protein. The only side effects were self-limiting infusion reactions, and no long-term toxicities were observed. Objective tumor regression responses were seen in 4 of 10 patients in an initial cohort with measurable, relapsed

follicular lymphoma, including two complete regressions lasting 44 and 57 months. In a second cohort of 23 patients who completed the vaccination series while in first partial or complete remission after chemotherapy, two-thirds developed anti-idiotype T-cell or antibody responses, and reduction in residual tumor was seen in 4 of 18 (22%) cases (responses seen in pleura, lymph nodes, and bone marrow). In a subset of patients, serum antibodies were elicited that could selectively bind to autologous tumor cells and induce signal transduction. The relapse-free survival of these first-remission patients appeared favorable, with 70% remaining progression free at a median follow-up of 43 months.

Six patients who had failed to have tumor regression or who had relapsed after the initial dendritic cell vaccine were treated with five booster injections of Id-KLH protein without dendritic cells (Timmerman *et al.*, 2002). Remarkably, three of these six patients experienced durable tumor regressions (two complete, lasting 24+ and 48+ months). This demonstrated for the first time that even patients with relatively large tumor burdens could have clinically meaningful responses to idiotype vaccines and that by simply reformulating and administering the same idiotype protein, one could achieve a clinical response even after resistance to the original vaccine. One case of vaccine-induced tumor regression is depicted in Fig. 2. A patient with follicular lymphoma was first vaccinated with idiotype-pulsed dendritic cells while in first remission following chemotherapy with cyclophosphamide, vincristine, and prednisone. An idiotype-specific T-cell proliferative response was measured postvaccination, yet recurrence was detected 15 months later. Subcutaneous booster injections of Id-KLH protein were administered at 33 months, by which time extensive lymphadenopathy had developed in the cervical, axillary, iliac, and inguinal regions, along with bone marrow involvement. Within 2 weeks of receiving the first Id-KLH injection, rapid regression of all palpable lymph nodes was noted, and a complete response was later documented by CT scans. The rapid clinical response in this case suggests that a memory immune response was invoked. At no time did this patient have detectable antiidiotype antibodies in the serum. However, tumor-specific cytolytic T cells were recovered from the peripheral blood, thus suggesting a mechanism for these striking antitumor effects. Direct observations of lymphoma regressions in this clinical trial represent some of the most convincing evidence for the potential efficacy of the idiotype vaccination (Timmerman *et al.*, 2002).

D. Granulocyte-Macrophage Colony-Stimulating Factor as a Vaccine Adjuvant

Another approach to increasing the potency of idiotype vaccines has been through the use of GM-CSF as a cytokine adjuvant. Investigators at the U.S. National Cancer Institute (NCI) found that among a panel of cytokines

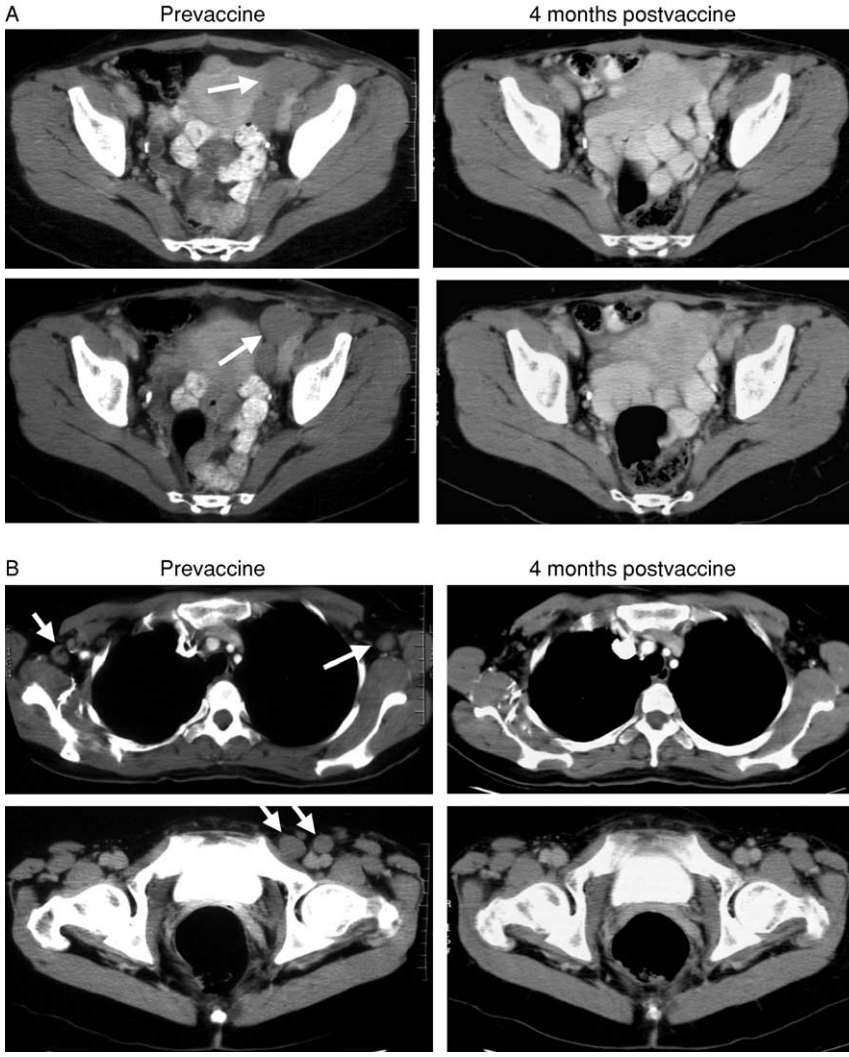

FIGURE 2 Regression of follicular lymphoma following vaccination with idiotype-loaded dendritic cells and Id-KLH protein. A patient with follicular lymphoma was initially treated with infusions of idiotype-pulsed autologous dendritic cells while in first remission postchemotherapy, but relapsed 15 months later. Widespread disease developed 18 months later, and subcutaneous booster injections of Id-KLH protein plus an immune adjuvant were administered. (A) CT scan images of the pelvis show that bulky left-sided pelvic lymph nodes have completely regressed 4 months after booster vaccinations. (B) Regression of axillary (top panels) and inguinal (bottom panels) lymph nodes following booster vaccinations. Arrows indicate sites of disease prior to vaccination. From Timmerman, J. *et al.* (2002b). Idiotype-pulsed dendritic cell vaccination for B cell lymphoma: clinical and immune responses in 35 patients. *Blood* **99,** 1517–1526, with permission.

coinjected with Id-KLH in a murine lymphoma model, GM-CSF was uniquely potent in eliciting T-cell-mediated antitumor immunity (Kwak *et al*., 1996). Similar results have been obtained in surveying the ability of various cytokine gene-transduced tumor cell vaccines to promote antitumor immunity (Dranoff *et al*., 1993). The efficacy of GM-CSF as a vaccine adjuvant is likely related to its stimulation of the growth, maturation, migration, and antigen-presenting properties of dendritic cells (Banchereau *et al*., 2000). Id-KLH plus GM-CSF was first tested in a group of 20 patients with follicular lymphoma in first clinical complete remission after combination chemotherapy (PACE; prednisone, doxorubicin, cyclophosphamide, and etoposide) (Bendandi *et al*., 1999). Vaccinations consisted of SC injections of Id-KLH mixed with GM-CSF on Day 1, with continued GM-CSF injections at the identical site for an additional 3 days to promote infiltration of antigen-presenting cells and T-cell stimulation. Five total vaccinations were administered on Months 1, 2, 3, 4, and 6. Once again, side effects were limited to injection site reactions, principally because of the coinjected GM-CSF. Following vaccination, peripheral blood lymphocytes from 19 of the 20 patients were found to secrete cytokines when cocultured with autologous tumor cells, and cytotoxicity by CD8+ T cells toward autologous tumor cells was demonstrated in 6 patients. Serum anti-idiotype antibodies were detected by ELISA in 75% of cases. As these patients had no radiographically measurable tumor at the time of vaccination, residual disease was quantified in the peripheral blood by the polymerase chain reaction (PCR) for the t(14;18) translocation characteristic of follicular lymphoma. In 8 of 11 PCR-evaluable cases, clearance of the bcl-2 PCR signal from the blood was achieved following vaccination. In addition, 18 of the 20 (90%) patients remained in continuous first clinical complete remission at a median of 42 months postchemotherapy. This study has served to establish GM-CSF as a preferred immune adjuvant for Id-KLH vaccines. Based on these results, a NCI-sponsored Phase III randomized, controlled trial of Id-KLH plus GM-CSF vaccination has been initiated for follicular lymphoma patients in first clinical complete remission following chemotherapy (Timmerman, 2002). (See Section V; this and other new trials of idiotype vaccination are listed in Table I.)

IV. Recombinant Idiotype Vaccines

Although the previous studies provide adequate rationale for formal efficacy evaluation of Id-KLH vaccines in lymphoma, there remains the need to simplify delivery of this individualized therapy. Should controlled trials demonstrate efficacy and lead to regulatory approval, the manufacturing of custom-made idiotype vaccines for thousands of lymphoma patients each year would represent an enormous challenge for the pharmaceutical

industry. Production of idiotype proteins by the rescue hybridoma method, though time-tested in the previously mentioned three studies, is tedious and time consuming, often requiring 6 or more months to complete, and fails to yield a vaccine product in 15–20% of cases. However, idiotype vaccine production can now be streamlined through the use of PCR techniques that allow rapid amplification and cloning of idiotype genes from B-cell tumor specimens (molecular rescue; see Fig. 1) (Hawkins *et al.*, 1994). The resulting idiotype DNA sequences can be inserted into a variety of vectors for either *in vitro* expression of protein or direct injection into the host as genetic vaccines (Timmerman and Levy, 2000).

Recombinant idiotype proteins are at present being produced in a colorful assortment of protein expression systems. Genitope, Inc. (Redwood City, CA) has developed procedures to produce full-length tetrameric idiotype proteins with a human IgG3 backbone in murine lymphoma cells, with a success rate of more than 95%. This recombinant Id-KLH (My Vax™/GTOP-99) plus GM-CSF was found to be safe and to have comparable immunogenicity to rescue hybrid-derived idiotype protein in a Phase I/II study in follicular lymphoma (Timmerman *et al.*, 2000). Anti-idiotype antibody or T-cell proliferative responses were detected in 60% and 50% of patients, respectively (40% positive for both response types). A critical question is whether recombinant idiotype protein vaccines retain the correct three-dimensional conformation and relevant immunogenicity of native, rescue hybridoma-derived tumor idiotype proteins. Importantly, antibodies and T cells reactive toward the recombinant idiotype displayed equivalent reactivity toward the autologous rescue hybridoma-derived idiotype proteins. In addition, postvaccine serum from patients could specifically stain autologous tumor cells, indicating that the induced humoral response was highly relevant to the idiotype as presented in its native conformation by tumor cells (Timmerman and Levy, unpublished observations). These results have led to Genitope initiating a randomized Phase III trial of this vaccine in follicular lymphoma and Phase II testing in aggressive lymphomas (see Sections V and VII).

Favrille, Inc. (San Diego, CA) has found that recombinant idiotype proteins can also be rapidly produced in sf9 insect cells, using the baculovirus vector system (Jones and Morikawa, 1996). This insect-derived Id-KLH (FavId™) plus GM-CSF has been found to be safe and immunogenic in a recent trial in follicular lymphoma. The vaccine was applied in the stringent setting of relapsed, measurable follicular lymphoma, and partial ($n = 1$) or minor ($n = 5$) tumor regression responses were seen in six of seven treated patients (Redfern *et al.*, 2002). In an ironic twist, Large Scale Biology (Vacaville, CA) is pursuing the use of tobacco plants to produce a cancer therapy. Idiotype genes are inserted into the genome of the tobacco mosaic virus, which when used to infect tobacco plants directs the synthesis of large amounts of idiotype protein as a single-chain variable region

TABLE I Current Idiotype Vaccine Trials for Lymphoma

Sponsor and type	*Disease/Situation*	*Vaccine*	*Accrual status/ contact info*	*Ref.*
NCI Phase III randomized	Follicular lymphoma 1st remission after PACE (CR only)	Id-KLH vs. KLH plus GM-CSF	Open for accrual: www.clinicaltrials.gov	Bendandi *et al.* (1999)
Genitope Phase III randomized	Follicular lymphoma 1st remission after CVP (CR or PR)	Id-KLH vs. KLH plus GM-CSF	Open for accrual: www.genitope.com	Timmerman (2003), Timmerman *et al.* (2000)
Favrille Phase II	Follicular lymphoma previously untreated or relapsed/measurable	Rituximab, then Id-KLH plus GM-CSF	Open for accrual: www.favrille.com	Redfern *et al.* (2002)
UCSD Phase II	Indolent or mantle cell lymphoma after HDC + HSCT	Id-KLH plus GM-CSF	Open for accrual: cancercto@ucsd.edu	Holman *et al.* (2002)
UCLA Phase II	Follicular lymphoma relapsed/measurable	Id-loaded dendritic cells, then boost with Id-KLH plus GM-CSF	Opening Fall 2004	Timmerman *et al.* (2002a); Timmerman and Levy (1999)
Large Scale Biology Phase I/II	Follicular lymphoma 1st remission after CVP	Id +/− GM-CSF	Accrual complete	Reddy *et al.*, (2002)
Genitope (Univ. of Nebraska) Phase II	Follicular lymphoma after HDC7 +HSCT	Id-KLH plus GM-CSF	Open for accrual	None

Genitope Phase II	Aggressive lymphomas 1st remission after CHOP	Id-KLH plus GM-CSF	Accrual complete	Leonard *et al*., (2002); Timmerman *et al*. (2001)
NCI Phase I/II	Mantle cell lymphoma 1st remission after EPOCH plus rituximab	Id-KLH plus GM-CSF	Accrual complete	Wilson *et al*. (2002); Neelapu *et al*. (2003)
Univ. of Navarra, Spain Phase II	Follicular lymphoma 1st relapse, measurable disease	CHOP, then Id-KLH plus GM-CSF	Open for accrual	None
CellGenix Freiburg, Germany Phase I	NHL relapse or progression after anthracycline chemotherapy	Recombinant Id Fab fragment plus MF59 and GM-CSF	Open for accrual	Veelken *et al*. (2002); Osterroth *et al*. (1999)
Tenovus Laboratory Southampton, England Phase I/II	Follicular lymphoma CR after chemotherapy, radiation, or HDC + HSCT	Plasmid DNA vaccine: scFv-FrC	Accrual complete	Zhu *et al*. (2001)

Note: CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CR, complete remission; CVP, cyclophosphamide, vincristine, and prednisone; EPOCH, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin; Fab, antigen-binding fragment; GM-CSF, granulocyte-macrophage colony-stimulating factor; HDC + HSCT, high-dose chemotherapy and hematopoietic stem cell transplantation; Id-KLH, idiotype coupled to keyhole limpet hemocyanin; MF59, adjuvant; NCI, U.S. National Cancer Institute; PACE, prednisone, doxorubicin, cyclophosphamide, etoposide; PR, partial remission; scFv-FrC, single-chain variable fragment of idiotype linked to fragment C of tetanus toxin; UCLA, University of California, Los Angeles; UCSD, University of California, San Diego.

immunoglobulin fragment (scFv). Immunization with this tobacco-derived idiotype has shown efficacy in a murine lymphoma model (McCormick *et al.*, 1999). This product has also recently been found to be safe and immunogenic in follicular lymphoma patients (Reddy *et al.*, 2002). Recombinant idiotype proteins produced in bacteria (CellGenix, Germany) have also been shown to be immunogenic *in vitro* for the induction of cytotoxic T lymphocytes (Osterroth *et al.*, 1999), and are now also being studied in Phase I clinical trials (Veelken *et al.*, 2002).

The simplest of all recombinant idiotype vaccines is the direct immunization with plasmid DNA encoding idiotype (Restifo *et al.*, 2000). Inoculation with antigen-encoding DNA results in *in situ* production of protein within the host, thereby bypassing the need for production and purification of recombinant idiotype protein. Bacterial plasmid DNA has been found to be immunogenic in a host of murine models, due in part to its content of immunostimulatory unmethylated CpG oligonucleotide sequences (Krieg *et al.*, 1999). Although vaccination with idiotype-encoding plasmid DNA has displayed efficacy in several murine lymphoma models (Biragyn *et al.*, 1999; King *et al.*, 1998; Syrengelas *et al.*, 1996), initial clinical experience with a first-generation idiotype-encoding vector has been disappointing (Timmerman *et al.*, 2002b). Plasmid DNA encoding tumor idiotype linked to xenogeneic (murine) constant region sequences induced a detectable anti-idiotype immune response in only 1 of 12 patients and was devoid of clinical activity at the relatively low doses tested. Nonetheless, DNA vaccination remains a potentially attractive platform for the further development of idiotype vaccines. DNA vaccine technology offers opportunities to easily manipulate antigen sequences to include additional helper epitopes, cellular targeting sequences, and immunostimulatory (i.e., cytokine, chemokine, or CpG) motifs. Preclinical studies have demonstrated the powerful effects of linking carrier protein (tetanus toxoid) (Zhu *et al.*, 2001) or chemokine (Biragyn *et al.*, 2001) sequences to the tumor antigen-encoding DNA. Freda Stevenson and colleagues in England are currently conducting a study by using idiotype DNA linked to fragment C of tetanus toxoid as a carrier protein (Zhu *et al.*, 2001), and immunologic and clinical results are awaited.

V. Phase III Idiotype Vaccine Trials

Based on the results cited previously, the NCI and Genitope have each initiated large Phase III, randomized double-blind controlled trials of idiotype vaccination for follicular lymphoma (see Table II for comparison). Both trials are aimed at definitive demonstration of idiotype vaccine efficacy and attainment of FDA approval. Eligible patients are those with previously untreated grades 1–3 follicular lymphoma (prior local radiotherapy

permitted). After collection of tumor for vaccine production, patients are treated with a uniform chemotherapy regimen. Those who achieve remission and do not progress during a 6-month immunologic recovery period will be randomized 2:1 to receive Id-KLH plus GM-CSF vs. KLH plus GM-CSF. The principal endpoint for both studies is prolongation of progression-free survival (PFS) after chemotherapy.

The NCI-sponsored trial is being carried out at eight medical centers in the United States, including the NCI Clinical Center. Patients will be treated with the PACE regimen to their best clinical response, and only those with complete clinical remissions will be eligible for vaccination (randomization). The study has a target enrollment of 563 patients and assumes that two-thirds of patients will achieve clinical complete remission [as in their Phase II study (Hsu *et al*., 1997)] to meet an accrual goal of 375 randomized patients. Idiotype proteins will be produced using the traditional rescue hybridoma method. Vaccines were manufactured at the NCI for the initial patients on this trial, but in 2003 Biovest International partnered with the NCI to carry out the manufacture of vaccines for the remainder of the study. A total of five vaccinations will be administered as previously described (Hsu *et al*., 1997).

The Genitope study is ongoing at 34 centers throughout the United States and Canada, with the goal of accruing 700 patients. Following tissue collection, patients receive eight cycles of cyclophosphamide, vincristine, and prednisone, and are then evaluated for clinical response. This study differs from that of the NCI in that all patients achieving (and maintaining for 6 months) at least a partial response will be eligible to proceed to vaccination (randomization). Recombinant idiotype proteins are produced by the molecular rescue method described previously. Patients will receive seven monthly vaccinations rather than the traditional five. It will be several years before efficacy results of these two trials are available. Accrual to the NCI study has lagged behind that of the Genitope study (as of July 2003, 145 vs. 472 subjects, respectively), in large part due to the greater number of clinical sites in the Genitope study. Accrual to the Genitope study will likely be completed in late 2004, with the first efficacy analysis expected in 2005 or 2006. Clinical results for the NCI trial are not expected until well after 2006.

VI. Pharmacologic Considerations and Integration with Standard Therapies

Despite the advanced stage of clinical development for idiotype vaccines, how their differing biochemical composition affects clinical potency remains unclear. For instance, the influence of the idiotype's molecular form (whole immunoglobulins vs. single-chain Fv fragment vs. Fab fragment, etc.), glycosylation patterns (expected to differ among recombinant proteins), and alternative carrier protein conjugations on the ability to elicit

humoral and T-cell-mediated immunity have not been subjected to direct comparative studies in preclinical model systems. Moreover, GM-CSF, now the favored adjuvant for idiotype vaccines, was initially chosen for clinical testing based on limited data in a single murine lymphoma model study. This raises the question of whether other adjuvant formulations, combinations of adjuvants, or delivery with dendritic cells might provide superior immunologic potency (Timmerman and Levy, 2000b). Detailed studies in clinically relevant model systems might help optimize some of these many variables and lead to second-generation idiotype protein vaccines with improved potency.

Like all immunizations, therapeutic idiotype vaccines depend on an intact host immune system for their activity. Thus, integration of vaccine therapies with standard lymphoma treatments must take into account the latter's potential immunosuppressive influences. The antitumor immune effector mechanisms operative after idiotype vaccination are incompletely understood, but can likely involve antibodies, as well as CD8+ and CD4+ T cells. Although anti-idiotypic antibodies might not be necessary for clinical antitumor effects in all patients (Bendandi *et al*., 1999; Timmerman *et al*., 2002), available evidence suggests that they should not be ignored as potential effectors. This evidence includes (1) the remarkable clinical activity of passive anti-idiotype monoclonal antibodies (Davis *et al*., 1998; Miller *et al*., 1982), (2) the finding that anti-idiotype antibodies alone can provide protection from lymphoma challenge in several murine models of idiotype vaccination (Campbell *et al*., 1987; George *et al*., 1987; Syrengelas and Levy, 1999; Timmerman and Levy, 2000), and (3) the positive correlation between antiidiotype antibody responses and improved long-term clinical outcome in vaccinated subjects. It is likely that the relative contributions of antibody vs. T-cell effectors differ from patient to patient, and that for some, a humoral antiidiotype response might be critical to vaccine efficacy. Rituximab, given its depletion of normal B cells (McLaughlin *et al*., 1998), markedly impairs the host's ability to mount primary and secondary humoral immune responses (Gonzales-Stawinski *et al*., 2001; van der Kolk *et al*., 2002). For this reason, prior therapy with rituximab is not permitted in most idiotype vaccine studies, including the current Phase III trials. Although B cells often begin to recover within 6–9 months of completing rituximab therapy, B-cell depletion can persist for several years in some cases (Timmerman and Levy, unpublished observations). To preserve the patient's ability to mount humoral responses to idiotype vaccines, it has been suggested that rituximab be held until after vaccinations are completed and the anti-idiotype humoral response is well established. However, augmentation of T-cell-mediated antitumor immunity has been described in B-cell-deficient mice (Qin *et al*., 1998). This has led to the hypothesis that cytoreduction with anti-CD20 monoclonal antibodies prior to idiotype vaccination might augment anti-idiotype T-cell responses, and this approach

is currently being tested in a study by Favrille (see later). Purine analogs such as fludarabine severely suppress T-cell immunity McLaughlin *et al.* (1996) and are also not advised prior to vaccine therapy.

VII. New and Ongoing Phase I/II Clinical Trials of Idiotype Vaccination

In addition to the Phase III trials described previously, there are at least 11 new or ongoing (unpublished) Phase I/II trials of idiotype vaccination in the United States and Europe (Table II). Favrille is investigating the efficacy of FavId™ plus GM-CSF vaccination 3 months after cytoreduction using rituximab in relapsed or untreated follicular lymphoma. Although rituximab will abrogate humoral anti-idiotype immune responses, T-cell antitumor responses should remain intact or possibly even be augmented (Qin *et al.*, 1998). Patients having objective responses or stable disease after the

TABLE II Current Phase III Trials of Idiotype Vaccination for Follicular Lymphoma

	Genitope	*NCI/Biovest*
Year opened	2000	1999
Target enrollment	$n = 700$	$n = 563$
Number of centers	34	8
Eligibility		
Histology	Grades 1–3	Grades 1–3
Stages	III, IV	Bulky II, III, IV
Prior therapy	Local XRT only	Local XRT only
Tumor sample required	LN, FNA, blood, or BM	LN, blood, or other
Method of Id production	Molecular rescue, in mammalian cells	Rescue hybridoma (traditional)
Prevaccine chemotherapy	CVP × 8	PACE to best response
Response requirement	CR, CRu, or PR (nonresponders and early progressors eligible for phase II trial of rituximab followed by Id-KLH)	CR or CRu
Randomization Id-KLH vs. KLH	2:1	2:1
Vaccine	Id-KLH + GM-CSF × 7	Id-KLH + GM-CSF × 5

Note: NCI, U.S. National Cancer Institute; XRT, radiotherapy; LN, lymph node; FNA, fine needle aspiration; BM, bone marrow; CVP, cyclophosphamide, vincristine, and prednisone; PACE, prednisone, doxorubicin, cyclophosphamide, and etoposide; CR, complete response; CRu, complete response unconfirmed; PR, partial response; Id-KLH, idiotype coupled to keyhole limpet hemocyanin; GM-CSF, granulocyte-macrophage colony-stimulating factor.

6-monthly Id-KLH injections will be eligible to continue immunizations until disease progression. With this prolonged immunization schedule, it is likely that humoral anti-idiotype responses will eventually develop as B cells recover. The primary endpoint of the study is PFS. If favorable PFS is observed in this study (relative to historical controls), a randomized, controlled study of rituximab followed by FavId™ vs. rituximab alone might be initiated (J. Gutheil, Favrille, personal communication).

At the University of California, Los Angeles, we are testing the ability of escalated doses of mature monocyte-derived dendritic cells loaded with idiotype to induce tumor regressions in relapsed follicular lymphoma, and whether subsequent boosting with Id-KLH can improve the response rate as observed in a small cohort of similarly treated patients (Timmerman *et al.*, 2002a). The setting of radiographically measurable tumor is a stringent test for a lymphoma vaccine, yet one in which clinically meaningful antitumor activity and its underlying immunologic mechanisms can be studied. There have been too few patients reported to estimate a response rate for Id-KLH protein vaccination in the setting of measurable tumor, though it appears to be less than 20% (Hsu *et al.*, 1997; Kwak *et al.*, 1992; Redfern *et al.*, 2002). Initial experience with idiotype-pulsed blood dendritic cells suggested a response rate of approximately 30% (Timmerman *et al.*, 2002a). Among 16 patients harboring untreated, measurable follicular lymphoma in the asymptomatic watch-and-wait setting, we observed only one partial and two mixed responses using Id-KLH plus GM-CSF (Timmerman *et al.*, 2002c). Together, these findings suggest that dendritic cell-based immunization might be more clinically potent than Id-KLH. Documentation of a tumor regression rate $\geq$30% in our current dendritic cell trial would suggest that the dendritic cell formulation is more active than Id-KLH alone, issuing a challenge for further comparative studies.

Idiotype vaccination is being studied at several centers as a consolidative immunotherapy following high-dose chemotherapy and hematopoietic stem cell transplantation (Davis *et al.*, 2001; Holman *et al.*, 2002). Stanford investigators have previously shown that patients with indolent or aggressive lymphomas who are vaccinated in this setting can successfully mount humoral and cellular anti-idiotype immune responses (Davis *et al.*, 2001). Further studies will be required to demonstrate clinical benefits in this situation.

Several groups of investigators are now studying idiotype vaccination following initial cytoreductive therapy in aggressive lymphomas. In a study sponsored by Genitope (Timmerman *et al.*, 2001), 14 patients with advanced-stage aggressive lymphoma (6 diffuse large B cell, 5 mantle cell, 3 follicular large cell; 11/14 with an international prognostic index score $\geq$2) were first treated with cyclophosphamide, adriamycin, vincristine, and prednisone (CHOP) to best clinical response, then with a series of five Id-KLH plus GM-CSF vaccinations over 6 months as described previously

(Timmerman *et al.*, 2000), beginning 14 weeks after chemotherapy. Half the subjects were found to mount idiotype-specific immune responses. However, most patients relapsed before completion of the vaccination series, thus prompting the study to be extended using an accelerated vaccination schedule designed to more rapidly achieve anti-idiotypic immunity (Leonard *et al.*, 2002). Final results of this study are pending.

Investigators at the NCI are studying Id-KLH vaccination in 26 subjects with previously untreated mantle cell lymphoma given 12 weeks following cytoreduction with etoposide, prednisone, vincristine, cyclophosphamide, adriamycin, and rituximab (EPOCH-R) (Wilson *et al.*, 2002). Id-KLH plus GM-CSF was administered as in their previous trial in follicular lymphoma (Bendandi *et al.*, 1999). Not unexpectedly, humoral responses were markedly impaired by the prior rituximab therapy, with anti-KLH antibody responses being delayed and diminished compared with those measured previously (Bendandi *et al.*, 1999). (Humoral anti-idiotype responses have not been reported on yet.) However, tumor-specific T-cell cytokine release responses remained intact (Neelapu *et al.*, 2003). With a median follow-up of 18 months, overall survival is 100% and progression-free survival 73%, which appears favorable compared with historical controls. Further clinical results in aggressive lymphomas are awaited. Documentation of favorable survival in these studies could prompt controlled trials in these patient populations.

VIII. Conclusions and Future Prospects

At the present time, all therapeutic lymphoma vaccines remain investigational. However, it is anticipated that clinical efficacy of idiotype vaccines will eventually be demonstrated in controlled trials, with first-generation vaccines now undergoing Phase III testing and new advances in tumor immunology being applied in preclinical and Phase I/II clinical studies to improve vaccine potency. Idiotype will likely continue as an attractive target in lymphoma vaccine development, given its high degree of tumor specificity and established therapeutic potential, yet a greater repertoire of lymphoma-associated antigens for therapeutic targeting is desired. A second major class of lymphoma vaccines now moving into clinical testing is based on antigens derived from whole autologous tumor cells (Timmerman, 2003). These include tumor cells transduced with the genes encoding GM-CSF (Borrello and Sotomayor, 2002; Borrello *et al.*, 2000; Levitsky *et al.*, 1996), CD40 ligand (Briones *et al.*, 2002; Wierda *et al.*, 2000), costimulatory molecules (Briones *et al.*, 2003), heat shock proteins purified from tumor cells (Younes *et al.*, 2003), or dendritic cells loaded with killed tumor cells (Dhodapkar *et al.*, 2002; Gatza and Okada, 2002; Selenko *et al.*, 2001). One powerful way to facilitate the uptake of killed tumor cells by dendritic cells

is opsonization through antitumor monoclonal antibodies such as rituximab (Dhodapkar *et al*., 2002; Selenko *et al*., 2001). We have recently found that immunization using dendritic cells loaded with irradiated tumor cells in the presence of an opsonizing antitumor antibody can evoke tumor-protective immunity in a murine lymphoma model (Frankil and J. Timmerman, unpublished observations). Preliminary evidence from animal studies also suggests that the B-cell differentiation antigens CD19 (Hooijberg *et al*., 1996) and CD20 (Roberts *et al*., 2002), or the T-cell receptor idiotypes of T-cell lymphomas (Okada *et al*., 1997; Wong *et al*., 1999), might be susceptible vaccine targets. Other appropriate antigenic targets can be identified using patient-derived T-cell clones (Rosenberg, 1999) or using genomic or proteomic technologies (Schultze and Vonderheide, 2001). To maximize the effectiveness of active vaccines directed at these antigens, strategies to overcome tumor-induced immunosuppression and tolerance will also need to be devised (Chouaib *et al*., 1997; Overwijk and Restifo, 2001). One such strategy, blockade of the T-cell negative regulatory molecule CTLA-4 (Chambers *et al*., 2001; Hodi *et al*., 2003; Phan *et al*., 2003), is soon to be explored in a new trial in follicular lymphoma being carried out at the University of California, Los Angeles, and the Mayo Clinic. Given the promise held by idiotype and other lymphoma vaccine therapies, the time might be near when these novel immunotherapeutic agents join the existing armamentarium of effective lymphoma treatments.

References

Abe, A., Emi, N., Taji, H., Kasai, M., Kohno, A., and Saito, H. (1996). Induction of humoral and cellular anti-idiotypic immunity by intradermal injection of naked DNA encoding a human variable region gene sequence of an immunoglobulin heavy chain in a B cell malignancy. *Gene Ther.* **3,** 988–993.

Anonymous (1983). Biologic response modifiers: A report. *Natl. Cancer Instit. Monograph* **63,** 57–59.

Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y. J., Pulendran, B., and Palucka, K. (2000). Immunobiology of dendritic cells. *Annu. Rev. Immunol.* **18,** 767–811.

Bendandi, M., Gocke, C. D., Kobrin, C. B., Benko, F. A., Sternas, L. A., Pennington, R., Watson, T. M., Reynolds, C. W., Gause, B. L., Duffey, P. L., Jaffe, E. S., Creekmore, S. P., Longo, D. L., and Kwak, L. W. (1999). Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma [see comments] *Nat. Med.* **5,** 1171–1177.

Biragyn, A., Surenhu, M., Yang, D., Ruffini, P. A., Haines, B. A., Klyushnenkova, E., Oppenheim, J. J., and Kwak, L. W. (2001). Mediators of innate immunity that target immature, but not mature, dendritic cells induce antitumor immunity when genetically fused with nonimmunogenic tumor antigens. *J. Immunol.* **167,** 6644–6653.

Biragyn, A., Tani, K., Grimm, M. C., Weeks, S., and Kwak, L. W. (1999). Genetic fusion of chemokines to a self tumor antigen induces protective, T-cell dependent antitumor immunity [see comments] *Nat. Biotechnol.* **17,** 253–258.

Borrello, I., Sotomayor, E. M., Rattis, F. M., Cooke, S. K., Gu, L., and Levitsky, H. I. (2000). Sustaining the graft-versus-tumor effect through posttransplant immunization with granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing tumor vaccines. *Blood* **95,** 3011–3019.

Borrello, I. M., and Sotomayor, E. M. (2002). Cancer vaccines for hematologic malignancies. *Cancer Control* **9,** 138–151.

Briones, J., Timmerman, J., and Levy, R. (2002). *In vivo* antitumor effect of CD40L-transduced tumor cells as a vaccine for B-cell lymphoma. *Cancer Res.* **62,** 3195–3199.

Briones, J., Timmerman, J. M., Panicalli, D. L., and Levy, R. (2003). Antitumor immunity after vaccination with B lymphoma cells overexpressing a triad of costimulatory molecules. *J. Natl. Cancer Inst.* **95,** 548–555.

Campbell, M. J., Carroll, W., Kon, S., Thielemans, K., Rothbard, J. B., Levy, S., and Levy, R. (1987). Idiotype vaccination against murine B cell lymphoma. Humoral and cellular responses elicited by tumor-derived immunoglobulin M and its molecular subunits. *J. Immunol.* **139,** 2825–2833.

Cao, W., Myers-Powell, B. A., and Braciale, T. J. (1994). Recognition of an immunoglobulin VH epitope by influenza virus-specific class I major histocompatibility complex-restricted cytolytic T lymphocytes. *J. Exp. Med.* **179,** 195–202.

Chakrabarti, D., and Ghosh, S. K. (1992a). Induction of syngeneic cytotoxic T lymphocytes against a B cell tumor. II. Characterization of anti-idiotypic CTL lines and clones. *Cell Immunol.* **144,** 443–454.

Chakrabarti, D., and Ghosh, S. K. (1992b). Induction of syngeneic cytotoxic T lymphocytes against a B cell tumor. III. MHC class I-restricted CTL recognizes the processed form(s) of idiotype. *Cell Immunol.* **144,** 455–464.

Chambers, C. A., Kuhns, M. S., Egen, J. G., and Allison, J. P. (2001). CTLA-4-mediated inhibition in regulation of T cell responses: Mechanisms and manipulation in tumor immunotherapy. *Annu. Rev. Immunol.* **19,** 565–694.

Chouaib, S., Asselin-Paturel, C., Mami-Chouaib, F., Caignard, A., and Blay, J. Y. (1997). The host-tumor immune conflict: From immunosuppression to resistance and destruction. *Immunol. Today* **18,** 493–497.

Davis, T. A., Hsu, F. J., Caspar, C. B., van Beckhoven, A., Czerwinsk, D. K., Liles, T. M., Taidi, B., Benike, C. J., Engleman, E. G., and Levy, R. (2001). Idiotype vaccination following ABMT can stimulate specific anti-idiotype immune responses in patients with B-cell lymphoma. *Biol. Blood Marrow Transplant* **7,** 517–522.

Davis, T. A., Maloney, D. G., Czerwinski, D. K., Liles, T. M., and Levy, R. (1998). Anti-idiotype antibodies can induce long-term complete remissions in non-Hodgkin's lymphoma without eradicating the malignant clone. *Blood* **92,** 1184–1190.

Dhodapkar, K. M., Krasovsky, J., Williamson, B., and Dhodapkar, M. V. (2002). Antitumor monoclonal antibodies enhance cross-presentation of cellular antigens and the generation of myeloma-specific killer T cells by dendritic cells. *J. Exp. Med.* **195,** 125–133.

Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H. *et al.* (1993). Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc. Natl. Acad. Sci. USA* **90,** 3539–3543.

Gatza, E., and Okada, C. Y. (2002). Tumor cell lysate-pulsed dendritic cells are more effective than TCR Id protein vaccines for active immunotherapy of T cell lymphoma. *J. Immunol.* **169,** 5227–5235.

George, A. J., Tutt, A. L., and Stevenson, F. K. (1987). Anti-idiotypic mechanisms involved in suppression of a mouse B cell lymphoma, BCL1. *J. Immunol.* **138,** 628–634.

Gonzalez-Stawinski, G. V., Yu, P. B., Love, S. D., Parker, W., and Davis, R. D., Jr. (2001). Hapten-induced primary and memory humoral responses are inhibited by the infusion of anti-CD20 monoclonal antibody (IDEC-C2B8, Rituximab). *Clin. Immunol.* **98,** 175–179.

Hawkins, R. E., Zhu, D., Ovecka, M., Winter, G., Hamblin, T. J., Long, A., and Stevenson, F. K. (1994). Idiotypic vaccination against human B-cell lymphoma. Rescue of variable region gene sequences from biopsy material for assembly as single-chain Fv personal vaccines. *Blood* **83,** 3279–3288.

Hodi, F. S., Mihm, M. C., Soiffer, R. J., Haluska, F. G., Butler, M., Seiden, M. V., Davis, T., Henry-Spires, R., MacRae, S., Willman, A., Padera, R., Jaklitsch, M. T., Shankar, S., Chen, T. C., Korman, A., Allison, J. P., and Dranoff, G. (2003). Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. *Proc. Natl. Acad. Sci. USA* **100,** 4712–4717.

Holman, P., Bashey, A., Carrier, E., Corringham, S., Davis, B., D., Chen, J., Gold, D., Mu, X., and Ball, E. D. (2002). Immune response to idiotype vaccination following high dose chemotherapy + autologous stem cell transplant in indolent and mantle cell lymphoma. *Blood* **100,** 315b (abstract 4792).

Hooijberg, E., Visseren, M. J., van den Berk, P. C., Jellema, A. P., Romeijn, P., Sein, J. J., van der Voort, E. I., Hekman, A., Ossendorp, F., and Melief, C. J. (1996). Lysis of syngeneic tumor B cells by autoreactive cytotoxic T lymphocytes specific for a CD19 antigen-derived synthetic peptide. *J. Immunother. Emphasis Tumor Immunol.* **19,** 346–356.

Horning, S. J., and Rosenberg, S. A. (1984). The natural history of initially untreated low-grade non-Hodgkin's lymphomas. *N. Engl. J. Med.* **311,** 1471–1475.

Hsu, F. J., Benike, C., Fagnoni, F., Liles, T. M., Czerwinski, D. *et al.* (1996). Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nat. Med.* **2,** 52–58.

Hsu, F. J., Caspar, C. B., Czerwinski, D., Kwak, L. W., Liles, T. M., Syrengelas, A., Taidi-Laskowski, B., and Levy, R. (1997). Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma–long-term results of a clinical trial. *Blood* **89,** 3129–3135.

Jones, I., and Morikawa, Y. (1996). Baculovirus vectors for expression in insect cells. *Curr. Opin. Biotechnol.* **7,** 512–516.

King, C. A., Spellerberg, M. B., Zhu, D., Rice, J., Sahota, S. S., Thompsett, A. R., Hamblin, T. J., Radl, J., and Stevenson, F. K. (1998). DNA vaccines with single-chain Fv fused to fragment C of tetanus toxin induce protective immunity against lymphoma and myeloma. *Nat. Med.* **4,** 1281–1286.

Krieg, A. M., Yi, A. K., and Hartmann, G. (1999). Mechanisms and therapeutic applications of immune stimulatory cpG DNA. *Pharmacol. Ther.* **84,** 113–120.

Krikorian, J. G., Portlock, C. S., Cooney, P., and Rosenberg, S. A. (1980). Spontaneous regression of non-Hodgkin's lymphoma: A report of nine cases. *Cancer* **46,** 2093–2099.

Kwak, L. W., Campbell, M. J., Czerwinski, D. K., Hart, S., Miller, R. A., and Levy, R. (1992). Induction of immune responses in patients with B-cell lymphoma against the surface-immunoglobulin idiotype expressed by their tumors. *N. Engl. J. Med* **327,** 1209–1215.

Kwak, L. W., Young, H. A., Pennington, R. W., and Weeks, S. D. (1996). Vaccination with syngeneic, lymphoma-derived immunoglobulin idiotype combined with granulocyte/macrophage colony-stimulating factor primes mice for a protective T-cell response. *Proc. Natl. Acad. Sci. USA* **93,** 10972–10977.

Lauritzsen, G. F., Weiss, S., Dembic, Z., and Bogen, B. (1994). Naive idiotype-specific CD4+ T cells and immunosurveillance of B-cell tumors. *Proc. Natl. Acad. Sci. USA* **91,** 5700–5704.

Leonard, J., Vose, J., Timmerman, J. M., Levy, R., Ingolia, D., Denney, D., Coleman, M., and Kunkel, L. (2002). Personalized recombinant Idiotype vaccination after chemotherapy as initial treatment for mantle cell lymphoma. *Blood* **100,** 312b (abstract 4792).

Levitsky, H. I., Montgomery, J., Ahmadzadeh, M., Staveley-O'Carroll, K., Guarnieri, F., Longo, D. L., and Kwak, L. W. (1996). Immunization with granulocyte-macrophage colony-stimulating factor-transduced, but not B7-1-transduced, lymphoma cells primes

idiotype-specific T cells and generates potent systemic antitumor immunity. *J. Immunol.* **156,** 3858–3865.

Levy, R., and Dilley, J. (1978). Rescue of immunoglobulin secretion from human neoplastic lymphoid cells by somatic cell hybridization. *Proc. Natl. Acad. Sci. USA* **75,** 2411–2415.

Lundin, K. U., Hofgaard, P. O., Omholt, H., Munthe, L. A., Corthay, A., and Bogen, B. (2003). Therapeutic effect of idiotype-specific CD4+ T cells against B-cell lymphoma in the absence of anti-idiotypic antibodies. *Blood* **102,** 605–612.

McCormick, A. A., Kumagai, M. H., Hanley, K., Turpen, T. H., Hakim, I., Grill, L. K., Tuse, D., Levy, S., and Levy, R. (1999). Rapid production of specific vaccines for lymphoma by expression of the tumor-derived single-chain Fv epitopes in tobacco plants. *Proc. Natl. Acad. Sci. USA* **96,** 703–708.

McLaughlin, P., Grillo-Lopez, A. J., Link, B. K., Levy, R., Czuczman, M. S., Williams, M. E., Heyman, M. R., Bence-Bruckler, I., White, C. A., Cabanillas, F., Jain, V., Ho, A. D., Lister, J., Wey, K., Shen, D., and Dallaire, B. K. (1998). Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: Half of patients respond to a four-dose treatment program. *J. Clin. Oncol.* **16,** 2825–2833.

McLaughlin, P., Robertson, L. E., and Keating, M. J. (1996). Fludarabine phosphate in lymphoma: An important new therapeutic agent. *Cancer Treat. Res.* **85,** 3–14.

Miller, R. A., Maloney, D. G., Warnke, R., and Levy, R. (1982). Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. *N. Engl. J. Med.* **306,** 517–522.

Neelapu, S. S., Wilson, W., Baskar, S., White, T., Frye, R., Pennington, R., and Kwak, L. W. (2003). Induction of T cell responses by tumor antigen vaccination in mantle cell lymphoma following rituximab-based treatment. *Proc. Amer. Soc. Clin. Oncol* **22,** 165 (abstract 663).

Okada, C. Y., Wong, C. P., Denney, D. W., and Levy, R. (1997). TCR vaccines for active immunotherapy of T cell malignancies. *J. Immunol.* **159,** 5516–5527.

Osterroth, F., Alkan, O., Mackensen, A., Lindemann, A., Fisch, P., Skerra, A., and Veelken, H. (1999). Rapid expression cloning of human immunoglobulin Fab fragments for the analysis of antigen specificity of B cell lymphomas and anti-idiotype lymphoma vaccination. *J. Immunol. Methods* **229,** 141–153.

Osterroth, F., Garbe, A., Fisch, P., and Veelken, H. (2000). Stimulation of cytotoxic T cells against idiotype immunoglobulin of malignant lymphoma with protein-pulsed or idiotype-transduced dendritic cells. *Blood* **95,** 1342–1349.

Overwijk, W. W., and Restifo, N. P. (2001). Creating therapeutic cancer vaccines: Notes from the battlefield. *Trends Immunol.* **22,** 5–7.

Phan, G. Q., Yang, J. C., Sherry, R. M., Hwu, P., Topalian, S. L., Schwartzentruber, D. J., Restifo, N. P., Haworth, L. R., Seipp, C. A., Freezer, L. J., Morton, K. E., Mavroukakis, S. A., Duray, P. H., Steinberg, S. M., Allison, J. P., Davis, T. A., and Rosenberg, S. A. (2003). Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc. Natl. Acad. Sci. USA* **100,** 8372–8377.

Qin, Z., Richter, G., Schuler, T., Ibe, S., Cao, X., and Blankenstein, T. (1998). B cells inhibit induction of T cell-dependent tumor immunity. *Nat. Med.* **4,** 627–630.

Reddy, S. A., Czerwinski, D. K., Rajapaksa, R., Reinl, S., Garger, S. J., Cameron, T., Barrett, J., Novak, J., Holtz, R. B., and Levy, R. (2002). Plant derived single chain Fv Idiotype vaccines are safe and immunogenic in patients with follicular lymphoma: Results of a Phase I study. *Blood* **100,** 163a (abstract #609).

Redfern, C., Guthrie, T. H., Adler, M., Holman, P., Smith, M. R., Levy, R., Janakiramaan, N., Leonard, J. P., Rosenfelt, F., Wiernik, P. H., Just, R., Densmore, J., Gold, D., Gutheil, J., and Bender, J. F. (2003). Single agent activity of FavId [Id-KLH vaccine] for indolent NHL *Blood* **102,** 898a (abstract #3341).

Restifo, N. P., Ying, H., Hwang, L., and Leitner, W. W. (2000). The promise of nucleic acid vaccines. *Gene. Ther.* **7,** 89–92.

Roberts, W. K., Livingston, P. O., Agus, D. B., Pinilla-Ibarz, J., Zelenetz, A., and Scheinberg, D. A. (2002). Vaccination with CD20 peptides induces a biologically active, specific immune response in mice. *Blood* **99,** 3748–3755.

Rosenberg, S. A. (1999). A new era for cancer immunotherapy based on the genes that encode cancer antigens. *Immunity* **10,** 281–287.

Schultze, J. L., and Vonderheide, R. H. (2001). From cancer genomics to cancer immunotherapy: Toward second-generation tumor antigens. *Trends Immunol.* **22,** 516–523.

Selenko, N., Maidic, O., Draxier, S., Berer, A., Jager, U., Knapp, W., and Stockl, J. (2001). CD20 antibody (C2B8)-induced apoptosis of lymphoma cells promotes phagocytosis by dendritic cells and cross-priming of CD8+ cytotoxic T cells. *Leukemia* **15,** 1619–1626.

Snozl, M. (1995). Biologic therapy with interleukin-2: Clinical applications, Section 10.4. *In* "Biologic Therapy of Cancer" (V. T. DeVita, S. Hellman, and S. A. Rosenberg, Eds.), p. 271. J. B. Lippincott, Philadelphia.

Syrengelas, A. D., Chen, T. T., and Levy, R. (1996). DNA immunization induces protective immunity against B-cell lymphoma. *Nat. Med* **2,** 1038–1041.

Syrengelas, A. D., and Levy, R. (1999). DNA vaccination against the idiotype of a murine B cell lymphoma: Mechanism of tumor protection. *J. Immunol.* **162,** 4790–4795.

Timmerman, J., Vose, J., Kunkel, L., Bierman, P., Czerwinski, D., Hohenstein, M., Ingolia, D., Denney, D., and Levy, R. (2001). A phase 2 study demonstrating recombinant Idiotype vaccine elicits specific anti-Idiotype immune responses in aggressive non-Hodgkin's lymphoma. *Blood* **98,** 341a (abstract#1440).

Timmerman, J. M. (2002). Vaccine Therapies for Non-Hodgkin's Lymphoma. *Curr. Treat. Options Oncol.* **3,** 307–315.

Timmerman, J. M. (2003). Immunotherapy for lymphomas. *Int. J. Hematol* **77,** 444–455.

Timmerman, J. M., Czerwinski, D., Taid, B., Van Beckhoven, A., Vose, J., Ingolia, D., Kunkel, L., Denney, D., and Levy, R. (2000). A phase I/II trial to evaluate the immunogenicity of recombinant Idiotype protein vaccines for the treatment of non-Hodgkin's lymphoma (NHL). *Blood* **96,** 578a.

Timmerman, J. M., Czerwinski, D. K., Davis, T. A., Hsu, F. J., Benike, C., Hao, Z. M., Taidi, B., Rajapaksa, R., Caspar, C. B., Okada, C. Y., van Beckhoven, A., Liles, T. M., Engleman, E. G., and Levy, R. (2002a). Idiotype-pulsed dendritic cell vaccination for B-cell lymphoma: Clinical and immune responses in 35 patients. *Blood* **99,** 1517–1526.

Timmerman, J. M., and Levy, R. (1999). Dendritic cell vaccines for cancer immunotherapy. *Annu. Rev. Med.* **50,** 507–529.

Timmerman, J. M., and Levy, R. (2000a). Linkage of foreign carrier protein to a self-tumor antigen enhances the immunogenicity of a pulsed dendritic cell vaccine. *J. Immunol.* **164,** 4797–4803.

Timmerman, J. M., Levy, R., Czerwinski, D. K., Ingolia, D., Denney, D., and Kunkel, L. (2002b). A phase 2 trial to evaluate the efficacy of recombinant idiotype vaccines in untreated follicular lymphoma in the "watch-and-wait" period *Proc. Amer. Soc. Clin. Oncol.* **21,** 4a.

Timmerman, J. M., and Levy, R. L. (2000b). The history of the development of vaccines for lymphoma. *Clinical Lymphoma* **1,** 129–139.

Timmerman, J. M., Singh, G., Hermanson, G., Hobart, P., Czerwinski, D. K., Taidi, B., Rajapaksa, R., Caspar, C. B., Van Beckhoven, A., and Levy, R. (2002c). Immunogenicity of a plasmid DNA vaccine encoding chimeric idiotype in patients with B-cell lymphoma. *Cancer Res.* **62,** 5845–5852.

Trojan, A., Schultze, J. L., Witzens, M., Vonderheide, R. H., Ladetto, M., Donovan, J. W., and Gribben, J. G. (2000). Immunoglobulin framework-derived peptides function as cytotoxic T-cell epitopes commonly expressed in B-cell malignancies. *Nat. Med.* **6,** 667–672.

van der Kolk, L. E., Baars, J. W., Prins, M. H., and van Oers, M. H. (2002). Rituximab treatment results in impaired secondary humoral immune responsiveness. *Blood* **100,** 2257–2259.

Veelken, H., Mauerer, K., Waller, C. F., Eckerman, E., Simon, F., and Osterroth, F. (2002). A phase I trial of a recombinant Idiotype vaccine for active immunization against refractory NHL. *Annals of Oncology* **13,** 40 (abstract#120).

Wen, Y. J., Barlogie, B., and Yi, Q. (2001). Idiotype-specific cytotoxic T lymphocytes in multiple myeloma: Evidence for their capacity to lyse autologous primary tumor cells. *Blood* **97,** 1750–1755.

Wierda, W. G., Cantwell, M. J., Woods, S. J., Rassenti, L. Z., Prussak, C. E., and Kipps, T. J. (2000). CD40-ligand (CD154) gene therapy for chronic lymphocytic leukemia. *Blood* **96,** 2917–2924.

Wilson, W. H., Neelapu, S., White, T., Hedge, U., Pittaluga, S., Hakim, F., and Kwak, L. (2002). Idiotype vaccine following EPOCH-Rituximab treatment in untreated mantle cell lymphoma. *Blood* **100,** 162a (abstract #608).

Wong, C. P., Okada, C. Y., and Levy, R. (1999). TCR vaccines against T cell lymphoma: QS-21 and IL-12 adjuvants induce a protective CD8+ T cell response. *J. Immunol.* **162,** 2251–2258.

Younes, A., Fayad, L. E., Pro, B., McLaughlin, P., Hagemeister, F. B., Mansfield, P., Clayman, G., Medeiros, L. J., Manning, J., Lewis, J., and Srivastava, P. (2003). Safety and efficacy of heat shock protein-peptide 96 complex (HSPPC-96) vaccine therapy in patients with relapsed or previously untreated in low-grade non-Hodgkin's lymphoma. *Blood* **102,** 898.

Younes, A., Robertson, M. J., Flinn, I., Romaguera, J., Hagemeister, F., Cabanillas, F., Rodriguez, M., Dang, N., and Samaniego, F. (2002). A phase II study of interleukin-12 in patients with relapsed non-Hodgkin lymphoma and Hodgkin's disease. *Blood* **100,** 364a (abstract#1408).

Zhu, D., Rice, J., Savelyeva, N., and Stevenson, F. K. (2001). DNA fusion vaccines against B-cell tumors. *Trends Mol. Med.* **7,** 566–572.

Sherif S. Farag and Michael A. Caligiuri

Division of Hematology and Oncology
Comprehensive Cancer Center
The Ohio State University
Columbus, Ohio 43210

Cytokine Modulation of the Innate Immune System in the Treatment of Leukemia and Lymphoma

I. Chapter Overview

Therapeutic immunomodulation of leukemia and lymphoma using cytokines has largely focused on harnessing innate immune effector cells, including natural killer (NK) cells and monocytes, against autologous tumor cells. These immune effectors do not require the recognition of tumor-specific antigens and, therefore, may have wider applications than do tumor-specific cytotoxic T-cells. This chapter will review the biological rationale and clinical experience with cytokines in the immune therapy of leukemia and lymphoma and will focus on novel directions for their future use.

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

II. Introduction

In spite of recent advances in understanding the molecular pathophysiology of leukemia and lymphoma, treatment outcome for the majority of patients has remained modest. The use of high-dose cytarabine, novel regimens, high-dose chemotherapy with stem cell transplantation, and the development of monoclonal antibodies are among the salient advances in therapy over the past 2 decades, but have improved outcome for only subsets of patients with these disorders. Resistance to cytotoxic chemotherapy, including the use of myeloablative doses with stem cell support, remains the main cause of treatment failure, suggesting the need to explore alternative strategies that target proapoptotic pathways not influenced by chemotherapy resistance. Immune-based therapies that exploit effectors of the innate and adaptive arms of the immune system represent such strategies that hold promise for the treatment of leukemia and lymphoma.

The use of cytokines in the immunomodulation of leukemia and lymphoma has mostly focused on harnessing innate immune effector cells, including NK cells and monocytes, against autologous tumor cells. Although cytotoxic T lymphocytes are able to exert potent antitumor effects, as evidenced by the graft vs. leukemia/lymphoma effect following allogeneic stem cell transplantation (Horowitz *et al.*, 1990), and can potentially be modulated by a number of cytokines to augment their effect, their role in immune responses against autologous leukemia and lymphoma cells remains uncertain. Furthermore, the requirement for tumor-specific antigens, which are poorly defined for the majority of patients, limits this strategy. Innate immune effector cells, however, do not require tumor-specific antigen recognition and have been shown to lyse tumor cells without prior antigen recognition. This chapter reviews the biologic rationale and clinical experience with cytokines in the immune therapy of leukemia and lymphoma and focuses on novel directions for their future use.

III. Effector Cells of the Innate Immune System: Relevance to Cytokine Therapy

A. Natural Killer Cells

NK cells are critical effector cells of the innate immune system that are important targets for cytokine therapy in cancer. Comprising approximately 10–15% of peripheral blood lymphocytes, these large granular cells are identified by the expression of the CD56 antigen, a neural cell adhesion molecule of unknown function, and the lack of expression of CD3 (Robertson and Ritz, 1990). Functionally, NK cells play a critical role in the first line of defense against invading pathogens and have direct

cytolytic activity against virus-infected cells, and are an important source of immunoregulatory cytokines [e.g., interferon-gamma (IFN-γ), tumor necrosis factor-α (TNF-α), TNF-β, interleukin (IL)-10, granulocyte-macrophage colony-stimulating factor (GM-CSF)] (Trinchieri, 1989). In addition, NK cells can also mediate antibody-dependent cellular cytotoxicity (ADCC) of target cells through receptors that bind to the Fc potion of antibody, FcγRIII (CD16), expressed on the majority of these cells (Trinchieri, 1989). Because of their ability to lyse tumor cells (Allavena *et al.*, 1989; Landay *et al.*, 1987; Oshimi *et al.*, 1986), there has been significant interest in the use of NK cells in the treatment of leukemia and lymphoma, as well as other human cancers.

Based on the extent of surface expression of CD56, two functionally distinct subsets of human NK cells have been identified (Table I). The majority (~90%) of NK cells express low levels of CD56 ($CD56^{dim}$) but high levels of CD16, whereas the remaining cells (~10%) are $CD56^{bright}$ and $CD16^{dim/neg}$. $CD56^{bright}$ NK cells appear to have immunoregulatory function and have less potential for direct cytotoxicity and ADCC than $CD56^{dim}$ cells do (Cooper *et al.*, 2001; Lanier *et al.*, 1986). In addition, $CD56^{bright}$ NK cells constitutively express the high-affinity IL-2 receptor (IL-2R$\alpha\beta\gamma$) and expand *in vitro* and *in vivo* in response to low (picomolar) concentrations of IL-2 (Baume *et al.*, 1992; Caligiuri *et al.*, 1990). In contrast, resting $CD56^{dim}$ NK cells express only the intermediate affinity IL-2 receptor (IL-2R$\beta\gamma$) and proliferate weakly in response to high doses of IL-2 (Baume *et al.*, 1992; Caligiuri *et al.*, 1993). Although resting $CD56^{dim}$ NK cells are more cytotoxic against NK-sensitive targets than are $CD56^{bright}$ NK cells, $CD56^{bright}$ cells exhibit similar or enhanced cytotoxicity against NK targets compared with $CD56^{dim}$ cells after IL-2 activation (Nagler *et al.*, 1990; Robertson *et al.*, 1992). As discussed later, these differences have important implications for the development of therapeutic cytokine schedules.

TABLE I Natural Killer Cell Subsets

	$CD56^{dim}$	$CD56^{bright}$
Cytokine receptors		
IL-2R$\alpha\beta\gamma$	–	++
IL-2R$\beta\gamma$	++	++
Adhesion molecules	–/+	++
NK cell receptors		
FcγRIII (CD16)	+++	–/+
KIR	+++	–/+
CD94/NKG2	–/+	+
Effector functions		
ADCC	+++	–/+
Natural cytotoxicity	+++	–/+
Cytokine production	–/+	+++

I. Human NK Cell Development

Although NK cells and T lymphocytes might share a common precursor, it is now established that NK cells originate in the bone marrow from $CD34^+$ hematopoietic progenitor cells under the influence of a number of cooperating bone marrow stromal cytokines, including ligands for the receptor tyrosine kinases, flt-3, and c-kit, and IL-15. NK cell progenitors respond to the early acting stromal growth factors flt-3 ligand (FL) and c-kit ligand (KL) to develop into an intermediate precursor ($CD34^+$ IL-15Rβ^+ $CD56^-$) capable of responding to IL-15 (Mrozek *et al.*, 1996). IL-15 is then able to induce terminal NK cell differentiation (Mrozek *et al.*, 1996). The IL-15 receptor shares common signaling receptor subunits with that of IL-2 (β and γ chains), which together form the intermediate heterodimeric receptor complex, IL-2/15R$\beta\gamma$, that is upregulated by FL and KL (Fehniger and Caligiuri, 2001a,b). However, IL-15 and IL-2 also each use specific alpha chains (IL-15Rα and IL-2Rα) that confer high-affinity specific binding to their respective high-affinity receptors IL-15R$\alpha\beta\gamma$ and IL-2R$\alpha\beta\gamma$ (Fehniger *et al.*, 2002). As discussed later, this understanding also has important implications for the use of cytokine combinations, including IL-2 (or IL-15) with KL (SCF; stem cell factor), for *in vivo* expansion of NK cells for the immunotherapy of leukemia and lymphoma (Fig. 1).

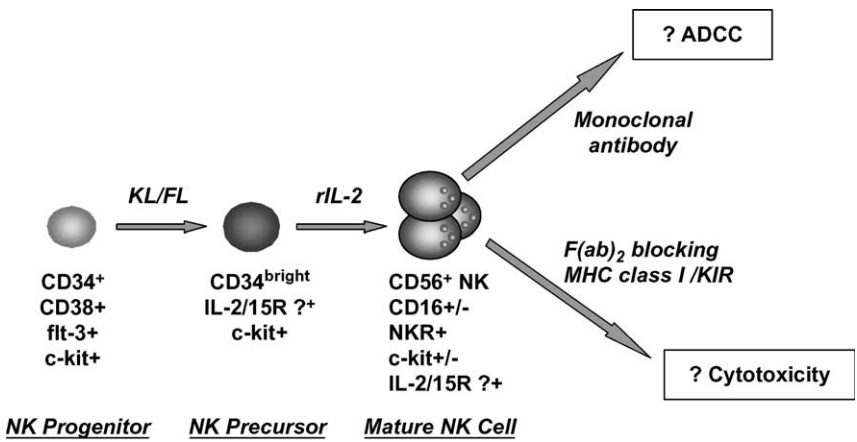

FIGURE 1 Natural killer (NK) cell development and therapeutic approaches based on cytokine immunomodulation. *In vivo* expansion of autologous NK cells used a combination KL (or FL) and rhuIL-2. Efficient tumor cell killing can then be achieved by either combination with a tumor-specific monoclonal antibody to induce ADCC or by using $F(ab)_2$ fragment antibodies to block the interaction of MHC class I molecules on the surface of tumor cells and inhibitory KIR and/or CD94/NKG2 receptors on NK cells, thereby enhancing natural cytotoxicity. Adapted from Farag, S. S., VanDeusen, J. B., Fehniger, T. A., and Caligiuri, M. A. (2003). Biology and clinical impact of human natural killer cells. *Int. J. Hematol.* **78**, 7–17, with permission.

2. NK Cell Receptors

An understanding of how NK cells recognize target cells provides important insight into the potential means of optimizing innate immune therapy for the treatment of leukemia and lymphoma. Unlike T and B cells, NK cells do not rearrange genes encoding receptors for antigen recognition in the context of major histocompatibility complex (MHC) class I or II molecules. However, MHC class I molecules remain important in modulating NK cell recognition and subsequent killing. Early studies indicated that self-major MHC class I molecules are critical for inhibiting NK cell-mediated lysis of normal autologous cells (Shimizu and DeMars, 1989; Storkus *et al*., 1989). This inhibition is mediated by a number of inhibitory receptors that specifically recognize groups of classical (e.g., HLA-A, -B, or -C) or nonclassical (e.g., HLA-E, -G) class I molecules (Lanier, 1998; Lopez-Botet *et al*., 2000a,b). Therefore, NK cells are tolerant to autologous cells that express normal levels of MHC class I, but can selectively lyse autologous cells that have altered or lost self-MHC class I expression (Ljunggren and Karre, 1990). Note, however, that MHC class I expression is not always necessary for protection from lysis by NK cells (Zijlstra *et al*., 1992) and inhibition by MHC class I is not always sufficient to prevent NK cytotoxicity (Malnati *et al*., 1993; Moretta *et al*., 1990), suggesting the presence of other functional receptors. It is now known that each NK cell expresses its own repertoire of activating and inhibitory receptors, and cytotoxicity is ultimately regulated by a balance of signals from these receptors (Fig. 2). The NK receptors include two families of paired inhibitory and activating receptors: (1) the killer immunoglobulin (Ig)-like receptor (KIR) family, which primarily recognize HLA-A, -B, and -C, and (2) the heterodimeric CD94/NKG2 C-type lectin receptor family, which recognizes HLA-E (Braud *et al*., 1998; Lanier, 1998; Lazetic *et al*., 1996; Lopez-Botet *et al*., 2000a,b; Winter *et al*., 1998). In addition, unpaired receptors important in mediating activating signals include the natural cytotoxicity receptors (NCRs) (Cantoni *et al*., 1999; Pende *et al*., 1999; Pessino *et al*., 1998), whose ligands are poorly defined, and the C-type lectin receptor NKG2D, which recognizes a number of MHC class I-like ligands, including MICA and MICB, and UL-16-like binding proteins (ULBP-1, -2, and -3), which are induced or upregulated on target cells during stress or neoplastic transformation (Bauer *et al*., 1999; Pende *et al*., 2001; Sutherland *et al*., 2002). A number of other receptors, including FcγRIII, function primarily as coreceptors, and in some cases their ligands remain unknown. The reader is referred to several excellent reviews on NK receptor biology that have recently been published (Farag *et al*., 2002a; Lanier, 1998; Middleton *et al*., 2002; Moretta *et al*., 2001). Table II lists the human NK cell activating and inhibitory receptors and their ligands.

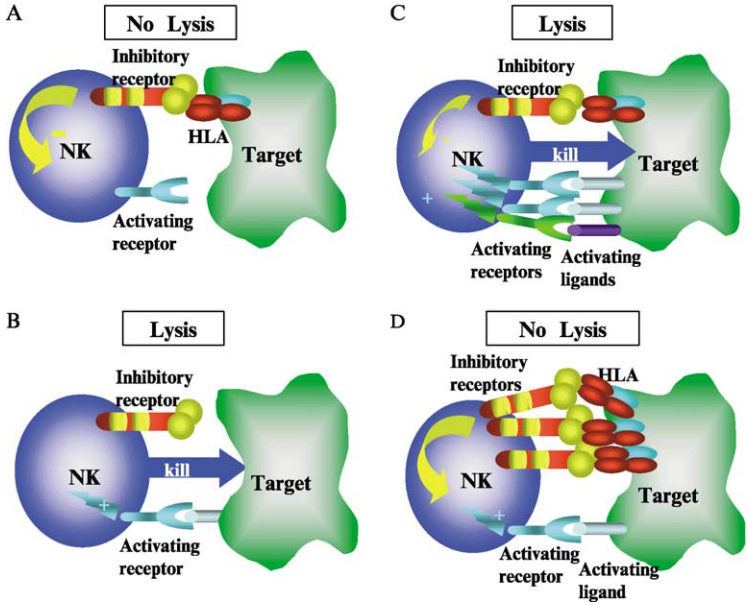

FIGURE 2 Regulation of NK cell response by activating and inhibitory receptors. The response of NK cells is determined by a balance of signals from activating and inhibitory NK cell receptors. Inhibitory receptors (e.g., inhibitory KIR, CD94/NKG2A) recognize and engage MHC class I molecules (HLA) on the target tumor cell and initiate an inhibitory signal. Activating receptors (e.g., activating KIR, CD94/NKG2C, NKG2D) bind ligands on the target cell surface and trigger NK cell activation and target cell lysis. (A) When inhibitory receptors engage HLA in the absence of an activating receptor–ligand interaction, a net negative signal is generated that results in no target cell lysis. (B) When activating receptors engage their ligands on target cells in the absence of inhibitory receptor–ligand interactions, a net activation signal is generated that results in target cell lysis. (C) The activating receptor–ligand interactions predominate over weaker inhibitory receptor–ligand signals with the net result of NK cell activation and target cell lysis. This can occur when activation receptors and ligands are upregulated (e.g., MICA/B and ULBPs on stressed or transformed cells), thereby amplifying the net activation signal to exceed the inhibitory signal. Alternatively, when expression of self-MHC class I ligands is decreased in the setting of viral infection or transformation, the net signal can be positive also, resulting in target cell lysis. (D) Inhibitory receptor–ligand interactions result in a net negative signal that prevents NK cell lysis of the target cell. This process can occur constantly as NK cells survey normal host tissues. The scenario of absence of both inhibitory and activating signals that results in no NK cell activation is not shown. From Farag S. S., Fehniger, T. A., Ruggeri, L., Velardi, A., and Caligiuri, M. A. (2002a). Natural killer cell receptors: New biology and insights into the graft-versus-leukemia effect. *Blood* **100**, 1935–1947, with permission.

B. Monocytes

Monocytes are also important cells of the innate immune system that might have potential for cytokine modulation in the treatment of cancer. Monocytes comprise 10–15% of circulating leukocytes, circulate transiently

TABLE II Inhibitory and Activating NK Cell Receptors

Receptor		*Ligand*
Paired activating and inhibitory NK cell receptors (MHC class I specific)		
Killer Ig receptors (KIR)[a]		
Inhibitory	Activating	
KIR2DL1	KIR2DS1	Group 2 HLA-C (w2, w4, w5, w6 and related alleles)
KIR2DL2	KIR2DS2	Group 1 HLA-C (w1, w3, w7, w8 and related alleles)
KIR2DL3	KIR2DL4	HLA-G
	KIR2DS4	Unknown
	KIR2DS5	Unknown
KIR2DL5	KIR3DS1	Unknown
		HLA-Bw4
KIR3DL1		HLA-A3,-A11
KIR3DL2		Unknown
KIR3DL7		
C-type lectin receptors		
Inhibitory	Activating	
CD94/NKG2A/B[b]	CD94/NKG2C	HLA-E (loaded with HLA-A, -B, -C leader peptides)
	CD94/NKG2E/H[b]	Unknown
Activating NK cell receptors (non-MHC class I specific)		
Natural cytotoxicity receptors		
NKp46	Unknown	
NKp44	Unknown	
NKp30	Unknown	
C-type lectin receptor		
NKG2D	MICA, MICB	
	ULBP-1, -2, -3	
Others		
Activating coreceptors		
CD16 (FcγRIII)	Fc portion of IgG	
CD2	CD58 (LFA-3)	
LFA-1	ICAM-1	
2B4	CD48	
NTB-A	Unknown	
NKp80	Unknown	
CD69	Unknown	
CD40 ligand	CD40	
Other inhibitory receptors		
ILT-2 (LIR-1)	HLA-G and other MHC class I molecules, CMV UL-18 protein (MHC class I-like molecule)	
P75/AIRM	Unknown	
IRp60	Unknown	
LAIR-1	Ep-CAM	

(continues)

in blood (8–72 h), and then egress into the tissues to become macrophages, where they function in defense against infectious agents. Importantly, monocytes/macrophages also recognize antibody-coated targets through Fc receptors, including activating (FcγRI, FcγRIIA, and FcγRIII) and inhibitory (FcγRIIB) Fc receptors (Daeron, 1997). Although their potential role in cancer immunotherapy is less well studied compared to that of NK cells, monocytes can exert direct antitumor activity and participate in ADCC against a variety of tumor cells (Chachoua *et al.*, 1994; Charak *et al.*, 1993). GM-CSF can expand monocyte numbers through differentiation of precursors and augment their function, including the potential for ADCC against tumors by upregulating activating Fc receptors (Chachoua *et al.*, 1994; Charak *et al.*, 1993).

IV. Cytokine Therapy: Exploiting Innate Immune Effectors

The improved understanding of the cytokines involved in modulating effector cells of the innate immune system, together with recent understanding of the NK cell recognition and killing of target cells, has provided a basis for the rational investigation of immunoregulatory cytokine combinations in the treatment of lymphoma and leukemia.

A. *In Vivo* Expansion and Activation of NK Cells with Interleukin-2

Recombinant human IL-2 (rhuIL-2) is the first immunoregulatory cytokine approved by the U.S. Food and Drug Administration (FDA) for the treatment of human cancer. IL-2 is a growth factor for T lymphocytes that is produced primarily by activated T-helper cells, but also has activity on B cells, NK cells, and monocytes. Although rhuIL-2 differs slightly from native IL-2 in not being glycosylated and in a one-amino-acid substitution (serine substituted for cysteine at position 125), the two have similar

Note: LAIR, leukocyte-associated immunoglobulin-like receptor; Ep-CAM, epithelial cellular adhesion molecule; CMV, cytomegalovirus.

Source: Farag, S. S., Fehniger, T. A., Becknell, B., Blaser, B. W., and Caligiuri, M. A. (2003). New directions in natural killer cell-based immunotherapy of human cancer. *Exp. Opin. Biol. Ther.* **3**, 237–250, with permission.

[a] KIR are classified according to the number of extracellular Ig domains (D) and the length of the intracytoplasmic tail. Those with long (L) cytoplasmic tails have immunotyrosine-inhibitory motifs (ITIMs) mediating inhibitory signals, whereas those with short (S) cytoplasmic tails have immunotyrosine-activating motifs (ITAMs) mediating activating signals. Although KIR2DL4 has ITIM motifs in its long cytoplasmic tail, functionally it is better classified as an activating KIR (see text).

[b] NKG2A and NKG2B, and NKG2E and NKG2H are splice variants, respectively.

immunomodulatory activities. rhuIL-2 has no direct antileukemic activity, and its therapeutic effect is entirely mediated by its ability to modulate immune reactions. In spite of its wide spectrum of activity on different immune effector cells, for the treatment of leukemia and lymphoma, investigation has mostly focused on the ability of IL-2 to modulate NK cells *in vivo*.

1. Treatment of Acute Leukemia with rhuIL-2

Numerous clinical trials have been conducted in patients with acute leukemia, predominantly acute myeloid leukemia (AML), using a variety of doses and schedules. Unfortunately, the majority of studies have been small pilot trials, which have only tested tolerability and feasibility of treatment, making assessment of efficacy difficult to ascertain. As high doses are required to activate NK cells *in vivo*, early clinical trials used high-dose IL-2, alone or in combination with IL-2-activated blood lymphocytes, for treating patients with relapsed and refractory acute leukemia with modest success (Lim *et al*., 1992; Maraninchi *et al*., 1998; Meloni *et al*., 1994, 1996; Olive *et al*., 1994). In a pilot trial of 24 patients with relapsed or refractory AML with $\leq$30% marrow blasts treated with a high dose (8–18 $\times$ 10^6 IU/m^2) of rhuIL-2 by continuous intravenous infusion, 13 achieved a complete response (CR), suggesting promising activity (Meloni *et al*., 1997). Based on these results, a randomized trial in AML patients in second CR was initiated (Meloni *et al*., 1997). In contrast, however, a multicenter Phase II trial of 39 AML and 19 acute lymphoblastic leukemia (ALL) patients with relapsed or refractory disease treated with high-dose intravenous bolus infusion (8×10^6 IU/m^2 every 8–12 h) of rhuIL-2 showed only modest responses (Maraninchi *et al*., 1998). Only two AML patients achieved complete remission, whereas no patient with ALL responded. Although significant biologic effects, in terms of an increase in cells with lymphokine activated killer (LAK) activity, were demonstrated, treatment was associated with severe hematologic, hemodynamic, and metabolic toxicity. Based on the demonstrated activity in patients with lower disease burden, and the toxicity associated with the high-dose schedules, lower doses of rhuIL-2 have been used to prevent disease relapse in AML patients already in complete remission (Bergmann *et al*., 1995; Sievers *et al*., 1998; Wiernik *et al*., 1994) or following autologous hematopoietic progenitor cell transplantation (Benyunes *et al*., 1993, 1995; Fefer *et al*., 1993; Massumoto *et al*., 1996; Meehan *et al*., 1997; Robinson *et al*., 1997). Generally, although feasibility was demonstrated in these trials, conclusions regarding efficacy have not been possible. A major limitation, however, has been the significant toxicity associated with high doses of IL-2, necessitating the investigation of lower dose regimens.

Caligiuri and colleagues demonstrated that low doses of IL-2 can induce *in vivo* selective expansion of $CD56^{bright}$ NK cells because of the binding of IL-2 to its high-affinity receptor (Caligiuri *et al*., 1990, 1991, 1993). Initial

Phase I/II trials in patients with human immunodeficiency virus (HIV) infection and malignancy demonstrated that low-dose rhuIL-2 (1×10^6 IU/m^2/day) is well tolerated with and consistently resulted in expansion of NK cells *in vivo* (Bernstein *et al.*, 1995, 1998). Significantly, the expanded CD56^{bright} NK cell population demonstrated significant cytotoxicity against NK-resistant cells only when incubated in higher concentrations of IL-2 that saturate the intermediate-affinity receptors (Caligiuri *et al.*, 1990, 1991, 1993). Based on these observations, a regimen of extended low-dose rhuIL-2 with interval intermediate-dose rhuIL-2 pulsing was developed and found to be well tolerated following intensive chemotherapy in AML patients (Farag *et al.*, 2002b). Of relevance to therapy, the expansion of NK cells following low-dose IL-2 results from enhanced NK cell differentiation from bone marrow progenitors, combined with an IL-2-dependent delay in NK cell death, rather than proliferation of mature NK cells in the periphery (Fehniger *et al.*, 2000). This suggests that use of rhuIL-2 with rhuSCF (stem cell factor) might be a more therapeutically effective combination for *in vivo* expansion of NK cells. Exogenous SCF would result in NK progenitor differentiation into NK precursors, which are in turn responsive to exogenous IL-2. We are currently investigating the safety and efficacy of low-dose rhuIL-2 with rhuSCF in patients with HIV-related malignancy.

Low doses of rhuIL-2 appear to be better tolerated (Cortes *et al.*, 1999; Farag *et al.*, 2002b), but the efficacy of strategies targeting *in vivo* expansion and activation of NK cells in acute leukemia patients remains uncertain. The reported *in vivo* antitumor activity of IL-2 therapy has generally been modest or difficult to convincingly demonstrate. A multicenter randomized trial comparing low-dose with high-dose rhuIL-2 in 110 patients with refractory anemia with excess blasts in transformation and secondary AML who were in first remission after induction and consolidation therapy showed no significant difference in relapse-free or overall survival (Ganser *et al.*, 2000). Results of randomized trials specifically testing the efficacy of rhuIL-2 compared with observation in AML patients after remission induction and consolidation are awaited. An interim analysis of a Cancer and Leukemia Group B (CALGB) study of AML patients $\geq$60 years of age randomized to receive low-dose IL-2 with intermediate-dose pulse IL-2 as maintenance therapy or observation following intensive induction and consolidation therapy has shown no benefit to IL-2 (Larson, personal communication). An additional large randomized trial in younger AML patients treated with low-dose rhuIL-2 and intermediate-dose pulse therapy is currently ongoing through CALGB, and the results are awaited. It is of interest that a randomized trial of intermediate-dose rhuIL-2 (12×10^6 IU/m^2/day) in 130 acute leukemia patients in remission following autologous bone marrow transplantation has shown no benefit (Blaise *et al.*, 2000).

2. Treatment of Lymphoma with rhuIL-2

Single-agent rhuIL-2 has also been investigated in patients with non-Hodgkin's lymphoma (NHL) and Hodgkin's disease as a therapy for patients with refractory disease or to prevent progression following autologous bone marrow transplantation. Similar to the studies in acute leukemia, higher-dose regimens have been less well tolerated, but in general the clinical results have been disappointing (Duggan *et al*., 1992; Gisselbrecht *et al*., 1994) or inconclusive (Gonzalez-Barca *et al*., 1999; Lauria *et al*., 1996; Raspadori *et al*., 1995; van Besien *et al*., 1997; Vey *et al*., 1996), despite consistent observations of a biologic effect on NK cell expansion and activation. No results of any randomized trials of rhuIL-2 treatment following conventional dose therapy or autologous hematopoietic progenitor cell transplantation in lymphoma patients have yet been reported.

3. Potential Reasons for Failure of IL-2 Immunotherapy

As discussed previously, in spite of the ability of IL-2 to expand and activate NK cells *in vivo*, the results have generally been disappointing regardless of the dose and schedule used. Furthermore, although addition of other cytokines, such as rhuSCF, might increase the ability to expand NK cells *in vivo*, it is also likely that the efficacy of this approach will remain modest for patients with leukemia and lymphoma (and likely other malignancies). Recent understanding of the biology of NK cell receptors in the recognition and killing of target cells offers a potential explanation for the observed failure of rhuIL-2 immunotherapy and suggests a novel direction for investigation to optimize treatment. The interaction of MHC class I molecules on tumor cells with inhibitory KIR on autologous NK cells is likely to mediate an important inhibitory signal to rhuIL-2 (with or without other cytokines) expanded NK cells and limit successful killing. The expression of MHC class I molecules by different cancer cell types is not well studied, but recent studies suggest that MHC class I expression might be normal in the vast majority of cases of tumors, including AML and multiple myeloma (Frohn *et al*., 2002; Igarashi *et al*., 2002; Wetzler *et al*., 2001). It is possible, however, that within a given disease type, differences in NK cell receptor ligand expression by the tumor cells exist, which might in turn affect the susceptibility to NK cell lysis and therefore cytokine immunomodulation. For example, differences in the expression of NKG2D ligands by AML blasts has recently been demonstrated (Farag *et al*., 2002b). To further test this hypothesis, we are currently correlating NKG2D ligand expression on AML cells with relapse-free survival of patients treated with IL-2 in an attempt to identify a potential subset of patients that might benefit from rhuIL-2 therapy. If a relationship between NKR-activating ligand expression and outcome following IL-2 therapy is observed, future studies of IL-2 therapy should prospectively target such patients whose cancer cells might be most susceptible to autologous NK cell lysis. As discussed

later, another potential strategy under investigation is the combination of rhuIL-2 with *in vivo* blockade of the inhibitory interactions of MHC class I molecules on tumor cells with KIR on cytokine-expanded NK cells.

V. Novel Approaches for the Immunomodulation of Leukemia and Lymphoma

A. Enhancing Antibody-Dependent Cellular Cytotoxicity

The recent availability of monoclonal antibodies for therapeutic use in lymphoma and leukemia provides the opportunity to harness the ADCC capacity of innate immune effector cells, including NK cells and monocytes. The combination of monoclonal antibodies that act through ADCC with rhuIL-2, for example, is potentially capable of enhancing antitumor killing by better directing immune effector cells to the cancer cells. In patients with AML, HuM195 (anti-CD33) is an available monoclonal antibody, although it is not currently approved for clinical use (Caron *et al*., 1992). In patients with lymphoproliferative diseases, a number of antibodies are currently available, including rituximab, alemtuzumab, anti-CD22, and Hu1D10.

1. IL-2 and Monoclonal Antibodies in the Treatment of Lymphoma and Leukemia

Recent work has provided the rationale for combining of IL-2 and monoclonal antibodies in the treatment of NHL. Rituximab is a chimeric monoclonal antibody directed against the CD20 antigen found on the vast majority of B-cell lymphomas and is currently FDA-approved for the treatment of lymphoma. Although multiple mechanisms have been proposed for the activity of rituximab, including complement-dependent cytotoxicity (CDC) (Golay *et al*., 2000; Harjunpaa *et al*., 2000) and a direct proapoptotic effect (Hofmeister *et al*., 2000), other works have recently established the importance of ADCC as a predominant mechanism of lymphoma cell clearance and that Fcγ receptors are critical for the *in vivo* actions of rituximab in NHL (Clynes *et al*., 2000). In a xenograft model of human lymphoma, knocking out the FcγR loci in mice showed complete abrogation of response to rituximab (Clynes *et al*., 2000). In addition, the recent demonstration in NHL patients that response to rituximab is dependent on specific FcγRIIIa polymorphisms supports the importance of ADCC in the *in vivo* activity of rituximab (Cartron *et al*., 2002). As discussed previously, the activating FcγR on NK cells and monocytes (FcγRIIIa) mediates ADCC. However, although NK cells are important effectors of ADCC, it has been suggested that their ability to function in ADCC might be reduced with advanced malignancy (Kono *et al*., 2002), possibly because of

defective expression of NK cell-triggering receptors (Costello *et al.*, 2002). Cytokines, including IL-2, IFN-α, GM-CSF, IL-15, and IL-12, have been shown to enhance ADCC when added to monoclonal antibodies *in vitro* (Carson *et al.*, 2001; Hank *et al.*, 1990) and *in vivo* in murine models (Berinstein *et al.*, 1988; Carrodeguas *et al.*, 1999).

Based on the previous data, Phase I trials of the combination of IL-2 and rituximab have been initiated, using a variety of IL-2 doses and schedules, as recently reviewed by Morgensztern *et al.* (2002). Phase II trials investigating the combination in rituximab-resistant indolent and aggressive NHL are currently in progress and should better define the efficacy of this approach. If confirmed, studies can be extended to investigation of rhuIL-2 (or other cytokines that modulate FcγR-bearing immune cells) with other clinically available monoclonal antibodies, including anti-CD22, Hu1D10, and alemtuzumab. Note, however, that recent data in patients with chronic lymphocytic leukemia suggest that ADCC might not be an important mechanism for the activity of rituximab in this disease compared with that in NHL (Farag *et al.*, 2004), suggesting that the strategy of combining cytokine with antibody might not be readily extrapolated to other diseases in which an antibody shows single-agent activity.

The combination of rhuIL-2 and HuM195 has been investigated in the treatment of AML (Kossman *et al.*, 1999). HuM195 has been shown to rapidly target and saturate AML cells after intravenous infusion and has induced responses in patients with relapsed AML (Aaron *et al.* 1994). Furthermore, HuM195 is capable of mediating ADCC (Caron *et al.*, 1992). In a Phase I trial involving 14 patients with relapsed or refractory AML and advanced myelodysplasia, patients received low-dose rhuIL-2 ($0.6–2.0 \times 106$ IU/m^{2}day) together with a fixed dose of HuM195 (Kossman *et al.*, 1999). Although the addition of rhuIL-2 caused expansion of NK cells *in vivo*, the antileukemic activity of the combination appeared modest, with no patient achieving remission (Kossman *et al.*, 1999). The modest activity and significant toxicity observed do not favor further evaluation of this combination.

2. *IL-2, GM-CSF, and Monoclonal Antibodies to Optimize ADCC*

As described previously, monocytes are also important FcγR-bearing immune effector cells capable of ADCC, which can be enhanced by GM-CSF. Although IL-2 and GM-CSF can individually expand (and activate) FcγR-bearing NK cells and monocytes, respectively, there is evidence that the combination might be synergistic. GM-CSF might indirectly increase the number and activity of NK cells *in vivo* by activating monocytes to release cytokines such as IL-12, IL-15, and IL-18, which in turn can enhance the function of NK cells (Carson *et al.*, 1994; Fehniger *et al.*, 1999). Significant numbers of immune cells with inducible antitumor activity mediated by both monocytes and NK cells are present in GM-CSF-mobilized stem cell

products, compared with those mobilized with G-CSF (Triozzi *et al*., 1996). LAK cell-mediated cytotoxicity derived from peripheral blood mononuclear cell cultures incubated with IL-2 and GM-CSF is significantly higher compared with that generated with IL-2 alone (Baxevanis *et al*., 1995). Finally, the combination of IL-2 and GM-CSF was also reported to increase the *in vitro* efficacy of ADCC by the monoclonal antibody mAb 17-1A against a colorectal carcinoma cell line compared with the effect of each cytokine alone (Masucci *et al*., 1990). Based on these observations, the ability of the combination of IL-2 and GM-CSF to act synergistically to enhance monoclonal antibody efficacy is currently under investigation in an ongoing Phase I trial sponsored by the National Cancer Institute of monoclonal antibody therapy with sargramostim (GM-CSF) and rhuIL-2 in children with neuroblastoma. In addition, we are investigating the feasibility of adoptive infusion of innate immune effector cells previously mobilized by the combination of rhuIL-2 and GM-CSF following high-dose chemotherapy and rituximab in patients with chemotherapy-refractory NHL, to maximally create an environment that promotes ADCC in an early posttransplant setting.

3. Interleukin-12

IL-12 is a cytokine that regulates the activity of T lymphocytes, dendritic cells, and NK cells. In particular, it facilitates the development of Th1 helper T cells, enhances the cytotoxic activity and ADCC potential of NK cells, and induces the secretion of IFN-γ by both T and NK cells (Brunda, 1995; Brunda *et al*., 1993). Although IL-12 has shown *in vivo* antitumor activity in a number of murine tumor models (Brunda *et al*., 1993; Nastala *et al*., 1994; Wajchman *et al*., 2002), the mechanism of its antitumor activity remains poorly defined. Cell depletion studies have suggested that NK cells, as well as $CD4^+$ and $CD8^+$ T cells, are important for its antitumor activity (Nastala *et al*., 1994; Wajchman *et al*., 2002).

Phase I trials have indicated that rhuIL-12 has modest single-agent activity against a variety of advanced tumors (Gollob *et al*., 2000; Ohno *et al*., 2000; Rook *et al*., 1999). Importantly, a correlation between clinical responses maintenance of induced IFN-γ levels, which in many patients drop with continuing therapy, has been demonstrated (Gollob *et al*., 2000). This paradoxical downmodulation of IFN-γ induction might limit the efficacy of rhuIL-12, possibly accounting for the modest response rates observed to date (Gollob *et al*., 2000; Ohno *et al*., 2000; Rook *et al*., 1999). Therefore, it has been suggested that the addition of rhuIL-2 might lengthen the duration of immune stimulation by rhuIL-12 and thereby augment its antitumor activity (Gollob *et al*., 2000; Rook *et al*., 1999). The combination of rhuIL-2 and rhuIL-12 awaits clinical investigation.

Because of the ability of IL-12 to promote ADCC by NK cells, it has recently been investigated in combination with rituximab in patients with

NHL. In a Phase I trial of rhuIL-12 (30–500 ng/kg SC biweekly) and rituximab (375 mg/m^2 weekly) in 43 patients with CD20$^+$ B-cell NHL, the combination was well tolerated with constitutional symptoms and dose-limiting elevation of liver enzymes occurring at 500 ng/kg of IL-12 (Ansell *et al*., 2002). A greater than 20-fold increase in the serum level of IFN-γ was seen at intermediate and higher doses ($\geq$100 ng/kg) of rhuIL-12. Remarkably, objective responses were demonstrated in 29 (69%) patients, with 8 of 11 complete responses observed at IL-2 doses of $\geq$300 ng/kg. A Phase II dose of IL-12 of 300 ng/kg is recommended for further study (Ansell *et al*., 2002). Although the true efficacy of the combination of IL-12 and rituximab in NHL remains to be determined, the responses seen in this population, which included many patients with poor-risk lymphoma, are encouraging. Of importance, the mechanism of activity of the combination remains uncertain because although increases in IFN-γ levels were noted, no significant increase in the numbers of NK cells was observed. In addition, ADCC activity was not reported. Further studies are required to determine the mechanism of action of this combination and whether this combination offers any advantage over that of IL-2 and rituximab.

B. Blockade of NK Cell Receptor–Ligand Interactions to Enhance Cytokine Activity

As noted previously, a major limitation to the success of cytokine immunomodulation of leukemia and lymphoma is the inhibition of autologous NK cells by MHC class I molecules expressed on tumor cells. Although cytokines, such as rhuIL-2 with or without rhuSCF, might successfully expand NK cells *in vivo*, these cells might be actively inhibited by their target cells (Fig. 3A) (Farag *et al*., 2003). Furthermore, such inhibition might also operate in the presence of monoclonal antibodies, since recent evidence suggests that Fcγ receptors expressed by NK cells likely function as coreceptors, with their activating signal modulated by other primary activating and inhibitory NK cell receptors (Fig. 3A). Although the ability of other activating (NKG2D and NCR) and inhibitory (KIR) NK receptors to modulate ADCC is currently unknown, it is possible that the reported variability in ADCC, and lack of correlation between the density of CD20 on lymphoma cells and the extent of rituximab-induced ADCC (Weng and Levy, 2002), might be due to modulation by MHC class I expression on lymphoma cells interacting with KIR on NK cells. The blockade of NK cell receptor-inhibitory ligand interaction *in vivo* might enhance the antitumor activity of NK cells expanded by cytokines. The feasibility of *in vivo* blockade of MHC class I-KIR interactions using F(ab$'$)$_2$ anti-Ly49C (mouse counterpart of human KIR) has been demonstrated in a mouse model with an enhancement of observed NK cell–mediated antitumor activity (Koh *et al*., 2001). Therefore, the addition of antibody, which can block *in vivo*

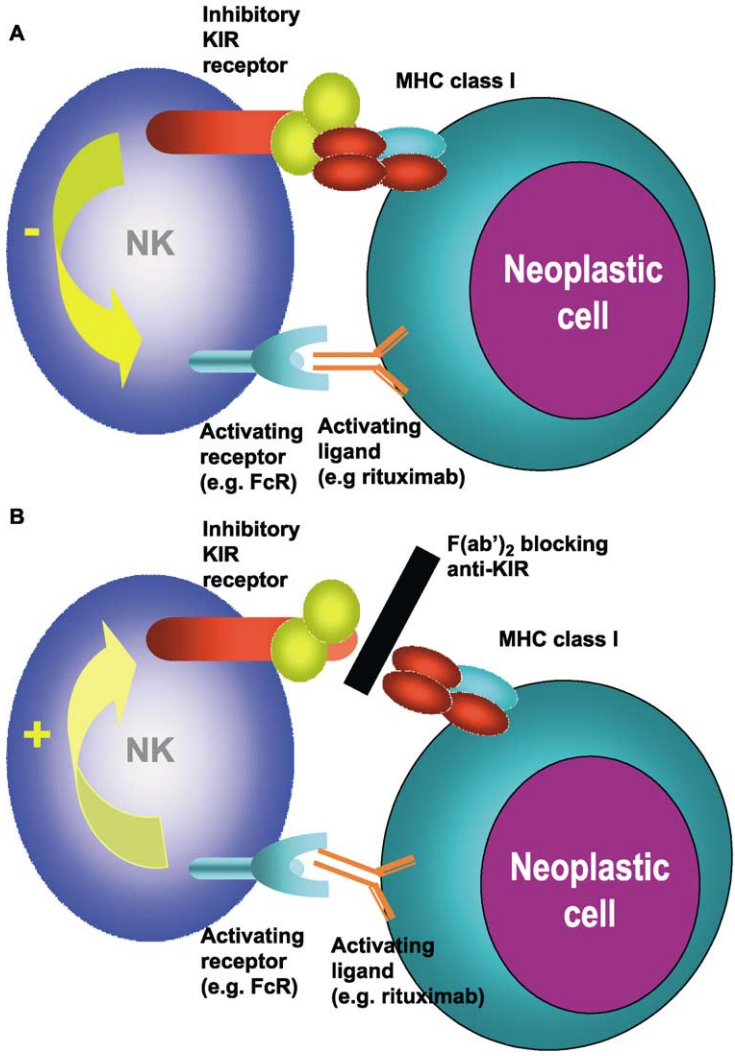

FIGURE 3 Potential significance of NK receptors for cytokine therapy of lymphoma. (A) Expression of MHC class I molecules on lymphoma cells that interact with inhibitory KIR on NK cells to mediate an inhibitory signal that might potentially mitigate the therapeutic effect of cytokines, such as IL-2 and IL-2, dependent on *in vivo* activation of NK cells. This effect might also limit the potential antilymphoma effect of cytokines used in combination with monoclonal antibodies, such as rituximab. (B) The use of $F(ab')_2$ antibody fragments that block MHC class I-KIR interactions in combination with cytokine therapy might counteract this effect and improve therapeutic effect. Adapted from Farag, S. S. (2003). Novel uses of immunomodulatory cytokines in the treatment of lymphoma. *Immune Enhancing Cytokines* **5**, 11–14, with permission.

MHC class I interactions with KIR, to rhuIL-2, rhuIL-2 and rhuSCF, or cytokines and monoclonal antibody, might increase antitumor efficacy and is a novel approach that should be further investigated (Fig. 3B). It is also possible that the simultaneous blockade of CD94/NKG2A/B interactions with HLA-E further enhances antitumor activity.

VI. Conclusion

Although this chapter has focused on the activity of immune-modulating cytokines on the innate immune system, it is likely that effects on other effector cells, particularly T lymphocytes, play an important part in the control of leukemia and lymphoma, although this is less well understood outside the area of allogeneic hematopoietic stem cell transplantation. Although cytokine therapy for leukemia and lymphoma over the past decade or more, particularly in the use of rhuIL-2, has yielded generally discouraging results, the recent understanding of effector cell biology has shed new insight into how these cytokines can be optimally used. Further study of the heterogeneity of expression of activating NK cell receptor ligands on tumor cells might identify which subsets of patients are best suited for cytokine-based therapy. In addition, the elucidation of NK cell receptor biology has suggested novel therapeutic strategies to investigate. Finally, the increasing availability of novel monoclonal antibodies directed against lineage-restricted antigens on leukemia and lymphoma cells should facilitate the testing of cytokine antibody combinations. The challenge over the coming years will be to translate recent knowledge of the biology of effector cells to properly harness the antitumor activity of cells of the innate immune system and determine the place of this treatment in the overall management of patients with hematologic malignancy.

References

Aaron, P. C., Jurcic, J. G., Scott, A. M., Finn, R. D., Divgi, C. R., Jureidini, I. M., Sgouros, G., Tyson, D., and Old, L. J. (1994). A phase Ib trial of humanized monoclonal antibody M195 (anti-CD33) in myeloid leukemia: specific targeting without immunogenecity. *Blood* **83,** 1760–1768.

Allavena, P., Damia, G., Colombo, T., Maggioni, D., D'Incalci, M., and Mantovani, A. (1989). Lymphokine-activated killer (LAK) and monocyte-mediated cytotoxicity on tumor cell lines resistant to antitumor agents. *Cell Immunol.* **120,** 250–258.

Ansell, S. M., Witzig, T. E., Kurtin, P. J., Sloan, J. A., Jelinek, D. F., Howell, K. G., Markovic, S. N., Habermann, T. M., Klee, G. G., Atherton, P. J., and Erlichman, C. (2002). Phase 1 study of interleukin-12 in combination with rituximab in patients with B-cell non-Hodgkin lymphoma. *Blood* **99,** 67–74.

Bauer, S., Groh, V., Wu, J., Steinle, A., Phillips, J. H., and Spies, T. (1999). Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* **285,** 727–729.

Baume, D. M., Robertson, M. J., Levine, H., Manley, T. J., Schow, P. W., and Ritz, J. (1992). Differential responses to interleukin 2 define functionally distinct subsets of human natural killer cells. *Eur. J. Immunol.* **22,** 1–6.

Baxevanis, C. N., Dedoussis, G. V., Papadopoulos, N. G., Missitzis, I., Beroukas, C., Stathopoulos, G. P., and Papamichail, M. (1995). Enhanced human lymphokine-activated killer cell function after brief exposure to granulocyte-macrophage colony stimulating factor. *Cancer* **76,** 1253–1260.

Benyunes, M. C., Higuchi, C., York, A., Lindgren, C., Thompson, J. A., Buckner, C. D., and Fefer, A. (1995). Immunotherapy with interleukin 2 with or without lymphokine-activated killer cells after autologous bone marrow transplantation for malignant lymphoma: A feasibility trial. *Bone Marrow Transplant.* **16,** 283–288.

Benyunes, M. C., Massumoto, C., York, A., Higuchi, C. M., Buckner, C. D., Thompson, J. A., Petersen, F. B., and Fefer, A. (1993). Interleukin-2 with or without lymphokine-activated killer cells as consolidative immunotherapy after autologous bone marrow transplantation for acute myelogenous leukemia. *Bone Marrow Transplant.* **12,** 159–163.

Bergmann, L., Heil, G., Kolbe, K., Lengfelder, E., Puzicha, E., Martin, H., Lohmeyer, J., Mitrou, P. S., and Hoelzer, D. (1995). Interleukin-2 bolus infusion as late consolidation therapy in 2nd remission of acute myeloblastic leukemia. *Leuk. Lymph.* **16,** 271–279.

Berinstein, N., Starnes, C. O., and Levy, R. (1988). Specific enhancement of the therapeutic effect of anti-idiotype antibodies on a murine B cell lymphoma by IL-2. *J. Immunol.* **140,** 2839–2845.

Bernstein, Z. P., Khatri, V., Poiesz, B., Gould, M., Jacob, S., Kunkel, L. A., and Caligiuri, M. A. (1998). Phase I/II study of daily subcutaneous (sc) low dose interleukin-2 (IL-2) in AIDS-associated lymphomas (AIDS-NHL). *Blood* **92**(Suppl 1), 625a (abstract 2576).

Bernstein, Z. P., Porter, M. M., Gould, M., Lipman, B., Bluman, E. M., Stewart, C. C., Hewitt, R. G., Fyfe, G., Poiesz, B., and Caligiuri, M. A. (1995). Prolonged administration of low-dose interleukin-2 in human immunodeficiency virus-associated malignancy results in selective expansion of innate immune effectors without significant clinical toxicity. *Blood* **86,** 3287–3294.

Blaise, D., Attal, M., Reiffers, J., Michallet, M., Bellanger, C., Pico, J. L., Stoppa, A. M., Payen, C., Marit, G., Bouabdallah, R., Sotto, J. J., Rossi, J. F., Brandely, M., Hercend, T., and Maraninchi, D. (2000). Randomized study of recombinant interleukin-2 after autologous bone marrow transplantation for acute leukemia in first complete remission. *Eur. Cytokine Netw.* **11,** 91–98.

Braud, V. M., Allan, D. S., O'Callaghan, C. A., Soderstrom, K., D'Andrea, A., Ogg, G. S., Lazetic, S., Young, N. T., Bell, J. I., Phillips, J. H., Lanier, L. L., and McMichael, A. J. (1998). HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* **391,** 795–799.

Brunda, M. J. (1995). Role of IL12 as an anti-tumour agent: Current status and future directions. *Res. Immunol.* **146,** 622–628.

Brunda, M. J., Luistro, L., and Warrier, R. R. (1993). Antitumor and antimetastatic activity of interleukin-12 against murine tumors. *J. Exp. Med.* **178,** 1223–1230.

Caligiuri, M. A., Murray, C., Robertson, M. J., Wang, E., Cochran, K., Cameron, C., Schow, P., Ross, M. E., Klumpp, T. R., Soiffer, R. J., and Ritz, J. (1993). Selective modulation of human natural killer cells *in vivo* after prolonged infusion of low dose recombinant interleukin 2. *J. Clin. Invest.* **91,** 123–132.

Caligiuri, M. A., Murray, C., Soiffer, R. J., Klumpp, T. R., Seiden, M., Cochran, K., Cameron, C., Ish, C., Buchanan, L., Perillo, D., and Ritz, J. (1991). Extended continuous infusion low-dose recombinant interleukin-2 in advanced cancer: Prolonged immunomodulation without significant toxicity. *J. Clin. Oncol.* **9,** 2110–2119.

Caligiuri, M. A., Zmuidzinas, A., Manley, T. J., Levine, H., Smith, K. A., and Ritz, J. (1990). Functional consequences of interleukin 2 receptor expression on resting human lymphocytes. Identification of a novel natural killer cell subset with high affinity receptors. *J. Exp. Med.* **171,** 1509–1526.

Cantoni, C., Bottino, C., Vitale, M., Pessino, A., Augugliaro, R., Malaspina, A., Parolini, S., Moretta, L., Moretta, A., and Biassoni, R. (1999). NKp44, a triggering receptor involved in tumor cell lysis by activated human natural killer cells, is a novel member of the immunoglobulin superfamily. *J. Exp. Med.* **189,** 787–796.

Caron, P. C., Co, M. S., Bull, M. K., Avdalovic, N. M., Queen, C., and Scheinberg, D. A. (1992). Biological and immunological features of humanized M195 (anti-CD33) monoclonal antibodies. *Cancer Res.* **52,** 6761–6767.

Caron, P. C., Jurcic, J. G., Scott, A. M., Finn, R. D., Divgi, C. R., Graham, M. C., Jureidini, I. M., Sgouros, G., Tyson, D., and Old, L. J. (1994). A phase 1B trial of humanized monoclonal antibody M195 (anti-CD33) in myeloid leukemia: Specific targeting without immunogenicity. *Blood* **83,** 1760–1768.

Carrodeguas, L., Baiocchi, R. A., Roychowdhury, S., Peng, R., Orosz, C. G., and Caligiuri, M. A. (1999). The combination of rituximab (anti-CD20 mAb) and daily low dose interleukin-2 (IL-2) therapy effectively treats established human Epstein-Barr virus-associated lymphoproliferative disorder (EBV- LPD) in a mouse model of human EBV-LPD. *Blood* **94**(Suppl 1), 90a (abstract 392).

Carson, W. E., Giri, J. G., Lindemann, M. J., Linett, M. L., Ahdieh, M., Paxton, R., Anderson, D., Eisenmann, J., Grabstein, K., and Caligiuri, M. A. (1994). Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. *J. Exp. Med.* **180,** 1395–1403.

Carson, W. E., Parihar, R., Lindemann, M. J., Personeni, N., Dierksheide, J., Meropol, N. J., Baselga, J., and Caligiuri, M. A. (2001). Interleukin-2 enhances the natural killer cell response to Herceptin-coated Her2/neu-positive breast cancer cells. *Eur. J. Immunol.* **31,** 3016–3025.

Cartron, G., Dacheux, L., Salles, G., Solal-Celigny, P., Bardos, P., Colombat, P., and Watier, H. (2002). Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. *Blood* **99,** 754–758.

Chachoua, A., Oratz, R., and Liebes, L. (1994). Phase Ib trial of granulocyte-marcrophage colony stimulating factor combined with murine monoclonal antibody R24 in patients with metastatic melanoma. *J. Immunother. Emphasis Tumor Immunol.* **16,** 132–141.

Charak, B. S., Sadowski, R. M., and Mazumdar, M. (1993). Granulocyte-macrophage colony stimulating factor in autologous bone marrow transplantation: Augmentation of graft-versus-tumor effect via antibody dependent cellular cytotoxicity. *Leuk. Lymph.* **9,** 453–457.

Clynes, R. A., Towers, T. L., Presta, L. G., and Ravetch, J. V. (2000). Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. *Nat. Med.* **6,** 443–446.

Cooper, M. A., Fehniger, T. A., Turner, S. C., Chen, K. S., Ghaheri, B. A., Ghayur, T., Carson, W. E., and Caligiuri, M. A. (2001). Human natural killer cells: A unique innate immunoregulatory role for the CD56(bright) subset. *Blood* **97,** 3146–3151.

Cortes, J. E., Kantarjian, H. M., O'Brien, S., Giles, F., Keating, M. J., Freireich, E. J., and Estey, E. H. (1999). A pilot study of interleukin-2 for adult patients with acute myelogenous leukemia in first complete remission. *Cancer* **85,** 1506–1513.

Costello, R. T., Sivori, S., Marcenaro, E., Lafage-Pochitaloff, M., Mozziconacci, M. J., Reviron, D., Gastaut, J. A., Pende, D., Olive, D., and Moretta, A. (2002). Defective expression and function of natural killer cell-triggering receptors in patients with acute myeloid leukemia. *Blood* **99,** 3661–3667.

Daeron, M. (1997). Fc Receptor Biology. *Annu. Rev. Immunol.* **15,** 203–234.

Duggan, D. B., Santarelli, M. T., Zamkoff, K., Lichtman, S., Ellerton, J., Cooper, R., Poiesz, B., Anderson, J. R., Bloomfield, C. D., and Peterson, B. A. (1992). A phase II study of recombinant interleukin-2 with or without recombinant interferon-beta in non-Hodgkin's lymphoma. A study of the Cancer and Leukemia Group B. *J. Immunother.* **12,** 115–122.

Farag, S. S., Fehniger, T., Becknell, B., Blaser, B. W., and Caligiuri, M. A. (2003). New directions in natural killer cell-based immunotherapy of human cancer. *Exp. Opin. Biol. Ther.* **3,** 237–250.

Farag, S. S., Fehniger, T. A., Ruggeri, L., Velardi, A., and Caligiuri, M. A. (2002a). Natural killer cell receptors: New biology and insights into the graft-versus-leukemia effect. *Blood* **100,** 1935–1947.

Farag, S. S., Flinn, I., Modali, R., Lehman, T. A., Young, D., and Byrd, J. C. (2004). FcγRIIIa an FcγIIa polymorphisms do not predict response to rituximab in B-cell chronic lymphocytic leukemia. *Blood* **103,** 1472–1474.

Farag, S. S., George, S. L., Lee, E. J., Baer, M., Dodge, R. K., Becknell, B., Fehniger, T. A., Silverman, L. R., Crawford, J., Bloomfield, C. D., Larson, R. A., Schiffer, C. A., and Caligiuri, M. A. (2002b). Postremission therapy with low-dose interleukin 2 with or without intermediate pulse dose interleukin 2 therapy is well tolerated in elderly patients with acute myeloid leukemia: Cancer and Leukemia Group B study 9420. *Clin. Cancer Res.* **8,** 2812–2819.

Fefer, A., Benyunes, M., Higuchi, C., York, A., Massumoto, C., Lindgren, C., Buckner, C. D., and Thompson, J. A. (1993). Interleukin-2 +/− lymphocytes as consolidative immunotherapy after autologous bone marrow transplantation for hematologic malignancies. *Acta Haematol.* **89,** 2–7.

Fehniger, T. A., Bluman, E. M., Porter, M. M., Mrozek, E., Cooper, M. A., VanDeusen, J. B., Frankel, S. R., Stock, W., and Caligiuri, M. A. (2000). Potential mechanisms of human natural killer cell expansion *in vivo* during low-dose IL-2 therapy. *J. Clin. Invest.* **106,** 117–124.

Fehniger, T. A., and Caligiuri, M. A. (2001a). Interleukin 15: Biology and Relevance to Human Disease. *Blood* **97**(1), 14–32.

Fehniger, T. A., and Caligiuri, M. A. (2001b). Ontogeny and expansion of human natural killer cells: Clinical implications. *Int. Rev. Immunol.* **20,** 503–534.

Fehniger, T. A., Cooper, M. A., and Caligiuri, M. A. (2002). Interleukin-2 and interleukin-15: Immunotherapy for cancer. *Cytokine Growth Factor Rev.* **13,** 169–183.

Fehniger, T. A., Shah, M. H., Turner, M. J., VanDeusen, J. B., Whitman, S. P., Cooper, M. A., Suzuki, K., Wechser, M., Goodsaid, F., and Caligiuri, M. A. (1999). Differential cytokine and chemokine gene expression by human NK cells following activation with IL-18 or IL-15 in combination with IL-12: Implications for the innate immune response. *J. Immunol.* **162,** 4511–4520.

Frohn, C., Hoppner, M., Schlenke, P., Kirchner, H., Koritke, P., and Luhm, J. (2002). Anti-myeloma activity of natural killer lymphocytes. *Br. J. Haematol.* **119,** 660–664.

Ganser, A., Heil, G., Seipelt, G., Hofmann, W., Fischer, J. T., Langer, W., Brockhaus, W., Kolbe, K., Ittel, T. H., Brack, N., Fuhr, H. G., Knuth, P., Hoffken, K., Bergmann, L., and Hoelzer, D. (2000). Intensive chemotherapy with idarubicin, ara-C, etoposide, and m-AMSA followed by immunotherapy with interleukin-2 for myelodysplastic syndromes and high-risk Acute Myeloid Leukemia (AML). *Ann. Hematol.* **79,** 30–35.

Gisselbrecht, C., Maraninchi, D., Pico, J. L., Milpied, N., Coiffier, B., Divine, M., Tiberghien, P., Bosly, A., Tilly, H., and Boulat, O. (1994). Interleukin-2 treatment in lymphoma: A phase II multicenter study. *Blood* **83,** 2081–2085.

Golay, J., Zaffaroni, L., Vaccari, T., Lazzari, M., Borleri, G. M., Bernasconi, S., Tedesco, F., Rambaldi, A., and Introna, M. (2000). Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab *in vitro*: CD55 and CD59 regulate complement-mediated cell lysis. *Blood* **95,** 3900–3908.

Gollob, J. A., Mier, J. W., Veenstra, K., McDermott, D. F., Clancy, D., Clancy, M., and Atkins, M. B. (2000). Phase I trial of twice-weekly intravenous interleukin 12 in patients with metastatic renal cell cancer or malignant melanoma: Ability to maintain IFN-gamma induction is associated with clinical response. *Clin. Cancer Res.* **6,** 1678–1692.

Gonzalez-Barca, E., Granena, A., Fernandez-Sevilla, A., Moreno, V., Salar, A., Rueda, F., and Garcia, J. (1999). Low-dose subcutaneous interleukin-2 in patients with minimal residual lymphoid neoplasm disease. *Eur. J. Haematol.* **62,** 231–238.

Hank, J. A., Robinson, R. R., Surfus, J., Mueller, B. M., Reisfeld, R. A., Cheung, N. K., and Sondel, P. M. (1990). Augmentation of antibody dependent cell mediated cytotoxicity following *in vivo* therapy with recombinant interleukin 2. *Cancer Res.* **50,** 5234–5239.

Harjunpaa, A., Junnikkala, S., and Meri, S. (2000). Rituximab (anti-CD20) therapy of B-cell lymphomas: Direct complement killing is superior to cellular effector mechanisms. *Scand. J. Immunol.* **51,** 634–641.

Hofmeister, J. K., Cooney, D., and Coggeshall, K. M. (2000). Clustered CD20 induced apoptosis: src-family kinase, the proximal regulator of tyrosine phosphorylation, calcium influx, and caspase 3-dependent apoptosis. *Blood Cells Mol. Dis.* **26,** 133–143.

Horowitz, M. M., Gale, R. P., Sondel, P. M., Goldman, J. M., Kersey, J., Kolb, H. J., Rimm, A. A., Ringden, O., Rozman, C., and Speck, B. (1990). Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* **75,** 555–562.

Igarashi, T., Srinivasan, R., Wynberg, J., Takahashi, B., Becknell, M., Caligiuri, W. M., Linehan, R., and Childs (2002). Generation of alloreactive NK cells with selective cytotoxicity to melanoma and renal cell carcinoma based on KIR-ligand incompatibility. *Blood* **100,** 73a.

Koh, C. Y., Blazar, B. R., George, T., Welniak, L. A., Capitini, C. M., Raziuddin, A., Murphy, W. J., and Bennett, M. (2001). Augmentation of antitumor effects by NK cell inhibitory receptor blockade *in vitro* and *in vivo*. *Blood* **97,** 3132–3137.

Kono, K., Takahashi, A., Ichihara, F., Sugai, H., Fujii, H., and Matsumoto, Y. (2002). Impaired antibody-dependent cellular cytotoxicity mediated by herceptin in patients with gastric cancer. *Cancer Res.* **62,** 5813–5817.

Kossman, S. E., Scheinberg, D. A., Jurcic, J. G., Jimenez, J., and Caron, P. C. (1999). A phase I trial of humanized monoclonal antibody HuM195 (anti-CD33) with low-dose interleukin 2 in acute myelogenous leukemia. *Clin. Cancer Res.* **5,** 2748–2755.

Landay, A. L., Zarcone, D., Grossi, C. E., and Bauer, K. (1987). Relationship between target cell cycle and susceptibility to natural killer lysis. *Cancer Res.* **47,** 2767–2770.

Lanier, L. L. (1998). Activating and inhibitory NK cell receptors. *Adv. Exp. Med. Biol.* **452,** 13–18.

Lanier, L. L., Le, A. M., Civin, C. I., Loken, M. R., and Phillips, J. H. (1986). The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *J. Immunol.* **136,** 4480–4486.

Lauria, F., Raspadori, D., Ventura, M. A., Rondelli, D., Zinzani, P. L., Gherlinzoni, F., Miggiano, M. C., Fiacchini, M., Rosti, G., Rizzi, S., and Tura, S. (1996). Immunologic and clinical modifications following low-dose subcutaneous administration of rIL-2 in non-Hodgkin's lymphoma patients after autologous bone marrow transplantation. *Bone Marrow Transplant.* **18,** 79–85.

Lazetic, S., Chang, C., Houchins, J. P., Lanier, L. L., and Phillips, J. H. (1996). Human natural killer cell receptors involved in MHC class I recognition are disulfide-linked heterodimers of CD94 and NKG2 subunits. *J. Immunol.* **157,** 4741–4745.

Lim, S. H., Newland, A. C., Kelsey, S., Bell, A., Offerman, E., Rist, C., Gozzard, D., Bareford, D., Smith, M. P., and Goldstone, A. H. (1992). Continuous intravenous infusion of high-dose recombinant interleukin-2 for acute myeloid leukaemia—a phase II study. *Cancer Immunol. Immunother.* **34,** 337–342.

Ljunggren, H. G., and Karre, K. (1990). In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol. Today* **11,** 237–244.

Lopez-Botet, M., Bellon, T., Llano, M., Navarro, F., Garcia, P., and de Miguel, M. (2000a). Paired inhibitory and triggering NK cell receptors for HLA class I molecules. *Hum. Immunol.* **61,** 7–17.

Lopez-Botet, M., Llano, M., Navarro, F., and Bellon, T. (2000b). NK cell recognition of non-classical HLA class I molecules. *Semin. Immunol.* **12,** 109–119.

Malnati, M. S., Lusso, P., Ciccone, E., Moretta, A., Moretta, L., and Long, E. O. (1993). Recognition of virus-infected cells by natural killer cell clones is controlled by polymorphic target cell elements. *J. Exp. Med.* **178,** 961–969.

Maraninchi, D., Vey, N., Viens, P., Stoppa, A. M., Archimbaud, E., Attal, M., Baume, D., Bouabdallah, R., Demeoq, F., Fleury, J., Michallet, M., Olive, D., Reiffers, J., Sainty, D., Tabilio, A., Tiberghien, P., Brandely, M., Hercend, T., and Blaise, D. (1998). A phase II study of interleukin-2 in 49 patients with relapsed or refractory acute leukemia. *Leuk. Lymph.* **31,** 343–349.

Massumoto, C., Benyunes, M. C., Sale, G., Beauchamp, M., York, A., Thompson, J. A., Buckner, C. D., and Fefer, A. (1996). Close simulation of acute graft-versus-host disease by interleukin-2 administered after autologous bone marrow transplantation for hematologic malignancy. *Bone. Marrow Transplant.* **17,** 351–356.

Masucci, G., Ragnhammar, P., Wersall, P., and Mellstedt, H. (1990). Granulocyte-monocyte colony-stimulating-factor augments the interleukin-2-induced cytotoxic activity of human lymphocytes in the absence and presence of mouse or chimeric monoclonal antibodies (mAb 17-1A). *Cancer Immunol. Immunother.* **31,** 231–235.

Meehan, K. R., Badros, A., Frankel, S. R., Cahill, R., Areman, E., Jenson, M., Sacher, R., and Mazumder, A. (1997). A pilot study evaluating interleukin-2-activated hematopoietic stem cell transplantation for hematologic malignancies. *J. Hematother.* **6,** 457–464.

Meloni, G., Foa, R., Vignetti, M., Guarini, A., Fenu, S., Tosti, S., Tos, A. G., and Mandelli, F. (1994). Interleukin-2 may induce prolonged remissions in advanced acute myelogenous leukemia. *Blood* **84,** 2158–2163.

Meloni, G., Vignetti, M., Andrizzi, C., Capria, S., Foa, R., and Mandelli, F. (1996). Interleukin-2 for the treatment of advanced acute myelogenous leukemia patients with limited disease: Updated experience with 20 cases. *Leuk. Lymph.* **21,** 429–435.

Meloni, G., Vignetti, M., Pogliani, E., Invernizzi, R., Allione, B., Mirto, S., Sica, S., Leoni, F., Selleri, C., and Mandelli, F. (1997). Interleukin-2 therapy in relapsed acute myelogenous leukemia. *Cancer J. Sci. Am.* **3,** S43–47.

Middleton, D., Curran, M., and Maxwell, L. (2002). Natural killer cells and their receptors. *Transpl. Immunol.* **10,** 147–164.

Moretta, A., Bottino, C., Pende, D., Tripodi, G., Tambussi, G., Viale, O., Orengo, A., Barbaresi, M., Merli, A., and Ciccone, E. (1990). Identification of four subsets of human CD3-CD16+ natural killer (NK) cells by the expression of clonally distributed functional surface molecules: correlation between subset assignment of NK clones and ability to mediate specific alloantigen recognition. *J. Exp. Med.* **172,** 1589–1598.

Moretta, A., Bottino, C., Vitale, M., Pende, D., Cantoni, C., Mingari, M. C., Biassoni, R., and Moretta, L. (2001). Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. *Annu. Rev. Immunol.* **19,** 197–223.

Morgensztern, D., Wolin, M., and Rosenblatt, J. (2002). Interleukin-2 and rituximab in lymphoma: Rationale and current trials. *Biol. Ther. Lymph.* **5,** 12–14.

Mrozek, E., Anderson, P., and Caligiuri, M. A. (1996). Role of interleukin-15 in the development of human CD56+ natural killer cells from CD34+ hematopoietic progenitor cells. *Blood* **87,** 2632–2640.

Nagler, A., Lanier, L. L., and Phillips, J. H. (1990). Constitutive expression of high affinity interleukin 2 receptors on human CD16-natural killer cells *in vivo*. *J. Exp. Med.* **171,** 1527–1533.

Nastala, C. L., Edington, H. D., and McKinney, T. G. (1994). Recombinant IL-12 administration induces tumor regression in association with IFN gamma production. *J. Immunol.* **53,** 1697–1706.

Ohno, R., Yamaguchi, Y., and Toge, T. (2000). A dose escalation and pharmacokinetic study of subcutaneously administered recombinant human interleukin-12 and its biological effects in Japanese patients with advanced maligancies. *Clin. Cancer Res.* **6,** 2661–2669.

Olive, D., Chambost, H., Sainty, D., Stoppa, A. M., Blaise, D., el Marsafy, S., Brandely, M., Mannoni, P., Mawas, C., and Maraninchi, D. (1994). Modifications of leukemic blast cells induced by *in vivo* high-dose recombinant interleukin-2. *Leukemia* **8,** 1230–1235.

Oshimi, K., Oshimi, Y., Akutsu, M., Takei, Y., Saito, H., Okada, M., and Mizoguchi, H. (1986). Cytotoxicity of interleukin 2-activated lymphocytes for leukemia and lymphoma cells. *Blood* **68,** 938–948.

Pende, D., Cantoni, C., Rivera, P., Vitale, M., Castriconi, R., Marcenaro, S., Nanni, M., Biassoni, R., Bottino, C., Moretta, A., and Moretta, L. (2001). Role of NKG2D in tumor cell lysis mediated by human NK cells: Cooperation with natural cytotoxicity receptors and capability of recognizing tumors of nonepithelial origin. *Eur. J. Immunol.* **31,** 1076–1086.

Pende, D., Parolini, S., Pessino, A., Sivori, S., Augugliaro, R., Morelli, L., Marcenaro, E., Accame, L., Malaspina, A., Biassoni, R., Bottino, C., Moretta, L., and Moretta, A. (1999). Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. *J. Exp. Med.* **190,** 1505–1516.

Pessino, A., Sivori, S., Bottino, C., Malaspina, A., Morelli, L., Moretta, L., Biassoni, R., and Moretta, A. (1998). Molecular cloning of NKp46: A novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity. *J. Exp. Med.* **188,** 953–960.

Raspadori, D., Lauria, F., Ventura, M. A., Tazzari, P. L., Ferrini, S., Miggiano, M. C., Rondelli, D., and Tura, S. (1995). Low doses of rIL2 after autologous bone marrow transplantation induce a "prolonged" immunostimulation of NK compartment in high-grade non-Hodgkin's lymphomas *Ann. Haematol.* **71,** 175–179.

Robertson, M. J., and Ritz, J. (1990). Biology and clinical relevance of human natural killer cells. *Blood* **76,** 2421–2438.

Robertson, M. J., Soiffer, R. J., Wolf, S. F., Manley, T. J., Donahue, C., Young, D., Herrmann, S. H., and Ritz, J. (1992). Response of human natural killer (NK) cells to NK cell stimulatory factor (NKSF): Cytolytic activity and proliferation of NK cells are differentially regulated by NKSF. *J. Exp. Med.* **175,** 779–788.

Robinson, N., Benyunes, M. C., Thompson, J. A., York, A., Petersdorf, S., Press, O., Lindgren, C., Chauncey, T., Buckner, C. D., Bensinger, W. I., Appelbaum, F. R., and Fefer, A. (1997). Interleukin-2 after autologous stem cell transplantation for hematologic malignancy: A phase I/II study. *Bone Marrow Transplant.* **19,** 435–442.

Rook, A. H., Wood, G. S., Yoo, E. K., Elenitsas, R., Kao, D. M., Sherman, M. L., Witmer, W. K., Rockwell, K. A., Shane, R. B., Lessin, S. R., and Vonderheid, E. C. (1999). Interleukin-12 therapy of cutaneous T-cell lymphoma induces lesion regression and cytotoxic T-cell responses. *Blood* **94,** 902–908.

Shimizu, Y., and DeMars, R. (1989). Demonstration by class I gene transfer that reduced susceptibility of human cells to natural killer cell-mediated lysis is inversely correlated with HLA class I antigen expression. *Eur. J. Immunol.* **19,** 447–451.

Sievers, E. L., Lange, B. J., Sondel, P. M., Krailo, M. D., Gan, J., Liu-Mares, W., and Feig, S. A. (1998). Feasibility, toxicity, and biologic response of interleukin-2 after consolidation chemotherapy for acute myelogenous leukemia: A report from the Children's Cancer Group. *J. Clin. Oncol.* **16,** 914–919.

Storkus, W. J., Alexander, J., Payne, J. A., Dawson, J. R., and Cresswell, P. (1989). Reversal of natural killing susceptibility in target cells expressing transfected class I HLA genes. *Proc. Natl. Acad. Sci. USA* **86,** 2361–2364.

Sutherland, C. L., Chalupny, N. J., Schooley, K., VandenBos, T., Kubin, M., and Cosman, D. (2002). UL16-binding proteins, novel MHC class I-related proteins, bind to NKG2D and activate multiple signaling pathways in primary NK cells. *J. Immunol.* **168,** 671–679.

Trinchieri, G. (1989). Biology of natural killer cells. *Adv. Immunol.* **47,** 187–376.

Triozzi, P. L., Tucker, F., and Benzies, T. (1996). Antitumor and accessory immune activities of peripheral blood stem cells mobilized with granulocyte-macrophage colcony stimulating factor. *Bone Marrow Transplant.* **18,** 47–52.

van Besien, K., Margolin, K., Champlin, R., and Forman, S. (1997). Activity of interleukin-2 in non-Hodgkin's lymphoma following transplantation of interleukin-2-activated autologous bone marrow or stem cells. *Cancer J. Sci. Am.* **3,** S54–58.

Vey, N., Blaise, D., Tiberghien, P., Attal, M., Pico, J. L., Reiffers, J., Harrousseau, J. L., Fiere, D., Tabilio, A., Gabus, R., Brandely, M., and Maraninchi, D. (1996). A pilot study of autologous bone marrow transplantation followed by recombinant interleukin-2 in malignant lymphomas. *Leuk. Lymph.* **21,** 107–114.

Wajchman, J., Simmons, W. J., Klein, A., Koneru, M., and Ponzio, N. M. (2002). Interleukin-12-induced cytotoxicity against syngeneic B cell lymphomas of SJL/J mice. *Leuk. Res.* **26,** 577–590.

Weng, W., and Levy, R. (2002). Rituximab-induced antibody-dependent cellular cytotoxicity (ADCC) in follicular non-Hodgkin's lymphoma. *Blood* **100,** 157a.

Wetzler, M., Baer, M. R., Stewart, S. J., Donohue, K., Ford, L., Stewart, C. C., Repasky, E. A., and Ferrone, S. (2001). HLA class I antigen cell surface expression is preserved on acute myeloid leukemia blasts at diagnosis and at relapse. *Leukemia* **15,** 128–133.

Wiernik, P. H., Dutcher, J. P., Todd, M., Caliendo, G., and Benson, L. (1994). Polyethylene glycolated interleukin-2 as maintenance therapy for acute myelogenous leukemia in second remission. *Am. J. Hematol.* **47,** 41–44.

Winter, C. C., Gumperz, J. E., Parham, P., Long, E. O., and Wagtmann, N. (1998). Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition. *J. Immunol.* **161,** 571–577.

Zijlstra, M., Auchincloss, H., Jr., Loring, J. M., Chase, C. M., Russell, P. S., and Jaenisch, R. (1992). Skin graft rejection by beta 2-microglobulin-deficient mice. *J. Exp. Med.* **175,** 885–893.

Vincent T. Ho and Edwin P. Alyea

Bone Marrow Transplant Program
Dana-Farber Cancer Institute
Boston, Massachusetts

Donor Lymphocyte Infusions

I. Chapter Overview

This chapter reviews the clinical applications and outcomes of donor lymphocyte infusion (DLI). We also discuss future strategies to enhance the GVL response mediated by DLI, with focus on potential targets of the GVL effect.

II. Introduction

The success of DLIs in inducing complete and long-lasting remissions in patients with chronic myeloid leukemia (CML) has provided direct evidence of the existence of a graft-versus-leukemia (GVL) effect. Since the initial successful reports by Kolb and Slavin, DLIs have emerged as an effective strategy to treat patients who have relapsed after allogeneic stem cell transplantation. In

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

the past decade, diseases responsive to DLI and a GVL effect have been identified and efforts to enhance the GVL response are being explored (Kolb *et al.*, 1990; Slavin *et al.*, 1996). Graft-versus-host disease (GVHD) remains the principle toxicity of DLIs, and strategies aimed at limiting this toxicity are the focus of intense investigation. Although the GVL effect induced by DLI can cure some patients who have relapsed after transplant, its greatest potential might, in fact, lie with its use as a prophylactic infusion early after transplantation, at a time of minimal or undetectable residual disease. The principles illustrated by successful induction of a GVL effect by DLI have also spurred the development of nonmyeloablative approaches for allogeneic transplantation, which primarily depend on the GVL response for success.

Extensive laboratory efforts are underway to identify the effector mechanisms of response and potential targets of the GVL reaction. Interestingly, despite the dramatic clinical responses that have been observed, the targets and effector mechanisms of the GVL effect remain unclear. Many potential targets have been proposed, including alloantigens, such as minor histocompatibility antigens, as well as tumor-specific antigens. Responses might be mediated either by cellular-mediated direct cytotoxicity or indirectly via inflammatory cytokines. The role of humoral immunity is also now receiving attention, and might also play a role in the response to DLI.

III. Graft-Versus-Leukemia: Experimental Models

Several early preclinical studies of transplantation suggested the presence of a GVL effect. Barnes and Loutit in 1956 observed that radiation alone was not sufficient to eliminate 100% of leukemic cells in murine transplant models and therefore proposed the existence of the GVL effect mediated by the allogeneic splenocyte graft (Barnes and Loutit, 1956, 1957). This initial observation was confirmed by other investigators who demonstrated in murine models that adoptively transferred lymphocytes given either prior to or following transplant are capable of eliminating residual leukemia cells (Truitt and Johnson, 1995). The effector cell population and the target of the GVL reaction in these models varied depending not only on human leukocyte antigen (HLA) disparity between donor and host cells but also on antigens expressed by the leukemic cells. Despite the recognition of a GVL effect in preclinical models, there was no direct evidence of a clinical GVL in human transplantation until the use of DLI in the late 1980s and early 1990s.

Indirect evidence pointing to the existence of a GVL effect in human transplantation included the observation of a higher relapse rate in recipients of syngeneic transplants compared with allogeneic transplants from sibling donors (Gale *et al.*, 1994; Horowitz *et al.*, 1990). Numerous studies demonstrated a higher relapse rate in recipients of T-cell-depleted transplants

compared with patients receiving T-cell-replete transplantation (Apperley *et al.*, 1988; Goldman *et al.*, 1988; Horowitz *et al.*, 1990; Marmont *et al.*, 1991). The differences in relapse rates between T-cell-depleted transplantation and T-cell-replete transplantation were most pronounced in patients with CML, the disease now recognized as most sensitive to the GVL effect. In addition, there appears to be a tight link between the GVL effect and the development of GVHD. Several studies demonstrated a reduced risk of relapse in patients who developed GVHD after BMT (Weiden *et al.*, 1979, 1981) More direct evidence of this link has come from observations that abrupt withdrawal of immune suppression in patients with disease relapse after allogeneic transplant can induce remissions, often concurrent with the development of GVHD (Collins *et al.*, 1992; Higano *et al.*, 1990; Odom *et al.*, 1978). Direct evidence of the existence of a GVL effect emerged when DLIs were successfully used to treat patients with CML who had relapsed after BMT.

IV. Donor Lymphocyte Infusions for Chronic Myeloid Leukemia

Since the initial report by Kolb in 1990, many groups have reported on the use of DLIs and confirmed the high remission rates with DLIs in patients with CML, who relapse early after allogeneic BMT (Drobyski *et al.*, 1993; Frassoni *et al.*, 1992; Helg *et al.*, 1993; Jiang *et al.*, 1991; Kolb *et al.*, 1990; Porter *et al.*, 1994). Registry data from Europe and North America on patients with relapsed CML after HLA-matched sibling donor transplants demonstrate a complete cytogenetic response rate of >70% when patients are treated in early phases of relapse (Collins *et al.*, 1997; Kolb *et al.*, 1995; (Table I). Unfortunately, these studies also consistently demonstrate that patients with CML in more advanced stages of relapse, such as accelerated

TABLE I Results of Chronic Myeloid Leukemia Treated with Donor Lymphocyte Infusion (D4)

Stage of disease	*North American (Collins* et al., *1997)*	%	*EBMTR (Kolb, 1998)*	%
Early relapse	27/38	71	126/164	78
Cytogenetic	3/3	100	40/50	80
Hematologic	24/35	74	88/114	77
Advanced phase	5/18	28	13/36	36
Accelerated	4/12	33		
Blast phase	1/6	17		

phase or blast crisis, have a much lower response rate following DLI (Collins *et al*., 1997; Porter *et al*., 1994). As an example, in a recent analysis of 593 DLIs from Italy, DLI responses in molecular relapse, cytogenetic relapse, chronic phase, and accelerated/blastic phase relapse were 100%, 90%, 75%, and 35%, respectively (Raiola *et al*., 2003) (Fig. 1).

The responses observed in patients with CML after DLI appear to be durable. Two studies have reported long-term follow-up of patients who achieved complete remission and showed that relapse of CML is uncommon following DLI. In one study, only 5 (13%) of 39 patients who achieved complete cytogenetic remission relapsed following DLI with extended follow-up (Porter *et al*., 1999). The 3-year overall survival for the 39 patients in this report was excellent (70%). An EBMTR study reported the outcome of 44 patients with CML who achieved a molecular remission after treatment with DLI (Dazzi *et al*., 2000). With extended follow-up, 4 of 44 (9%) patients developed evidence of recurrent disease by PCR. The 3-year overall survival for this group of patients was also excellent (95%). Although these initial results are encouraging, 5- and 10-year follow-ups will be needed to fully assess the durability of this treatment modality. Interestingly, when relapse does occur, isolated extramedullary involvement without evidence of systemic disease can be observed. The mechanism of immune escape for cells in these myeloblastomas is not clear.

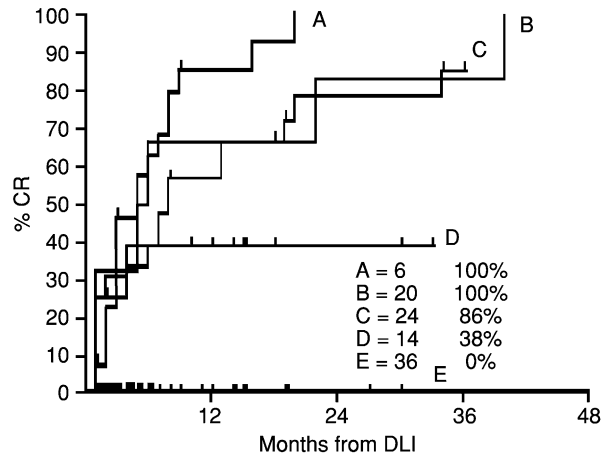

FIGURE 1 Actuarial probability of complete response (CR) to donor lymphocyte infusion (DLI) according to phase of disease at relapse. A: molecular relapses; B: cytogenetic relapses; C: CML chronic phase + CR postreinduction therapy of other diseases; D: CML accelerated phase + blastic phase; E: resistant diseases. From Raiola, A. M. (2003). Factors predicting response and graft-versus-host disease after donor lymphocyte infusions: a study of 593 infusions. *Bone Marrow Transplant*. **18**(5), 975–980.

DLI is also effective in treating patients with CML relapsing after unrelated donor transplant (Porter *et al.*, 2000). Eleven (46%) of 24 patients with CML treated with DLI from unrelated donors achieved a complete response. Similar to studies of patients with related donors, a high response rate was noted in those treated in early phases of relapse, in which 7 of 12 (58%) patients achieved cytogenetic remission. Consistent with prior studies, the response to DLI in advanced-stage CML was poor, with only 4 of 13 (31%) patients achieving remission. All four responders from this study were in accelerated phase, and no responses were observed in patients who received DLI in blast crisis. Note that the results of unrelated donor DLI and related donor DLI are similar despite greater antigenic differences in unrelated donor–recipient pairs.

Many investigators of DLIs were initially surprised by the significant delay after DLI before clinical response is evident. The time to complete cytogenetic response in patients with CML after DLI is often 8–16 weeks following the initial infusion of donor cells (Alyea *et al.*, 1998). Time to complete molecular response, as defined by elimination of the BCR/ABL transcript as detected by PCR, can be 6 months or greater after cell infusion (Fig. 2). Several studies have demonstrated that interferon-α is not required to achieve a response in patients with CML treated with DLI. Both the number of cells infused and the time after transplant when cells are infused appear to be important factors in limiting GVHD.

Stage of disease at the time of DLI is the most significant predictor of response in patients with CML. As noted previously, studies consistently demonstrate that patients receiving DLI in cytogenetic or hematologic relapse have a much higher response rate than patients treated in more advanced phases of the disease. The dose of T cells infused also appears

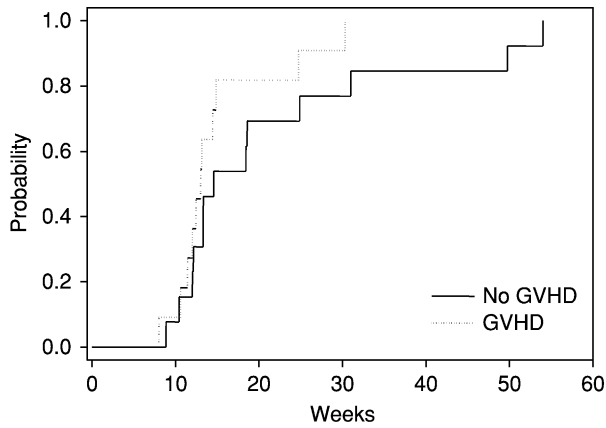

FIGURE 2 Impact of the development graft-versus-host disease (GVHD) on response in patients with CML treated with CD4+ DLI.

to impact both response rate and risk of development of GVHD. Although the impact of cell dose on response has been assessed in the large registry studies and no clear correlation was noted, the doses of cells infused in the majority of these patients might have been so high that the beneficial effects of low cell dose infusion were not apparent (Collins *et al.*, 1997; Kolb *et al.*, 1995). Smaller trials have also suggested a link between cell dose with response and toxicity.

Two prospective trials of unmanipulated DLIs have analyzed the relationship between T-cell number with response and GVHD. MacKinnon *et al.* (1995) have reported a high response rate and low incidence of GVHD in patients receiving 1×10^7 CD3+ cells/kg. Of eight patients receiving this dose, only one developed GVHD. GVL activity appeared to be diminished at cell doses lower than this threshold, as no responses were seen in patients receiving less than 1×10^7 CD3+ cells/kg. A subsequent trial from a European group compared a single intensive dose regimen with infusion of escalating doses of T cells (Dazzi *et al.*, 2000a). In this study, patients in the single infusion arm received a median infusion of 1.5×10^8 T cells/kg, whereas patients in the escalating regimen arm received 1×10^7, 5×10^7, and 1×10^8 T cells/kg if no response or toxicity was observed after each infusion. The incidence of GVHD was significantly lower with the escalating dose regimen (10%) compared with the single high cell dose infusion (44%; $p = 0.011$). There was no difference in the remission rate.

The effect of initial cell dose on outcome of DLI for CML has also been retrospectively evaluated in a recent multicenter study from Europe (Guglielmi *et al.*, 2002). In 298 patients who received DLI, responses rates were similar among the cohorts that received initial DLI doses of $<0.2 \times 10^8$ mononuclear cells/kg, $0.21–2.0 \times 10^8$ cells/kg, and $>2.0 \times 10^8$ cells/kg. The majority (62%) of patients at the lowest initial dose had additional infusions, compared with 5% in the highest initial dose. Most importantly, there was significantly less GVHD (26% vs. 53% vs. 62%) and myelosuppression in the lowest initial cell dose group compared to the mid-dose and high-dose groups, thus affirming earlier reports that repeated low cell dose infusions are as effective as a single higher-dose infusion, but with less GVHD and DLI-associated toxicity. In studies of DLI from unrelated donors, no correlation between cell dose and response rate or incidence of GVHD has been reported; however, large trials are not available to adequately address this issue.

V. Multiple Myeloma

Several studies of allogeneic transplantation in patients with myeloma had suggested the presence of a graft-versus myeloma (GVM) effect (Bensinger *et al.*, 1996; Bjorkstrand *et al.*, 1996; Le Blanc *et al.*, 2001).

DLI studies have provided direct evidence of the GVM effect, with DLI inducing significant responses in patients with multiple myeloma who have relapsed after transplantation (Table II). The overall response rate to DLI in patients with myeloma approaches 45%, with complete responses noted in about 25% of patients. Durable complete responses are noted in half the patients who obtain a complete remission, with follow-up more than 7 years in some patients.

Both dose of cells infused and timing of DLI after transplantation might influence response rates. The optimal dose of cells to be infused and timing of DLI have yet to be determined. Lokhorst *et al.* (2001) reported that patients receiving $>1 \times 10^8$ CD3+ cells/kg had an improved response; however, responses were noted in patients with infusion of doses as low as 1×10^7 CD3+ cells/kg. Early administration of DLI after allogeneic transplantation might improve response rates and improve GVM after transplantation.

Researchers have explored the use of T-cell depletion to reduce GVHD and transplant toxicity early after allogeneic hematopoietic stem cell transplantation (HSCT), followed by DLI to restore the GVM effect. Investigators at the Dana-Farber Cancer Institute administered prophylactic DLI to 14 myeloma patients 6–9 months after T-cell-depleted myeloablative allogeneic transplantation (Alyea *et al.*, 2001). Of the 14 patients receiving DLI, 11 had evidence of residual disease at the time of DLI. Of these 11 patients, 10 had significant clinical responses, with 6 obtaining complete remission. Although a significant GVM effect was observed with prophylactic DLI, only 58% of myeloma patients in this study were able to receive DLI after transplantation, because many patients had developed complications such as GVHD or infections, which precluded DLI administration. Investigators at Johns Hopkins reported on 16 patients who received DLI for relapsed or persistent myeloma 1 year after T-cell-depleted myeloablative BMT (Huff *et al.*, 2003). Ten (63%) developed GVHD after DLI, and 8 of 16 (50%) had disease response (6 CRs, 2 PRs). Investigators from Europe have also administered DLI to patients with residual or progressive myeloma after *in vivo* T-cell-depleted allogeneic HSCT, using reduced-intensity conditioning

TABLE II Results of Multiple Myeloma Treated with DLI

	N	*Prior chemotherapy*	*CR (%)*	*PR (%)*	*Overall RR (%)*
Salama *et al.*, 2000	25	3	7 (33)	2 (8)	9 (36)
Lokhorst *et al.*, 2000	27	13	6 (22)	8 (29)	14 (52)
DFCI	21	0	9 (43)	6 (29)	15/21 (71)
Huff *et al.*, 2003	16	0	6 (38)	2 (13)	8/15 (50)
Peggs *et al.*, 2003	14	0	1 (7)	6 (43)	7/14 (50)

(Peggs *et al.*, 2003). Of 20 patients, 14 received escalating-dose DLI for residual/progressive disease >6 months posttransplant. Seven (50%) patients had a clinical response. Demonstrating the close link between the GVM effect and GVHD, five of the seven patients who developed GVHD after DLI developed either PR or a CR. Unlike CML, these myeloma responses were less durable, and most in this study had progression of disease within 1 year despite persistent full donor chimerism.

VI. Myelodysplastic Syndrome and Acute Leukemia

Patients with myelodysplasic syndrome (MDS) and acute leukemia have also been treated with DLI (Table III). In the North American registry report, complete responses were noted in two of five patients with MDS treated with DLI, whereas in the European experience three of nine patients with MDS achieved a remission (Collins *et al.*, 1997; Kolb, 1998a).Response rates in acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL) are low and are similar to response rates noted in patients with advanced-stage CML. The complete response rate to DLI in patients with AML is 15–29% and in ALL 5–18%. The durability of response in patients with acute leukemia is less that that seen in patients with CML. In a study assessing the long-term outcome of patients treated with DLI, 36% of patients with acute leukemia who achieved remission after DLI relapsed, including 4 of 15 with AML and 3 of 4 with ALL (Porter *et al.*, 1999) The median time to relapse was 10 months (range 1–37 months).

DLI from unrelated donors in patients with acute leukemia is associated with a higher response rate than that seen with DLI from related donors. In one analysis, 8 of 19 (42%) patients with relapse acute leukemia achieved a complete response after unrelated DLI (Porter *et al.*, 2000). Despite the responses, outcomes for these patients were poor, as 30% died of treatment-related complications, 30% relapsed, and median survival was only 11 weeks.

TABLE III Results of MDS and Acute Leukemia Treated with DLI Alone

Disease	*North American experience (Collins* et al., *1997)*	*European experience (Kolb, 1998)*
Myelodysplasia	2/5 (40)	3/9 (33)
Acute myeloid leukemia	6/39 (15)	12/42 (29)
Acute lymphocytic leukemia	2/15 (13)	1/22 (5)

Many patients with relapsed acute leukemia after allogeneic transplantation have been treated with chemotherapy followed by DLI. In some cases, chemotherapy was administered because of rapidly progressive disease or in an attempt to debulk patients prior to DLI. Although the overall response rate to chemotherapy plus DLI is higher than DLI alone, long-term outcome does not appear significantly improved. A clinical trial that combined chemotherapy and DLI demonstrated an overall complete response rate of 47% (Levine *et al.*, 2002). Unfortunately, the toxicity associated with this approach was high, with a treatment-related mortality of 23% and a disappointing 2-year overall survival of 19% for all patients.

VII. Chronic Lymphocytic Leukemia and Lymphoma

Although there is indirect evidence of the existence of a GVL effect (Jones *et al.*, 1991), the DLI experience in patients with chronic lymphocytic lymphoma (CLL) and low-grade lymphoma is limited. Patients with CLL have obtained a complete response following DLI. The time to complete response might be prolonged, with one patient being followed 12 months after a single infusion of donor lymphocytes before obtaining a remission (Alyea *et al.*, 1999). There are case reports of patients with follicular lymphoma responding to DLI (Mandigers *et al.*, 1998). In the report from the North American registry, no responses were noted in patients with non-Hodgkin's lymphoma or in two patients with Hodgkin's disease (Collins *et al.*, 1997). Future reports of DLI will no doubt contain additional information about the response rate in these patients.

VIII. EBV-Associated Lymphoproliferative Disorders After Bone Marrow Transplantation

DLI and the infusion of Epstein-Barr virus (EBV) specific cytotoxic T cells is a highly effective treatment for posttransplant EBV-associated lymphoproliferative disorders (EBV-LPD) and represent a paradigm of success for the use of adoptive immunotherapy. In the early 1990s, Papadapoulos *et al.* (1994) treated five patients with post-BMT EBV-LPD, using infusions of unirradiated donor leukocytes at a dose of 1.0×10^6 CD3+ T cells body weight (one tenth the usual CD3+ cell dose used for treating relapsed disease), and observed a 100% pathologic and clinical response without significant GVHD. The therapeutic effect was attributed to the high concentration of EBV-specific CTLs normally present in the circulating lymphocyte pool and their transfer through the DLI, resulting in the development of EBV-specific immunity. As an extension of this strategy, researchers have now demonstrated that administration of *in vitro*

cultivated EBV-specific CTLs alone is sufficient to eradicate EBV-LPD (Gustafsson *et al*., 2000; Heslop *et al*., 1994; Rooney *et al*., 1995, 1998). PCR tests are now available that can quantify EBV DNA and offer a method of diagnosing patients prior to the onset of clinically evident EBV-LPD (Rooney *et al*., 1995). With this tool for early detection, prophylactic administration of EBV-specific CTLs can now be used as preemptive therapy against EBV-LPD after BMT (Gustafsson *et al*., 2000; Rooney *et al*., 1998).

IX. Complications of DLI

A. Graft-Versus-Host Disease

GVHD is the principle complication of DLI. GVHD occurs in 45–100% of patients with CML who achieve a complete cytogenetic response (Antin, 1993; Collins *et al*., 1997; Kolb *et al*., 1995) The GVHD that develops after DLI often has characteristics of chronic GVHD, involving the liver and skin; GVHD with characteristics of acute GVHD has also been noted. A variant of hepatic GVHD manifesting primarily as elevated serum transaminases and lobular hepatitis histologically has been described in patients after DLI (Akpek *et al*., 2002). Complications related to GVHD and its treatment are the primary reason for the 10–20% treatment-related mortality associated with DLI. The association between response to DLI and the development of GVHD suggests that GVL and GVHD might be closely related. However, clinical responses can be seen in some patients in the absence of GVHD, suggesting that these processes are distinct (Fig. 2). The separation of GVL and GVHD, both experimentally and clinically, remains an area of active investigation. Efforts to diminish GVHD without compromising GVL have included reducing T-cell doses, adjusting the timing of cell infusion after BMT, and selectively depleting T cells from the infusion product.

As previously discussed, infusion of low doses of T cells results in high response rates in patients with CML with minimal GVHD. This has led to a strategy of using escalating doses of lymphocytes, with infusion of higher doses of cells being reserved for patients who do not respond to the initial DLI (Dazzi *et al*., 2000a). To minimize toxicity associated with the infusion of larger number of cells, patients must be followed for prolonged periods, because response to DLI might be delayed. Responses have been noted up to 9 months after a single course of DLI. The relationship among cell dose, response, and toxicity is not well established in other diseases.

Administration of DLI very early after transplantation is associated with significant GVHD. In an early study by Sullivan *et al*. (1989), a high incidence of GVHD was noted with DLI given within the first weeks after BMT. While examining DLI at a later time point after transplantation, Barrett noted an increased risk of GVHD associated with early T-cell infusion at Day 30 after BMT compared with infusions at Day 45 after T-cell-depleted allogeneic BMT

(Barrett *et al.*, 1998). Larger registry studies of DLI did not demonstrate an increased risk GVHD when DLI was administered either within the first year or beyond 1 year after transplantation (Collins *et al.*, 1997; Kolb *et al.*, 1995).

Two strategies that use selective T-cell infusion have been explored to limit GVHD while preserving GVL: DLI depleted of CD8+ cells or DLI in which a suicide gene has been transduced into the infused cells. In clinical transplantation, evidence suggests that CD8+ cells play a role in the development of GVHD in humans. This evidence includes the observation that patients with a higher number of circulating CD8+ T cells during the period of early lymphoid reconstitution have an increased risk of developing GVHD (Soiffer *et al.*, 1993). In a clinical transplant, selective T-cell depletion of donor marrow with an anti-CD8 monoclonal antibody was found to be capable of reducing the incidence of GVHD without leading to an increased risk of relapse (Nimer *et al.*, 1994).

Two trials of CD8+ cell depletion prior to DLI have been performed (Alyea *et al.*, 1998; Giralt *et al.*, 1995). The incidence of GVHD noted in these trials was low when compared with that in trials using unmanipulated donor cell infusions. In one study, approximately 50% of patients with CML who achieved a complete cytogenetic response did not develop evidence of clinical GVHD. In addition, no patient receiving CD8-depleted donor lymphocytes developed GVHD in the absence of a response. GVHD has been noted to occur in some patients who have not achieved a response when treated with unmanipulated DLI. These two studies suggest that CD4+ donor cell infusions are capable of inducing a GVL effect while reducing the risk of GVHD. The responses to CD4+ DLI also appear durable (Shimoni *et al.*, 2001). A direct comparison of CD4+ DLI with unmanipulated DLI administered 6 months after T-cell-depleted DLI has been performed, and a significantly lower incidence of GVHD was noted in patients receiving CD4+ DLI. Larger comparative trials are necessary to confirm this finding.

As an alternative strategy to separate GVHD from GVL, investigators have designed donor T cells transduced with a suicide gene, the herpes simplex virus thymidine kinase (HStk), which could be capitalized on if the patient develops GVHD after DLI (Bonini *et al.*, 1994; Verzeletti *et al.*, 1994, 1995). These transduced cells retain their full immunologic potential (Marktel *et al.*, 2003), but are now amenable to selective destruction by the administration of ganciclovir in the event that unwanted GVHD occurs (Glazier *et al.*, 1983). This strategy offers the potential for inducing GVL response while having an easy mechanism for abolishing GVHD when it develops. Bordignon, Bonini, and coworkers reported on eight patients with relapsed leukemia or EBV-LPD after allogeneic BMT who were treated with HStk-tranduced lymphocyte infusions (Bonini *et al.*, 1997; Bordignon *et al.*, 1995). No toxicity or complication attributable to the gene transfer procedure was observed, and the transduced T cells remained detectable 12 months after infusion. Three of the eight patients developed GVHD (two

acute and one chronic GVHD) after the infusion, which was effectively controlled by eliminating the transgenic cells by ganciclovir. Similar results were reported by Tiberghien *et al.* (2001) in 12 patients who were given prophylactic DLI consisting of 2–20 $\times$ 10^5 HStk-transduced lymphocytes/kg following T-cell-depleted alloBMT. No acute toxicity related to the gene-modified cells was observed, and circulating HStk-transduced cells could be detected beyond 1 year in evaluable patients. Four patients developed clinically significant GVHD and were treated with ganciclovir, two achieved complete GVHD resolution with ganciclovir alone, and a third patient had resolution of GVHD after addition of glucocorticoid to ganciclovir.

X. Methods to Enhance the GVL Response after DLI

Strategies to enhance the GVL effect mediated by DLI have included activation of the infused cells, as well as methods to improve potential target antigen presentation. Slavin *et al.* (1996) administered IL-2 to patients following DLI. In addition, some patients received allogeneic cells that had been activated *ex vivo* by IL-2. Five of six patients with advanced hematologic malignancies who did not respond to DLI alone achieved remissions with the addition of IL-2 to DLI. In a trial at the Dana-Farber Cancer Institute, low-dose IL-2 was given for 12 weeks following DLI to patients with MDS, acute leukemia, and advanced-phase CML. Low-dose IL-2 administration was well tolerated and did not result in increased GVHD or additional toxicity. Responses were observed in some patients, but were rarely durable.

Several groups have attempted to prime donor cells prior to infusion. One approach has been to prime donor T cells by immunization of donors with immunoglobulin idiotype, as in multiple myeloma. This approach has been used in patients with myeloma undergoing conventional transplantation (Kwak *et al.*, 1995). A second approach has been to infuse *in vitro* generated and T-cell clones that have antileukemic activity. Falkenburg *et al.* (1999) reported the successful treatment of a patient with accelerated-phase CML using this approach. A similar approach by Slavin (2001) employed *in vitro* primed donor lymphocytes that had been incubated with irradiated lymphocytes obtained from the recipient in an attempt to immunize the donor cells. Future efforts to improve the response to DLI should focus on methods to augment tumor antigen presentation and identification of mediators and targets of the GVL effect.

XI. Mediators of the GVL Effect

The majority of evidence suggests that donor T cells mediate the GVL effect in animal models. In murine models, the relative contribution of either CD8+ or CD4+ T-cell subsets in mediating the GVL effect depends on the

HLA and minor antigen relationship between donor and host as well as the target antigens expressed by the malignant cell. CD8+ cells appear to mediate the GVL effect in the majority of models through direct cytotoxicity of the target cell. To highlight the importance of CD8+ cells, investigators demonstrated that mice receiving bone marrow depleted of CD8+ cells had an increased risk of leukemia relapse compared with mice receiving marrow depleted of CD4+ cells (Truitt and Atasoylu, 1991). In contrast, infusion of CD8-depleted marrow with the addition of CD4+ T cells leads to a low incidence of GVHD while preserving GVL in other models (Korngold and Sprent, 1987). The mechanism by which CD4+ cells mediate a GVL response is not clear.

Indirect evidence suggests that T cells mediate GVL in humans. Clinical trials have demonstrated that T-cell-depleted BMT results in the loss of significant GVL. This loss of GVL is responsible for the increased relapse rate seen in CML patients after T-cell-depleted BMT, which approaches 40–60% as compared with 10–20% after non-T-cell-depleted BMT. Both CD4+ and CD8+ T-cell subsets with antileukemic activity have been generated *in vitro* (Faber *et al.*, 1995; Jiang *et al.*, 1996a; Oettel *et al.*, 1994; van Lochem *et al.*, 1992). CD4+ T cells with selective cytotoxicity of Ph+ clones have been identified *in vivo*; however, with prolonged culture specificity appears to wane (Oettel *et al.*, 1994).

Serial phenotypic analysis has not revealed the *in vivo* expansion of either a population of CD8+ or CD4+ T cells in patients responding to DLI. T-cell repertoire analysis has also been employed as a more sensitive method to assess changes in the T-cell compartment following DLI. In some patients with CML and myeloma who respond to DLI, selective T-cell clonal expansion has been noted at the time of response (Claret *et al.*, 1997; Orsini *et al.*, 2000), and expansion of tumor-specific CD8+ T-cell clones has been demonstrated in the peripheral blood of multiple myeloma patients after DLI response (Orsini *et al.*, 2003).

Natural killer (NK) cells have also been identified as potential mediators of GVL. NK cells appear during hematopoietic recovery after allogeneic BMT and are able to recognize differences in the target's MHC class I (Kurago *et al.*, 1995; Malnati *et al.*, 1995) and class II molecules (Jiang *et al.*, 1996). Activated NK cells mediate cytotoxicity through MHC unrestricted killing. A correlation between high number of circulating NK cells and remission status has been noted in patients after BMT (Jiang *et al.*, 1997). Murine models do not support the role of NK cells in the GVL reaction mediated by DLI (Johnson *et al.*, 2001).

Three pathways by which the GVL effect might eliminate tumor cells have been suggested (Grogg *et al.*, 1992; Susskind *et al.*, 1996; Ziegler *et al.*, 1992) Direct killing of leukemia cells by perforin and granzyme attack mediated by CD4+ or CD8+ cytotoxic lymphocytes or NK cells has been postulated. T cells might also mediate killing through cytokines such as

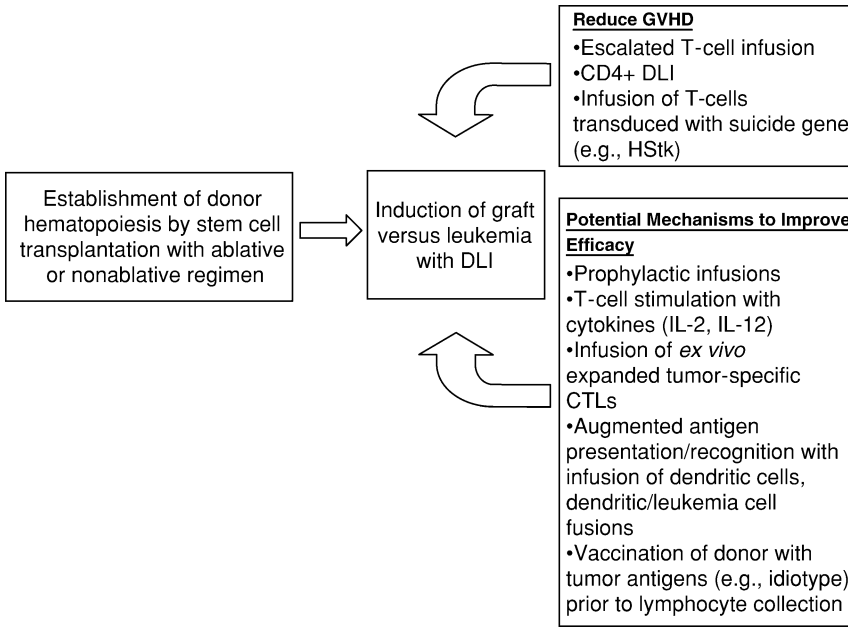

FIGURE 3 Strategies to improve response and reduce toxicity to DLI.

TNF-α and INF-γ, which have been shown to inhibit hematopoiesis (Zoumbos *et al.*, 1984). The involvement of Fas–Fas ligand interactions and the induction of apoptosis have also been implicated in the GVL effect. The Fas–Fas ligand appears to be an important pathway for T cells to mediate antigen-specific killing. Both chronic and acute leukemias have been shown to express the Fas antigen (Munker *et al.*, 1995). A more thorough understanding of the mechanisms of the GVL reaction will lead to targeted strategies that enhance the GVL effect and limit toxicity (Fig. 3).

XII. Potential Targets of the GVL Effect

Potential targets of the GVL effect can be generally divided into disease-specific and non-disease-specific antigens (Table IV). Non-disease-specific targets of the GVL response after HLA-matched allogeneic transplantation include minor histocompatibility antigens (mHAg) (Dolstra *et al.*, 1997) and gender-specific H-Y proteins in sex-mismatched donor–recipient pairs (James *et al.*, 2003; Vogt *et al.*, 2002) Disease-specific antigens include proteins restricted to malignant cells. For example, in CML, the protein product of the BCR/ABL gene fusion has been described as a potential GVL target (Bocchia *et al.*, 1996), as have other leukemia-specific proteins, including the

TABLE IV Antigenic Targets of the Graft-Versus-Leukemia Response

Non-disease-specific targets	*Tumor-specific targets*
Tissue-specific HLA minor antigens	Chronic myelogenous leukemia
HA-1	BCR/ABL
HA-2	PR1 peptide
	CML28
	CML66
Gender-specific proteins	Lymphoma/myeloma
HY antigens: SMCY, DBY	Immunoglobulin idiotype
	Broad tumor antigens
	Survivin
	Wilms' tumor protein (WT-1)

proteinase 3-derived peptide PR-1 (Molldrem *et al.*, 2000), CML28 (Yang *et al.*, 2002), CML66 (Yang *et al.*, 2001), and the antiapoptosis protein survivin (Andersen *et al.*, 2001; Schmidt *et al.*, 2003).

A. Minor Histocompatibility Antigens

In HLA-identical donor–recipient pairs, GVH and GVL can arise when alloreactive donor T cells respond to mismatched mHAgs expressed on recipient cells (Goulmy, 1997; Goulmy *et al.*, 1996). Some of these antigens are ubiquitous, including HA-3, HA-4, HA-6, and HA-7, whereas other minor antigens are specific for tissues of hematopoietic origin, including HA-1 and HA-2. Leukemia cells express a large number of mHAgs on their surface, and mHAg-specific CTLs have been demonstrated to recognize and lyse leukemia cells *in vitro* (Faber *et al.*, 1995; van der Harst *et al.*, 1994). Ubiquitously expressed mHAgs such as H-Y, HA-3, HA-4, HA-6, HA-7, and HA-8 might be targets for both GVH and GVL reactions. However, lineage-specific mHAgs, such as the HLA-A_2-restricted HA-1 and HA-2, which are expressed on hematopoietic-derived tissue only, could theoretically result in GVL activity without GVHD (Falkenburg *et al.*, 2002). This specificity would also explain the frequent conversion from mixed chimerism to complete donor hematopoiesis after response to DLI (Porter *et al.*, 1994, 1996). Despite this restriction, however, HA-1 was the first mHAg demonstrated to be associated with acute GVHD after HSCT (Goulmy *et al.*, 1996; Tseng *et al.*, 1999). The discordance between hematopoietic-restricted expression of HA-1 and the occurrence of GVHD in nonhematopoietic tissues that do not express HA-1 could potentially be explained by a cross-priming phenomenon. In this model, recipient HA-1-positive

antigen-presentation cells (e.g., Langerhans cells and dendritic cells in skin or gut) are lysed by the allospecific donor T-cell clones, and their destruction leads to release of inflammatory cytokines and tissue antigens, which stimulates influx of T cells with other specificities to the area and triggers GVHD.

Consistent with this hypothesis, Kircher and coworkers recently reported a patient with relapsed BCR/ABL-positive ALL after SCT who achieved complete remission and concomitantly developed extensive chronic GVHD 2 months after DLI. They generated seven CD8+ alloreactive T-cell clones by stimulating the post-DLI remission peripheral blood mononuclear cells (PBMCs) with the patient's pretransplant mature dendritic cells, and demonstrated that the target of the alloreactive cells was restricted to HA-1 (Kircher *et al*., 2002). In another recent report, Marijt and coworkers treated three HA-1-and/or HA-2-positive patients with relapsed disease (two CML, one MM) after alloSCT, using DLI from their HA-1- and/or HA-2-negative donors. They were able to demonstrate the emergence of HA-1- and HA-2-specific CD8+ T cells in the blood of the recipients 5–7 weeks after DLI, and emergence of these cells was immediately followed by complete remission of disease and conversion to full donor chimerism in all three patients. All three also developed mild GVHD in association with their disease response. These investigators were able to demonstrate *in vitro* that HA-1- or HA-2-specific CTL clones isolated during clinical response strongly inhibited, in a dose-dependent manner, growth of HA-1- and HA-2-expressing malignant recipient cells, but not normal donor hematopoietic progenitor cells. These results prove the direct involvement of HA-1- and HA-2-specific CTLs in the GVL response and imply that *in vitro*–generated HA-1- or HA-2-specific CTLs might be used as adoptive immunotherapy against relapsed hematologic malignancies after HSCT (Marijt *et al*., 2003).

In gender-mismatched allogeneic transplantation using female donors, male-specific minor antigens can be important targets for GVHD and perhaps for GVL responses as well. CTL clones specific for the H-Y antigen *SMCY* have been identified in male patients with acute and chronic GVHD after sex-mismatched BMT (Mutis *et al*., 1999; Rufer *et al*., 1998). Another H-Y antigen, DBY, which is HLA-DQ5 (class II) restricted, has also been shown to be the target of a CD4+ CTL clone isolated from a male patient with acute GVHD after transplantation of stem cells from an HLA genotypically identical female donor (Vogt *et al*., 2002). Recent evidence suggests that B-cell responses against DBY might be important in the pathogenesis of chronic GVHD as well. In an analysis by Miklos *et al*. (2002) involving 60 male patients who received stem cell grafts from female donors, 29 (48%) were found to have anti-DBY antibody at 4–8 months after transplant, as compared to 2 of 39 (5%) in male patients with male donors. Furthermore, this antibody response appears to correlate with decreased relapse rates. This observation is consistent with a recently reported series involving 3238 sibling transplants, in which F→M donor–recipient pairs were found to

have a significantly lower hazard ratio for leukemia relapse, but higher odds for GVHD, compared with all other gender combinations (Randolph *et al.*, 2003).

XIII. Tumor-Specific Targets of the DLI/GVL Response

A. BCR/ABL-Derived Peptides

Junctional p210 peptides derived from the BCR/ABL fusion gene are immunogenic *in vitro* and are putative leukemia-specific targets for the potent GVL effect associated with CML. In support of this, investigators have generated T-cell clones *in vitro* that are capable of recognizing proteins created by the BCR/ABL fusion gene product (Bocchia *et al.*, 1995; Cullis *et al.*, 1994; Greco *et al.*, 1996). Four peptides specific for the b3a2 fusion in CML have been identified as having high or intermediate binding efficiency to HLA A3, A11, and B8 (Bocchia *et al.*, 1996). In addition, a p210-specific CD4+CD3+ T-cell clone of donor origin has also been generated *in vitro* by stimulating PBMCs obtained post DLI with BCR/ABL junctional peptides (Zorn *et al.*, 2001). BCR /ABL peptide-specific T-cell proliferative responses and DTH responses have also been observed *in vivo* through vaccination of chronic-phase CML patients with b3a2 fusion peptides (Pinilla-Ibarz *et al.*, 2000). Despite these reports, there has not been any conclusive evidence to demonstrate that CML cells naturally process and present this epitope or that any *in vivo* clinical response in CML patients after DLI is directed against this antigen.

B. CML66 and CML28

Assessment of humoral responses after DLI has also led to the identification of potential targets of GVL. Immunophenotyping often demonstrates an expansion of B cells after DLI, suggesting a role for humoral immunity in the GVL reaction. Using SEREX, 13 leukemia-associated target antigens have been identified by high antibody titers in patients with CML 1 year after response to DLI (Wu *et al.*, 2000). Each of these antibodies appeared only after DLI and correlated temporally with the clinical disappearance of CML. Within this panel of 13 antigens, two represented novel genes, which have since been identified as CML66 and CML28 (Yang *et al.*, 2001, 2002). Northern blot analyses of CML66 have revealed that it is a broadly expressed tumor antigen in hematopoietic malignancies and in solid tumors such as lung cancer, prostate cancer, and melanoma (Yang *et al.*, 2001). Similarly, CML28 has been identified as a tumor antigen that is widely expressed in highly proliferative malignancies, including AML and CML blast crisis. Elevated

titers of IgG specific for CML28 has also been detected in 10–33% of patients with melanoma, lung, or prostate cancer (Yang *et al.*, 2002).

C. PR1 Peptide

PR1, a peptide from the primary granule enzyme proteinase 3, is aberrantly overexpressed in myeloid leukemias and might serve as a specific target for the GVL response. By using this HLA, A2.1-restricted nine-amino-acid peptide, investigators have successfully generated PR1-specific CTL clones from healthy donors and demonstrated that these CTLs can lyse leukemia blasts (Molldrem *et al.*, 1999) and CFU-GM from HLA-A2.1 patients with CML (Molldrem *et al.*, 1997). Tetramer staining techniques have shown that PR1-specific CTLs are present in the peripheral blood of patients with CML. In one analysis, PR1-specific CTLs were found in 11 of 12 CML patients who responded to interferon therapy, compared to zero of the seven who did not respond. Furthermore, six of eight CML patients who responded to allogeneic BMT had PR1-specific CTLs in their blood, but in the one patient who relapsed after BMT, no PR-1-specific CTL could be detected (Molldrem *et al.*, 2000). Finally, it has been shown that PR1-specific CTLs exist in high TCR avidity and low TCR avidity forms, and that CML target cell killing correlates with TCR avidity. High-avidity PR1-specific CTLs could be identified in patients in cytogenetic remission after interferon therapy, but is undetectable with IFN-resistant or untreated CML patients (Molldrem *et al.*, 2003). These observations suggest that resistance and outgrowth of CML might result, in part, from the selective deletion of high-avidity CTLs. Clinical trials with PR1 peptide and adoptive cellular therapy using PR1-specific CTLs are planned, and might replace DLI as a method for eradicating leukemia cells without GVHD after allogeneic transplantation.

With continued advances in laboratory techniques and understanding of cancer biology, we will undoubtedly find many other tumor antigens that could be exploited clinically in the future. Pan-cancer antigens such as survivin and the Wilms' tumor protein (WT-1), which are overexpressed in many solid and hematologic malignancies, are increasingly recognized and could perhaps be exploited through the use of DLI (Oka *et al.*, 2003; Schmidt *et al.*, 2003). Efforts to stimulate specific immune response against these and similar tumor antigens might lead to novel vaccine strategies effective against a broad range of cancers.

XIV. Future Directions

Efforts are focused on methods to make DLI both more effective and less toxic. Current trials are defining the appropriate timing of DLI as well as the number of cells to be infused. An improved understanding of both the effector cells and targets of the GVL response will allow for more

selected therapies to be developed in the future. Ultimately, for DLI to be a viable treatment option and available to a large number of patients, DLI must be separated from conventional stem cell transplantation and its toxicities. Nonmyeloablative transplant strategies, which markedly reduce treatment-related toxicity, might provide the appropriate platform for DLI.

References

Akpek, G., Boitnott, J. K., Lee, L. A., Hallick, J. P., Torbenson, M., Jacobsohn, D. A., Arai, S., Anders, V., and Vogelsang, G. B. (2002). Hepatitic variant of graft-versus-host disease after donor lymphocyte infusion. *Blood* **100**(12), 3903–3907.

Alyea, E., Canning, C., Houde, H., Soiffer, R., Giralt, S., Webb, I., Donovan, J., Gee, A., Gribben, J., Champlin, R., and Atkinson, K. (1999). A Pilot Study of CD8+ Cell Depletion of Donor Lymphocyte Infusions (DLI) using CD8 Monoclonal Antibody coated High Density Microparticles (HDM). *Blood* **94**(10), 161a.

Alyea, E., Weller, E., Schlossman, R., Canning, C., Webb, I., Doss, D., Mauch, P., Marcus, K., Fisher, D., Freeman, A., Parikh, B., Gribben, J., Soiffer, R., Ritz, J., and Anderson, K. (2001). T-cell–depleted allogeneic bone marrow transplantation followed by donor lymphocyte infusion in patients with multiple myeloma: Induction of graft-versus-myeloma effect. *Blood* **98**(4), 934–939.

Alyea, E. P., Soiffer, R. J., Canning, C., Neuberg, D., Schlossman, R., Pickett, C., Collins, H., Wang, Y., Anderson, K. C., and Ritz, J. (1998). Toxicity and efficacy of defined doses of CD4(+) donor lymphocytes for treatment of relapse after allogeneic bone marrow transplant. *Blood* **91**(10), 3671–3680.

Andersen, M. H., Pedersen, L. O., Becker, J. C., and Straten, P. T. (2001). Identification of a cytotoxic T lymphocyte response to the apoptosis inhibitor protein survivin in cancer patients. *Cancer Res.* **61**(3), 869–872.

Antin, J. H. (1993). Graft-versus-leukemia: No longer an epiphenomenon. *Blood* **82,** 2273–2277.

Apperley, J. F., Mauro, F. R., Goldman, J. M., Gregory, W., Arthur, C. K., Hows, J., Arcese, W., Papa, A., Mandelli, F., Wardle, D., Gravett, P., Franklin, I. M., Bandini, G., Ricci, P., Tura, S., Iacone, d., Torlontano, G., Heit, W., Champlin, R., and Gale, R. P. (1988). Bone marrow transplantation for chronic myeloid leukaemia in first chronic phase, Importance of a graft-versus-leukaemia effect. *Br. J. Haematol.* **69,** 239–245.

Barnes, D. W. H., and Loutit, J. F. (1956). Immunological and histological response following spleen treatment in irradiated mice. *In* "Progress in Radiobiology" (J. S. Mitchel, B. E. Holmes, and S. C. D., Eds.). Oliver Boyd, Edinburg, 291.

Barnes, D. W. H., and Loutit, J. F. (1957). Treatment of murine leukaemia with X-rays and homologous bone marrow: II. *Br. J. Haematol.* **3,** 241–252.

Barrett, A. J., Mavroudis, D., Tisdale, J., Molldrem, J., Clave, E., Dunbar, C., Cottler-Fox, M., Phang, S., Carter, C., Okunnieff, P., Young, N. S., and Read, E. J. (1998). T cell-depleted bone marrow transplantation and delayed T cell add-back to control acute GVHD and conserve a graft-versus-leukemia effect. *Bone Marrow Transplant.* **21**(6), 543–551.

Bensinger, W. I., Buckner, C. D., Anasetti, C., Clift, R., Storb, R., Barnett, T., Chauncey, T., Shulman, H., and Appelbaum, F. R. (1996). Allogeneic marrow transplantation for multiple myeloma: An analysis of risk factors on outcome. *Blood* **88,** 2787–2793.

Bjorkstrand, B., Ljungman, P., Svensson, H., Hermans, J., Alegre, A., Apperley, J., Blade, J., Carlson, K., Cavoo, M., Perrant, A., Goldstone, A., de Laurenzi, A., Majolino, A., marcus, R., Prentice, H., Remes, K., Samson, D., Sureda, A., Verdonck, L., Volin, L., and Gahrton, G. (1996). Allogeneic bone marrow transplantation versus autologous stem cell

transplantation in multiple myeloma: A retrospective case-matched study from the European Group for Blood and Marrow Transplantation. *Blood* **88,** 4711–4718.

Bocchia, M., Korontsvit, T., Xu, Q., Mackinnon, S., Yang, S., Sette, A., and Scheinberg, D. (1996). Specific human cellular immunity to bcr-abl oncogene derived peptides. *Blood* **87,** 3587–3592.

Bocchia, M., Wentworth, P., Southwood, S., Sidney, J., McGraw, K., Scheinberg, D., and Sette, A. (1995). Specific binding of leukemia oncogene fusion protein peptides to HLA class I molecules. *Blood* **85,** 2680–2684.

Bonini, C., Ferrari, G., Verzeletti, S., Servida, P., Zappone, E., Ruggieri, L., Ponzoni, M., Rossini, S., Mavilio, F., Traversari, C., and Bordignon, C. (1997). HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. *Science* **276**(5319), 1719–1724.

Bonini, C., Verzeletti, S., Servida, P., Rossini, S., Traversari, C., Ferrari, G., Nobili, N., Mavilio, F., and Bordignon, C. (1994). Transfer of the HSV-TK gene into donor peripheral blood lymphocytes for *in vivo* immunomodulation of donor antitumor immunity after ALLO-BMT (Meeting abstract). *Blood* **84,** 110a.

Bordignon, C., Bonini, C., Verzeletti, S., Nobili, N., Maggioni, D., Traversari, C., Giavazzi, R., Servida, P., Zappone, E., Benazzi, E. *et al.* (1995). Transfer of the HSV-tk gene into donor peripheral blood lymphocytes for *in vivo* modulation of donor anti-tumor immunity after allogeneic bone marrow transplantation. *Hum. Gene. Ther.* **6,** 813–819.

Claret, E. J., Alyea, E. P., Orsini, E., Pickett, C. C., Collins, H., Wang, Y., Neuberg, D., Soiffer, R. J., and Ritz, J. (1997). Characterization of T cell repertoire in patients with graft-versus-leukemia after donor lymphocyte infusion. *J. Clin. Invest.* **100**(4), 855–866.

Collins, R., Shpilberg, O., Drobyski, W., Porter, D., Giralt, S., Champlin, R., Goodman, S., Wolff, S., Hu, W., Verfaillie, C., List, A., Dalton, W., Ognoskie, N., Chetrit, A., Antin, J., and Nemunaitis, J. (1997). Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J. Clin. Oncol.* **15,** 433–444.

Collins, R. H., Rogers, Z. R., Bennett, M., Kumar, V., Nikein, A., and Fay, J. W. (1992). Hematologic relapse of chronic myelogenous leukemia following allogeneic bone marrow transplantation. Apparent graft-versus-leukemia effect following abrupt discontinuation of immunosuppression. *Bone Marrow Transplant.* **10,** 391–395.

Cullis, J., Barrett, A., Goldman, J., and Lechter, R. (1994). Binding of BCR/ABL junctional peptides to major histocompatibility complex (MHC) class I molecules: Studies in antigen-processing defective cell lines. *Leukemia* **8,** 165–170.

Dazzi, F., Szydlo, R. M., Craddock, C., Cross, N. C., Kaeda, J., Chase, A., Olavarria, E., van Rhee, F., Kanfer, E., Apperley, J. F., and Goldman, J. M. (2000). Comparison of single-dose and escalating-dose regimens of donor lymphocyte infusion for relapse after allografting for chronic myeloid leukemia. *Blood* **95**(1), 67–71.

Dazzi, F., Szydlo, R. M., Cross, N. C., Craddock, C., Kaeda, J., Kanfer, E., Cwynarski, K., Olavarria, E., Yong, A., Apperley, J. F., and Goldman, J. M. (2000). Durability of responses following donor lymphocyte infusions for patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. *Blood* **96**(8), 2712–2716.

Dolstra, H., Fredrix, H., Preijers, F., Goulmy, E., Figdor, C. G., de Witte, T. M., and van de Wiel-van Kemenade, E. (1997). Recognition of a B cell leukemia-associated minor histocompatibility antigen by CTL. *J. Immunol.* **158,** 560–565.

Drobyski, W. R., Keever, C. A., Roth, M. S., Koethe, S., Hanson, G., McFadden, P., Gottschall, J. L., Ash, R. C., van Tuinen, P., Horowitz, M. M., and Flomenberg, N. (1993). Salvage immunotherapy using donor leukocyte infusions as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation: Efficacy and toxicity of a defined T-cell dose. *Blood* **82,** 2310–2318.

Falkenburg, J. H., Marijt, W. A., Heemskerk, M. H., and Willemze, R. (2002). Minor histocompatibility antigens as targets of graft-versus-leukemia reactions. *Curr. Opin. Hematol.* **9**(6), 497–502.

Falkenburg, J. H., Wafelman, A. R., Joosten, P., Smit, W. M., van Bergen, C. A., Bongaerts, R., Lurvink, E., van der Hoorn, M., Kluck, P., Landegent, J. E., Kluin-Nelemans, H. C., Fibbe, W. E., and Willemze, R. (1999). Complete remission of accelerated phase chronic myeloid leukemia by treatment with leukemia-reactive cytotoxic T lymphocytes. *Blood* **94**(4), 1201–1208.

Frassoni, F., Fagioli, F., Sessarego, M., Gualandi, F., vanLint, M. T., Lamparelli, T., Occhini, D., Figari, O., Valbonesi, M., and Bacigalupo, A. (1992). The effect of donor leucocyte infusion in patients with leukemia following allogeneic bone marrow transplantation. *Exp. Hemato.* **20,** 712.

Gale, R. P., Horowitz, M. M., Ash, R. C., Champlin, R. E., Goldman, J. M., Rimm, A. A., Ringd,n, O., Veum Stone, J. A., and Bortin, M. M. (1994). Identical-twin bone marrow transplants for leukemia. *Ann. Intern. Med.* **120,** 646–652.

Giralt, S., Hester, J., Huh, Y., Hirsch-Ginsberg, C., Rondcn, G., Seong, D., Lee, M., Gajewski, J., Van Besien, K., Khouri, I., Mehra, R., Przepiorka, D., K"rbling, M., Talpaz, M., Kantarjian, H., Fischer, H., Deisseroth, A., and Champlin, R. (1995). CD8-depleted donor lymphocyte infusion as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation. *Blood* **86,** 4337–4343.

Glazier, A., Tutschka, P. J., Farmer, E. R., and Santos, G. W. (1983). Graft versus host disease in cyclosporine treated rats after syngeneic and autologous bone marrow reconstitution. *J. Exp. Med.* **158,** 1.

Goldman, J. M., Gale, R. P., Horowitz, M. M., Biggs, J. C., Champlin, R. E., Gluckman, E., Hoffmann, R. G., Jacobsen, S. J., Marmont, A. M., McGlave, P. B., Messner, H. A., Rimm, A. A., Rozman, C., Speck, B., Tura, S., Weiner, R. S., and Bortin, M. M. (1988). Bone marrow transplantation for chronic myelogenous leukemia in chronic phase. Increased risk for relapse associated with T-cell depletion. *Ann. Intern. Med.* **108,** 806–814.

Goulmy, E. (1997). Human minor histocompatibility antigens: new concepts for marrow transplantation and adoptive immunotherapy. *Immunol. Rev.* **157,** 125–140.

Goulmy, E., Schipper, R., Pool, J., Blokland, E., Falkenburg, J. H., Vossen, J., Grathwohl, A., Vogelsang, G. B., van Houwelingen, H. C., and van Rood, J. J. (1996). Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. *N. Engl. J. Med.* **334**(5), 281–285.

Greco, G., Fruci, D., Accapezzato, D., Barnaba, V., Nisini, R., Alimena, G., Montefusco, E., Vigneti, E., Butler, R., Tanigaki, N., and Tosi, R. (1996). Two brc-abl junction peptides bind HLA-A3 molecules and allow specific induction of human cytotoxic T lymphocytes. *Leukemia* **10**(4), 693–699.

Grogg, D., Hahn, S., and Erb, P. (1992). CD4+ T cell-mediated killing of major histocompatibility complex class II-positive antigen-presenting cells (APC). III. CD4+ cytotoxic T cells induce apoptosis of APC. *Eur. J. Immunol.* **22**(1), 267–272.

Guglielmi, C., Arcese, W., Dazzi, F., Brand, R., Bunjes, D., Verdonck, L. F., Schattenberg, A., Kolb, H. J., Ljungman, P., Devergie, A., Bacigalupo, A., Gomez, M., Michallet, M., Elmaagacli, A., Gratwohl, A., Apperley, J., and Niederwieser, D. (2002). Donor lymphocyte infusion for relapsed chronic myelogenous leukemia: Prognostic relevance of the initial cell dose. *Blood* **100**(2), 397–405.

Gustafsson, A., Levitsky, V., Zou, J. Z., Frisan, T., Dalianis, T., Ljungman, P., Ringden, O., Winiarski, J., Ernberg, I., and Masucci, M. G. (2000). Epstein-Barr virus (EBV) load in bone marrow transplant recipients at risk to develop posttransplant lymphoproliferative disease: Prophylactic infusion of EBV-specific cytotoxic T cells. *Blood* **95**(3), 807–814.

Helg, C., Roux, E., Beris, P., Cabrol, C., Wacker, P., Darbellay, R., Wyss, M., Jeannet, M., Chapuis, B., and Roosnek, E. (1993). Adoptive immunotherapy for recurrent CML after BMT. *Bone Marrow Transplant.* **12,** 125–129.

Heslop, H. E., Brenner, M. K., Rooney, C., Krance, R. A., Roberts, W. M., Rochester, R., Smith, C. A., Turner, V., Sixbey, J., Moen, R. *et al.* (1994). Administration of neomycin-resistance-gene-marked EBV-specific cytotoxic T lymphocytes to recipients of mismatched-related or phenotypically similar unrelated donor marrow grafts. *Hum. Gene. Ther.* **5**(3), 381–397.

Higano, C. S., Brixey, M., Bryant, E. M., Durnam, D. M., Doney, K., Sullivan, K. M., and Singer, J. W. (1990). Durable complete remission of acute nonlymphocytic leukemia associated with discontinuation of immunosuppression following relapse after allogeneic bone marrow transplantation. A case report of a probable graft-versus-leukemia effect. *Transfusion* **50,** 175–177.

Horowitz, M. M., Gale, R. P., Sondel, P. M., Goldman, J. M., Kersey, J., Kolb, H. J., Rimm, A. A., Ringdén, O., Rozman, C., Speck, B., Truitt, R. L., Zwaan, F. E., and Bortin, M. M. (1990). Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* **75,** 555–562.

Huff, C. A., Fuchs, E. J., Noga, S. J., O'Donnell, P. V., Ambinder, R. F., Diehl, L., Borrello, I., Vogelsang, G. B., Miller, C. B., Flinn, I. A., Brodsky, R. A., Marcellus, D., and Jones, R. J. (2003). Long-term follow-up of T cell-depleted allogeneic bone marrow transplantation in refractory multiple myeloma: Importance of allogeneic T cells. *Biol. Blood Marrow Transplant.* **9**(5), 312–319.

James, E., Chai, J. G., Dewchand, H., Macchiarulo, E., Dazzi, F., and Simpson, E. (2003). Multiparity induces priming to male-specific minor histocompatibility antigen, HY, in mice and humans. *Blood* **102**(1), 388–393.

Jiang, Y., Mavroudis, D., Dermime, S., Hensel, N., Couriel, D., Molldrem, J., and Barrett, A. (1996). Alloreactive CD4+ T lymphocytes can exert cytotoxicity to chronic myeloid leukaemia cells processing and presenting exogenous antigen. *Br. J. Haematol.* **93,** 606–612.

Jiang, Y. Z., Barrett, A. J., Goldman, J. M., and Mavroudis, D. A. (1997). Association of natural killer cell immune recovery with a graft-versus-leukemia effect independent of graft-versus-host disease following allogeneic bone marrow transplantation. *Ann. Hematol.* **74**(1), 1–6.

Jiang, Y. Z., Couriel, D., Mavroudis, D. A., Lewalle, P., Malkovska, V., Hensel, N. F., Dermime, S., Molldrem, J., and Barrett, A. J. (1996). Interaction of natural killer cells with MHC class II: Reversal of HLA-DR1-mediated protection of K562 transfectant from natural killer cell-mediated cytolysis by brefeldin-A. *Immunology* **87,** 481–486.

Jiang, Y. Z., Kanfer, E. J., Macdonald, D., Cullis, J. O., Goldman, J. M., and Barrett, A. J. (1991). Graft-versus-leukaemia following allogeneic bone marrow transplantation: Emergence of cytotoxic T lymphocytes reacting to host leukaemia cells. *Bone Marrow Transplant.* **8,** 253–258.

Johnson, B. D., Dagher, N., Stankowski, W. C., Hanke, C. A., and Truitt, R. L. (2001). Donor natural killer (NK1.1+) cells do not play a role in the suppression of GVHD or in the mediation of GVL reactions after DLI. *Biol. Blood Marrow Transplant.* **7**(11), 589–595.

Jones, R. J., Ambinder, R. F., Piantadosi, S., and Santos, G. W. (1991). Evidence of a graft-versus-lymphoma effect associated with allogeneic bone marrow transplantation. *Blood* **77,** 649–653.

Kircher, B., Stevanovic, S., Urbanek, M., Mitterschiffthaler, A., Rammensee, H. G., Grunewald, K., Gastl, G., and Nachbaur, D. (2002). Induction of HA-1-specific cytotoxic T-cell clones parallels the therapeutic effect of donor lymphocyte infusion. *Br. J. Haematol.* **117**(4), 935–939.

Kolb, H. (1998). Donor Lleukocyte transfusions for treatment of leukemic relapse after bone marrow transplantation. *Vox. Sang.* **74,** 321–329.

Kolb, H., Mittermuller, J., Clemm, C., Holler, E., Ledderose, G., Brehm, G., Heim, M., and Wikmanns, W. (1990). Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* **76,** 2462–2465.

Kolb, H. J. (1998). Donor leukocyte transfusions for treatment of leukemic relapse after bone marrow transplantation. EBMT Immunology and Chronic Leukemia Working Parties. *Vox. Sang.* **74**(Suppl. 2), 321–329.

Kolb, H. J., Schattenberg, A., Goldman, J. M., Hertenstein, B., Jacobsen, N., Arcese, W., Ljungman, P., Ferrant, A., Verdonck, L., Niederwieser, D., van Rhee, F., Mittermüller, J., de Witte, T., Holler, E., Ansari, H., and the European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia, f. (1995). Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood* **86,** 2041–2050.

Korngold, R., and Sprent, J. (1987). T cell subsets and graft versus host disease. *Transplant.* **44,** 335.

Kurago, Z. B., Smith, K. D., and Lutz, C. T. (1995). NK cell recognition of MHC class I. NK cells are sensitive to peptide-binding groove and surface alpha-helical mutations that affect T cells. *J. Immunol.* **154**(6), 2631–2641.

Kwak, L. W., Taub, D. D., Duffey, P. L., Bensinger, W. I., Bryant, E. M., Reynolds, C. W., and Longo, D. L. (1995). Transfer of myeloma idiotype-specific immunity from an actively immunised marrow donor. *Lancet* **345,** 1016–1020.

Le Blanc, R., Montminy-Metivier, S., Belanger, R., Busque, L., Fish, D., Roy, D. C., Kassis, J., Boileau, J., Lavallee, R., Belanger, D., Letendre, F., Hebert, J., Sauvageau, G., Perreault, C., and Roy, J. (2001). Allogeneic transplantation for multiple myeloma: Further evidence for a GVHD-associated graft-versus-myeloma effect. *Bone Marrow Transplant.* **28**(9), 841–848.

Levine, J. E., Braun, T., Penza, S. L., Beatty, P., Cornetta, K., Martino, R., Drobyski, W. R., Barrett, A. J., Porter, D. L., Giralt, S., Leis, J., Holmes, H. E., Johnson, M., Horowitz, M., and Collins, R. H., Jr. (2002). Prospective trial of chemotherapy and donor leukocyte infusions for relapse of advanced myeloid malignancies after allogeneic stem-cell transplantation. *J. Clin. Oncol.* **20**(2), 405–412.

Lokhorst, H. M., Schattenberg, A., Cornelissen, J. J., van Oers, M. H., Fibbe, W., Russell, I., Donk, N. W., and Verdonck, L. F. (2000). Donor lymphocyte infusions for relapsed multiple myeloma after allogeneic stem-cell transplantation: predictive factors for response and long-term outcome. *J. Clin. Oncol.* **18**(16), 3031–3037.

Mackinnon, S., Papadapoulos, E. B., Carabasi, M. H., Reich, L., Collins, N. H., Boulad, F., Castro-Malaspina, H., Childs, B. H., Gillio, A. P., Kernan, N. A., Small, T. N., Young, J. W., and O'Reilly, R. J. (1995). Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: Separation of graft-versus-leukemia responses from graft-versus-host disease. *Blood* **86,** 1261–1268.

Malnati, M. S., Peruzzi, M., Parker, K. C., Biddison, W. E., Ciccone, E., Moretta, A., and Long, E. O. (1995). Peptide specificity in the recognition of MHC class I by natural killer cell clones. *Science* **267**(5200), 1016–1018.

Mandigers, C. M., Meijerink, J. P., Raemaekers, J. M., Schattenberg, A. V., and Mensink, E. J. (1998). Graft-versus- lymphoma effect of donor leucocyte infusion shown by real-time quantitative PCR analysis of t(14;18). *Lancet* **352**(9139), 1522–1523.

Marijt, W. A., Heemskerk, M. H., Kloosterboer, F. M., Goulmy, E., Kester, M. G., van der Hoorn, M. A., van Luxemburg-Heys, S. A., Hoogeboom, M., Mutis, T., Drijfhout, J. W., van Rood, J. J., Willemze, R., and Falkenburg, J. H. (2003). Hematopoiesis-restricted

minor histocompatibility antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. *Proc. Natl. Acad. Sci. USA* **100**(5), 2742–2747.

Marktel, S., Magnani, Z., Ciceri, F., Cazzaniga, S., Riddell, S. R., Traversari, C., Bordignon, C., and Bonini, C. (2003). Immunologic potential of donor lymphocytes expressing a suicide gene for early immune reconstitution after hematopoietic T-cell-depleted stem cell transplantation. *Blood* **101**(4), 1290–1298.

Marmont, A., Horowitz, M. M., Gale, R. P., Sobocinski, K., Ash, R. C., van Bekkum, D. W., Champlin, R. E., Dicke, K. A., Goldman, J. M., Good, R. A., Herzig, R. H., Hong, R., Masaoka, T., Rimm, A. A., Ringd,n, O., Speck, B., Weiner, R., and Bortin, M. M. (1991). T-cell depletion of HLA-identical transplants in leukemia. *Blood* **78**, 2120–2130.

Miklos, D., Kim, H., Zorn, E., Hochberg, E., Guo, L., Mattes-Ritz, A., Soiffer, R. J., Antin, J., and Ritz, J. (2002). Antibody response to H-Y minor histocompatibility antigen DBY is detected in male patients after allogeneic stem cell transplantation and in normal female donors. *Blood* **100**(11), 213a (abstr 802).

Molldrem, J. J., Clave, E., Jiang, Y. Z., Mavroudis, D., Raptis, A., Hensel, N., Agarwala, V., and Barrett, A. J. (1997). Cytotoxic T lymphocytes specific for a nonpolymorphic proteinase 3 peptide preferentially inhibit chronic myeloid leukemia colony-forming units. *Blood* **90**(7), 2529–2534.

Molldrem, J. J., Lee, P. P., Kant, S., Wieder, E., Jiang, W., Lu, S., Wang, C., and Davis, M. M. (2003). Chronic myelogenous leukemia shapes host immunity by selective deletion of high-avidity leukemia- specific T cells. *J. Clin. Invest.* **111**(5), 639–647.

Molldrem, J. J., Lee, P. P., Wang, C., Champlin, R. E., and Davis, M. M. (1999). A PR1-human leukocyte antigen-A2 tetramer can be used to isolate low-frequency cytotoxic T lymphocytes from healthy donors that selectively lyse chronic myelogenous leukemia. *Cancer Res.* **59**(11), 2675–2681.

Molldrem, J. J., Lee, P. P., Wang, C., Felio, K., Kantarjian, H. M., Champlin, R. E., and Davis, M. M. (2000). Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. *Nat. Med.* **6**(9), 1018–1023.

Munker, R., Lubbert, M., Yonehara, S., Tuchnitz, A., Mertelsmann, R., and Wilmanns, W. (1995). Expression of the Fas antigen on primary human leukemia cells. *Ann. Hematol.* **70**(1), 15–17.

Mutis, T., Gillespie, G., Schrama, E., Falkenburg, J. H., Moss, P., and Goulmy, E. (1999). Tetrameric HLA class I-minor histocompatibility antigen peptide complexes demonstrate minor histocompatibility antigen-specific cytotoxic T lymphocytes in patients with graft-versus-host disease. *Nat. Med.* **5**(7), 839–842.

Nimer, S. D., Giorgi, J., Gajewski, J. L., Ku, N., Schiller, G. J., Lee, K., Territo, M., Ho, W., Feig, S., Selch, M., Isacescu, V., Reichert, T. A., and Champlin, R. E. (1994). Selective depletion of CD8+ cells for prevention of graft-versus-host disease after bone marrow transplantation. A randomized controlled trial. *Transplant.* **57**, 82–87.

Odom, L. F., August, C. S., Githens, J. H., Humbert, J. R., Morse, H., Peakman, D., Sharma, B., Rusnak, S. L., and Johnson, F. B. (1978). Remission of relapsed leukaemia during a graft-versus-host reaction. A graft-versus-leukaemia reaction in man? *Lancet* **2**, 537–540.

Oettel, K. R., Wesly, O. H., Albertini, M. R., Hank, J. A., Iliopolis, O., Sosman, J. A., Voelkerding, K., Wu, S. Q., Clark, S. S., and Sondel, P. M. (1994). Allogeneic T-cell clones able to selectively destroy Philadelphia chromosome-bearing (Ph1+) leukemia leines can also recognize Ph1-cells from the same patient. *Blood* **83**, 3390–3402.

Oka, Y., Tsuboi, A., Murakami, M., Hirai, M., Tominaga, N., Nakajima, H., Elisseeva, O. A., Masuda, T., Nakano, A., Kawakami, M., Oji, Y., Ikegame, K., Hosen, N., Udaka, K., Yasukawa, M., Ogawa, H., Kawase, I., and Sugiyama, H. (2003). Wilms tumor gene peptide-based immunotherapy for patients with overt leukemia from myelodysplastic syndrome (MDS) or MDS with myelofibrosis. *Int. J. Hematol.* **78**(1), 56–61.

Orsini, E., Alyea, E. P., Schlossman, R., Canning, C., Soiffer, R. J., Chillemi, A., Neuberg, D., Anderson, K. C., and Ritz, J. (2000). Changes in T cell receptor repertoire associated with graft-versus-tumor effect and graft-versus-host disease in patients with relapsed multiple myeloma after donor lymphocyte infusion. *Bone Marrow Transplant.* **25**(6), 623–632.

Orsini, E., Bellucci, R., Alyea, E. P., Schlossman, R., Canning, C., McLaughlin, S., Ghia, P., Anderson, K. C., and Ritz, J. (2003). Expansion of tumor-specific CD8+ T cell clones in patients with relapsed myeloma after donor lymphocyte infusion. *Cancer Res.* **63**(10), 2561–2568.

Papadapoulos, E. B., Ladanyi, M., Emmanuel, D., Mackinnon, S., Boulad, F., Carabasi, M. H., Castro-Malaspina, H., Childs, B. H., Gillio, A. P., Small, T. N., Young, J. W., Kernan, N. A., and O'Reilly, R. J. (1994). Infusions of donor leukocytes to treat Epstein-Barr-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. *N. Engl. J. Med.* **330,** 1185–1191.

Peggs, K. S., Mackinnon, S., Williams, C. D., D'sa, S., Thuraisundaram, D., Kyriakou, C., Morris, E. C., Hale, G., Waldmann, H., Linch, D. C., Goldstone, A. H., and Yong, K. (2003). Reduced-intensity transplantation with *in vivo* T-cell depletion and adjuvant dose-escalating donor lymphocyte infusions for chemotherapy-sensitive myeloma: Limited efficacy of graft-versus-tumor activity. *Biol. Blood Marrow Transplant.* **9**(4), 257–265.

Pinilla-Ibarz, J., Cathcart, K., Korontsvit, T., Soignet, S., Bocchia, M., Caggiano, J., Lai, L., Jimenez, J., Kolitz, J., and Scheinberg, D. A. (2000). Vaccination of patients with chronic myelogenous leukemia with bcr-abl oncogene breakpoint fusion peptides generates specific immune responses. *Blood* **95**(5), 1781–1787.

Porter, D. L., Collins, R. H., Jr., Hardy, C., Kernan, N. A., Drobyski, W. R., Giralt, S., Flowers, M. E., Casper, J., Leahey, A., Parker, P., Mick, R., Bate-Boyle, B., King, R., and Antin, J. H. (2000). Treatment of relapsed leukemia after unrelated donor marrow transplantation with unrelated donor leukocyte infusions. *Blood* **95**(4), 1214–1221.

Porter, D. L., Collins, R. H., Jr., Shpilberg, O., Drobyski, W. R., Connors, J. M., Sproles, A., and Antin, J. H. (1999). Long-term follow-up of patients who achieved complete remission after donor leukocyte infusions. *Biol. Blood Marrow Transplant.* **5**(4), 253–261.

Porter, D. L., Roth, M. S., Lee, S. J., McGarigle, C., Ferrara, J. L., and Antin, J. H. (1996). Adoptive immunotherapy with donor mononuclear cell infusions to treat relapse of acute leukemia or myelodysplasia after allogeneic bone marrow transplantation. *Bone Marrow Transplant.* **18**(5), 975–980.

Porter, D. L., Roth, M. S., McGarigle, C., Ferrara, J. L. M., and Antin, J. H. (1994). Induction of graft-vs-host disease as immunotherapy for relapsed chronic myelogenous leukemia. *N. Engl. J. Med.* **330,** 100–106.

Raiola, A. M., Van Lint, M. T., Valbonesi, M., Lamparelli, T., Gualandi, F., Occhini, D., Bregante, S., di Grazia, C., Dominietto, A., Soracco, M., Romagnani, C., Vassallo, F., Casini, M., Bruno, B., Frassoni, F., and Bacigalupo, A. (2003). Factors predicting response and graft-versus-host disease after donor lymphocyte infusions: A study on 593 infusions. *Bone Marrow Transplant.* **31**(8), 687–693.

Randolph, S. S., Gooley, T. A., Warren, E. H., Appelbaum, F. R., and Riddell, S. R. (2003). Female donors contribute to a selective graft versus leukemia effect in male recipients of HLA matched related hematopoietic cell transplants. *Blood* **103**(1), 347–352.

Rooney, C. M., Loftin, S. K., Holladay, M. S., Brenner, M. K., Krance, R. A., and Heslop, H. E. (1995). Early identification of Epstein-Barr virus-associated post-transplantation lymphoproliferative disease. *Br. J. Haematol.* **89**(1), 98–103.

Rooney, C. M., Smith, C. A., Ng, C. Y., Loftin, S., Li, C., Krance, R. A., Brenner, M. K., and Heslop, H. E. (1995). Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. *Lancet* **345**(8941), 9–13.

Rooney, C. M., Smith, C. A., Ng, C. Y., Loftin, S. K., Sixbey, J. W., Gan, Y., Srivastava, D. K., Bowman, L. C., Krance, R. A., Brenner, M. K., and Heslop, H. E. (1998). Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. *Blood* **92**(5), 1549–1555.

Rufer, N., Wolpert, E., Helg, C., Tiercy, J. M., Gratwohl, A., Chapuis, B., Jeannet, M., Goulmy, E., and Roosnek, E. (1998). HA-1 and the SMCY-derived peptide FIDSYICQV (H-Y) are immunodominant minor histocompatibility antigens after bone marrow transplantation. *Transplant.* **66**(7), 910–916.

Salama, M., Nevill, T., Marcellus, D., Parker, P., Johnson, M., Kirk, A., Porter, D., Giralt, S., Levine, J. E., Drobyski, W., Barrett, A. J., Horowitz, M., and Collins, R. H. (2000). Donor leukocyte infusions for multiple myeloma. *Bone Marrow Transplant.* **26**(11), 1179–1184.

Schmidt, S. M., Schag, K., Muller, M. R., Weck, M. M., Appel, S., Kanz, L., Grunebach, F., and Brossart, P. (2003). Survivin is a shared tumor-associated antigen expressed in a broad variety of malignancies and recognized by specific cytotoxic T cells. *Blood* **102**(2), 571–576.

Shimoni, A., Gajewski, J. A., Donato, M., Martin, T., O'Brien, S., Talpaz, M., Cohen, A., Korbling, M., Champlin, R., and Giralt, S. (2001). Long-Term follow-up of recipients of CD8-depleted donor lymphocyte infusions for the treatment of chronic myelogenous leukemia relapsing after allogeneic progenitor cell transplantation. *Biol. Blood Marrow Transplant.* **7**(10), 568–575.

Slavin, S. (2001). Immunotherapy of cancer with alloreactive lymphocytes. *Lancet Oncol.* **2**(8), 491–498.

Slavin, S., Naparstek, E., Nagler, A., Ackerstein, A., Samuel, S., Kapelushnik, J., Brautbar, C., and Or, R. (1996). Allogeneic cell therapy with donor peripheral blood cells and recombinant human interleukin-2 to treat leukemia relapse after allogeneic bone marrow transplantion. *Blood* **87**(6), 2195–2204.

Soiffer, R. J., Gonin, R., Murray, C., Robertson, M. J., Cochran, K., Chartier, S., Cameron, C., Daley, J., Levine, H., Nadler, L. M. *et al.* (1993). Prediction of graftversus-host disease by phenotypic analysis of early immune reconstitution after CD6-depleted allogeneic bone marrow transplantation. *Blood* **82**(7), 2216–2223.

Sullivan, K. M., Storb, R., Buckner, C. D., Fefer, A., Fisher, L., Weiden, P. L., Witherspoon, R. P., Appelbaum, F. R., Banaji, M., Hansen, J., Martin, P., Sanders, J. E., Singer, J., and Thomas, E. D. (1989). Graft-versus-host disease as adoptive immunotherapy in patients with advanced hematologic neoplasms. *N. Engl. J. Med.* **320,** 828–834.

Susskind, B., Iannotti, M. R., Shornick, M. D., Steward, N. S., Gorka, J., and Mohanakumar, T. (1996). Indirect allorecognition of HLA class I peptides by CD4+ cytolytic T lymphocytes. *Hum. Immunol.* **46**(1), 1–9.

Tiberghien, P., Ferrand, C., Lioure, B., Milpied, N., Angonin, R., Deconinck, E., Certoux, J. M., Robinet, E., Saas, P., Petracca, B., Juttner, C., Reynolds, C. W., Longo, D. L., Herve, P., and Cahn, J. Y. (2001). Administration of herpes simplex-thymidine kinase-expressing donor T cells with a T-cell-depleted allogeneic marrow graft. *Blood* **97**(1), 63–72.

Truitt, R. L., and Atasoylu, A. A. (1991). Contribution of CD4+ and CD8+ T cells to graft-versus-host disease and graft-versus-leukemia reactivity after transplantation of MHC-compatible bone marrow. *Bone Marrow Transplant.* **8**(1), 51–58.

Truitt, R. L., and Johnson, B. D. (1995). Principles of graft-vs.-leukemia reactivity. *Biol. Blood Marrow Transplant.* **1,** 61–68.

Tseng, L. H., Lin, M. T., Hansen, J. A., Gooley, T., Pei, J., Smith, A. G., Martin, E. G., Petersdorf, E. W., and Martin, P. J. (1999). Correlation between disparity for the minor histocompatibility antigen HA-1 and the development of acute graft-versus-host disease after allogeneic marrow transplantation. *Blood* **94**(8), 2911–2914.

van der Harst, D., Goulmy, E., Falkenburg, J. H., Kooij-winkelaar, Y. M., van Luxemburg-Heijs, S. A., Goselink, H. M., and Brand, A. (1994). Recognition of minor histocompatibility antigens on lymphocytic and myeloid leukemic cells by cytotoxic T-cell clones. *Blood* **83**(4), 1060–1066.

van Lochem, E., de Gast, B., and Goulmy, E. (1992). *In vitro* separation of host specific graft-versus-host and graft-versus- leukemia cytotoxic T cell activities. *Bone Marrow Transplant.* **10**(2), 181–183.

Verzeletti, S., Bonini, C., Traversari, C., Rossini, S., Ferrari, G., Nobili, N., Servida, P., Mavilio, F., and Bordignon, C. (1994). Transfer of the HSV-tK gene into donor peripheral blood lymphocytes for *in vivo* immunomodulation of donor antitumor immunity after allo- BMT (Meeting abstract). *Gene. Ther.* **1,** S24.

Verzeletti, S., Bonini, C., Traversari, C., Rossini, S., Ferrari, G., Nobili, N., Servida, P., Mavilio, F., and Bordignon, C. (1995). Retroviral vector gene transfer into donor peripheral blood lymphocytes for *in vitro* selection and *in vivo* immunomodulation of donor antitumor immunity after allo-BMT (Meeting abstract). *J. Cell. Biochem.* **59** (Suppl. 21A), 356.

Vogt, M. H., van den Muijsenberg, J. W., Goulmy, E., Spierings, E., Kluck, P., Kester, M. G., van Soest, R. A., Drijfhout, J. W., Willemze, R., and Falkenburg, J. H. (2002). The DBY gene codes for an HLA-DQ5-restricted human male-specific minor histocompatibility antigen involved in graft-versus-host disease. *Blood* **99**(8), 3027–3032.

Weiden, P. L., Flournoy, N., Thomas, E. D., Prentice, R., Buckner, C. D., and Storb, R. (1979). Antileukemic effect of graft-versus-host disease in recipients of allogeneic-marrow grafts. *N. Engl. J. Med.* **300,** 1068–1073.

Weiden, P. L., Sullivan, K., Flournoy, N., Storb, R., Thomas, E. D., and Team, t.S. M. T. (1981). Antileukemic effect of chronic graft-versus-host disease. Contribution to improved survival after allogeneic marrow transplantation. *N. Engl. J. Med.* **304,** 1529–1533.

Wu, C. J., Yang, X. F., McLaughlin, S., Neuberg, D., Canning, C., Stein, B., Alyea, E. P., Soiffer, R. J., Dranoff, G., and Ritz, J. (2000). Detection of a potent humoral response associated with immune-induced remission of chronic myelogenous leukemia. *J. Clin. Invest.* **106**(5), 705–714.

Yang, X. F., Wu, C. J., Chen, L., Alyea, E. P., Canning, C., Kantoff, P., Soiffer, R. J., Dranoff, G., and Ritz, J. (2002). CML28 Is a Broadly Immunogenic Antigen, Which Is Overexpressed in Tumor Cells. *Cancer Res.* **62**(19), 5517–5522.

Yang, X. F., Wu, C. J., McLaughlin, S., Chillemi, A., Wang, K. S., Canning, C., Alyea, E. P., Kantoff, P., Soiffer, R. J., Dranoff, G., and Ritz, J. (2001). CML66, a broadly immunogenic tumor antigen, elicits a humoral immune response associated with remission of chronic myelogenous leukemia. *Proc. Natl. Acad. Sci. USA* **98**(13), 7492–7497.

Ziegler, T. R., Young, L. S., Benfell, K., Scheltinga, M., Hortos, K., Bye, R., Morrow, F. D., Jacobs, D. O., Smith, R. J., Antin, J. H., and Wilmore, D. W. (1992). Clinical and metabolic efficacy of glutamine-supplemented parenteral nutrition after bone marrow transplantation. A randomized, double-blind, controlled study. *Ann. of Intern. Med.* **116,** 821–828.

Zorn, E., Orsini, E., Wu, C. J., Stein, B., Chillemi, A., Canning, C., Alyea, E. P., Soiffer, R. J., and Ritz, J. (2001). A CD4+ T cell clone selected from a CML patient after donor lymphocyte infusion recognizes BCR-ABL breakpoint peptides but not tumor cells. *Transplant.* **71**(8), 1131–1137.

Zoumbos, N. C., Djeu, J. Y., and Young, N. S. (1984). Interferon is the suppressor of hematopoiesis generated by stimulated lymphocytes *in vitro*. *J. Immunol.* **113**(2), 769–774.

Renier J. Brentjens and Michel Sadelain

Department of Medicine and Clinical Laboratories
Leukemia Service
Memorial Sloan-Kettering Cancer Center
New York, New York 10021

Somatic Cell Engineering and the Immunotherapy of Leukemias and Lymphomas

I. Chapter Overview

Most patients with leukemia or lymphoma either die from their disease or remain incurable. For this reason, novel strategies are needed to treat these diseases. Tumor cells, including those of hematologic origin, have developed multiple mechanisms to escape detection by the host immune system. This lack of immunogenicity has long hampered investigators in their attempts to harness the immune system to eradicate cancers. In recent years, advances in the field of tumor immunology have given investigators insights into these mechanisms of immune escape. With the advent of efficient gene transfer techniques, these insights are now being translated into novel treatment strategies. Poorly immunogenic tumor cells can now be engineered to recruit and activate host immune cells through the transfer of cytokine genes or directly stimulate effector immune cells through the transfer of genes encoding costimulatory molecules. Immunization with dendritic

Advances in Pharmacology, Volume 51

1054-3589/04 $35.00

cells modified to express tumor antigens could potentially induce a potent host tumor-specific immunity as well. Alternatively, the development of efficient T-cell transduction methodologies has enabled investigators to alter patient T-cell specificity to recognize antigens present on tumor cells, thus bypassing the need to prime and expand T cells within the tumor-bearing host. It is now possible to efficiently expand these T cells *ex vivo* to clinically relevant numbers prior to subsequent adoptive transfer back into the host. To date, a great deal of promising preclinical data has been published on these immune-based approaches for the treatment of hematologic malignancies. What remains largely unknown at this time is the efficacy of these strategies in patients.

II. Introduction

Despite current effective therapies for hematologic malignancies, most patients with leukemia or lymphoma either die of their disease or are incurable (Laport and Larson, 1997; Mayer *et al*., 1994; Schultze, 1997). For this reason, novel strategies to treat these diseases are required. Recent advances in the understanding of how the immune system functions in relation to malignant cells have enabled investigators to propose and test theories of how tumor cells escape detection by the host immune system and proliferate. Many mechanisms of tumor immune escape have been described to date. Notably, tumor cells might either fail to express or express at very low levels tumor-associated antigens (TAs) that could otherwise be targeted by the host immune system (Dunn *et al*., 2002; Vasmel *et al*., 1989). Furthermore, tumor cells might downregulate expression of major histocompatibility complex (MHC) molecules necessary for the presentation of TAs on the cell surface (Garrido *et al*., 1997). As a result, tumor cells become invisible to effector cells of the host immune system. Tumor cells further fail to generate inflammatory environments wherein cytokines that can enhance the recruitment of host immune cells to the site of the tumor are secreted (Leroy *et al*., 1998). More significantly, tumor cells might express cytokines, including IL-10 (Blay *et al*., 1993) and TGFβ (Letterio and Roberts, 1998), that are able to suppress the host immune response. Finally, most hematologic tumor cells fail to express costimulatory ligands able to bind costimulatory receptors on tumor-specific T cells (Hirano *et al*., 1996). A lack of costimulatory signals to the T cell during activation results in T-cell anergy or apoptosis (Schultze *et al*., 1996; Sotomayor *et al*., 1996). Lack of costimulatory ligands on the tumor cell might therefore result in abrogating, rather than activating, tumor-specific T-cell immunity.

One major goal of cancer immunotherapy is to circumvent the many mechanisms whereby tumor cells avoid detection in order to generate immune effector cells capable of recognizing and eradicating malignant cells.

To date, several immune-based therapies have already demonstrated efficacy against a select group of hematologic malignancies. Specifically, low- and intermediate-grade B-cell malignancies respond well to therapy with monoclonal antibodies targeting CD20 when used as a single agent or in combination with standard chemotherapies (Czuczman *et al*., 1999; Grillo-Lopez *et al*., 1999; Hainsworth *et al*., 2000). Alternatively, patients who relapse with chronic myeloid leukemia (CML) or, to a lesser degree, acute myeloid leukemia (AML) after allogeneic bone marrow transplantation can be cured through the infusion of donor T cells that recognize the host tumor cells (Collins *et al*., 1997; Kolb *et al*., 1990). Similarly, patients in the posttransplant setting who develop Epstein-Barr Virus (EBV)-associated lymphoproliferative disease can be cured with EBV-specific donor leukocyte (lymphocyte) infusions (DLIs; see Papadopoulos *et al*., 1994).

Infusion of both the tumor-targeted antibodies and tumor-specific T cells (DLIs) are examples of passive immunity, that is, adoptively transferred tumor immunity generated outside the host. Active tumor immunity, the generation of tumor-specific immunity within the host itself, is classically achieved through immunization of the host with tumor-derived protein extracts, plasmid DNA, or autologous antigen-presenting cells (APCs) consisting of either tumor cells or tumor antigen-loaded specialized APCs. In principle, this approach is superior to passive immunization strategies in that it generates a long-lasting antitumor protection capable of containing or eradicating relapsed disease indefinitely. However, active immunity assumes that competent tumor-specific effector cells are present within the host. This approach further presumes that these otherwise impotent immune cells can be stimulated in such a manner as to allow for the efficient generation of antitumor activity. To date, successful active immunization for hematologic malignancies in the clinical setting has been primarily limited to anti-idiotype vaccines in the setting of low-grade follicular lymphomas (Hsu *et al*., 1996, 1997; Nelson *et al*., 1996).

Through the recent advances in gene transfer technology, it is now possible to apply our knowledge of tumor immunology to generate potentially effective immunotherapies for hematologic malignancies. Specifically, the ability to genetically modify tumor cells to express either costimulatory ligands or immunoregulatory cytokines, or both, might result in more efficient tumor cell vaccines capable of inducing a clinically significant active cellular immune response. Genetically modified professional APCs that stably express TAs can serve as potential vaccines as well. Alternatively, the genetic modification of immune effector cells to recognize tumor cells allows for the rapid *ex vivo* generation of autologous tumor-specific T cells. In this review, we highlight some of the more promising approaches to the treatment of hematologic malignancies that specifically use gene transfer as a means to generate cell-mediated antitumor immunity.

III. Immune Effector Cell Activation

Cellular immune recognition and killing of tumor cells is generally mediated by two different effector cell types. T cells are the primary effector cells of the adaptive immune system, whereas natural killer (NK) cells are the primary effector cells of the innate immune system.

An efficient antitumor T-cell immune response is dependent on several factors. First, the host must possess T cells able to recognize antigens presented on the tumor cell. Second, these resting effector cells require productive activation to ensure proliferation and differentiation into potent effector cells. Effective activation is mediated through the interaction of T cells with either professional APCs, which include dendritic cells, macrophages, and B cells, or target tumor cells. APCs activate T cells through presentation of tumor antigen on class I and class II MHC molecules in conjunction with the expression of costimulatory ligands that bind costimulatory receptor molecules on the T cell. T cells recognize foreign antigens through the T-cell receptor (TCR), which binds specific peptides when presented by the target cell in the context of an MHC molecule. The TCR of $CD8^+$ cytotoxic T cells recognizes peptides in the context of MHC class I molecules, whereas $CD4^+$ helper T cells bind peptides in the context of MHC class II molecules. T-cell recognition of peptide through the TCR in this manner results in an initial activation termed *signal 1*. However, T-cell recognition through the TCR–peptide–MHC complex alone can result in T-cell anergy or apoptosis (Jenkins *et al*., 1991; Staveley-O'Carroll *et al*., 1998). To overcome this fate, T cells require a second signal, termed *signal 2*, mediated through the interaction of a costimulatory ligand on the APC or tumor cell, with a costimulatory receptor on the T cell. Classically, this second signal is mediated by the interaction of CD28 on the T cell with B7.1 or B7.2 on the APC. Stimulation of T cells through the TCR–peptide–MHC complex in the context of costimulation results in both T-cell activation and T-cell proliferation. Because tumor cells frequently fail to induce an inflammatory response to induce the recruitment of APCs to the tumor site and often do not express sufficient amounts of costimulatory ligands, an efficient T-cell antitumor response does not develop. In this setting, effective presentation of TAs would therefore require cross-presentation to T cells by professional APCs in a favorable context such as the lymph node.

NK cells, the effector cells of the innate immune system, bind MHC class I molecules through killer inhibitory receptors (KIRs), thereby inhibiting cytotoxic activity (Costello *et al*., 2003; Smyth *et al*., 2002). When the NK cell encounters a cell lacking MHC class I molecules, such as a tumor cell, this inhibitory signal is lost. Subsequently, cytotoxicity signals mediated through the binding of NK cell activation receptors, including NKG2D, natural cytotoxicity receptors, and costimulatory receptors, to ligands on the tumor cell activate NK cell cytotoxic activity (Costello *et al*., 2003;

Smyth *et al*., 2002). However, the lack of both an appropriate inflammatory cytokine milieu at the tumor site and MHC class I expression in the setting of limited or no expression of activating ligands on the tumor cell inhibits the NK cell antitumor activity (Costello *et al*., 2003).

IV. Immunization Against Hematologic Malignancies: Tumor Cell Vaccines

For many years, and with little success, investigators have attempted to generate a host antitumor immunity through vaccination strategies that use autologous tumor cells. In light of our current understanding of tumor immunology, it is now clear that the failure to generate a potent antitumor immunity in these early studies was largely due to the lack of tumor cell immunogenicity. With the advent of efficient gene transfer technology, generation of tumor cells with enhanced immunogenicity is now possible. Multiple reports have shown that both aggressive and indolent hematologic tumor cells can be genetically modified. Successful gene transfer in primary tumor cells, as well as tumor cell lines, has been achieved by using both viral vectors (retrovirus, lentivirus, herpes virus, adenovirus, and adeno-associated virus) (Koya *et al*., 2002; Mascarenhas *et al*., 1998; Naldini *et al*., 1996; Roddie *et al*., 2000; Stripecke *et al*., 2000; Tolba *et al*., 2001; Wendtner *et al*., 2002) and nonviral strategies such as plasmid electroporation (Mascarenhas *et al*., 1998).

A. Genetic Enhancement of Immunogenicity: Tumor Cells as Antigen-Presenting Cells

1. Costimulatory Molecules: B7.1

The lack of costimulatory ligands on most hematologic tumor cells sets the stage for tumor escape from cell-mediated immunity through the induction of anergy or apoptosis of tumor-specific T cells. Introduction of a gene encoding a costimulatory ligand into the tumor cell is perhaps the most direct approach to overcoming this mechanism of immune escape by transforming the malignant cell into an APC. The encounter of tumor-specific T cells with these modified tumor cells could theoretically result in active and sustained T-cell-specific antitumor activity. This theory is supported by several *in vitro* studies wherein primary AML tumor cells and tumor cell lines transduced to express B7.1 were able to elicit activation of both allogeneic (Hirst *et al*., 1997; Matsumoto and Anasetti, 1999; Mutis *et al*., 1998) and autologous (Boyer *et al*., 1997) tumor-reactive T cells through coculture with donor T cells. Furthermore, the adoptive transfer of a cytotoxic T-cell clone generated in this manner eradicated wild-type leukemic

tumor when infused in irradiated tumor-bearing mice after syngeneic bone marrow transplant (Boyer *et al*., 1997). This T-cell-mediated antitumor effect was sustained for up to 3 months.

Work by Matulonis *et al*. (1995) demonstrated the ability of vaccination with B7.1-modified tumor cells to generate antitumor immunity *in vivo*. In this model, vaccination of immune-competent C3H/HeJ mice with the myeloid 32Dp210 tumor cell line modified to express B7.1 resulted in eradication of the modified tumor and subsequent immunity to challenge with the parental 32Dp210 cell line. Furthermore, repeated immunizations with the 32Dp210 B7.1$^+$ clone cured mice with preestablished wild-type tumor. Similar results were not obtained when mice were vaccinated with 32Dp210 tumor cells modified to express the related B7.2 costimulatory ligand (Matulonis *et al*., 1996). Consistent with these findings, Dunussi-Joannopoulos *et al*. (1996) demonstrated that mice immunized with an irradiated murine AML cell line transduced to express B7.1 resulted in a 5- to 6-month CD8$^+$ T-cell-mediated immune protection against subsequent challenge with the wild-type AML tumor. In this model, vaccination with B7.1$^+$ irradiated tumor cells soon after inoculation with wild-type tumor cells also resulted in eradication of the wild-type tumor.

The mechanism whereby B7.1-modified tumor cells generate T-cell tumor immunity remains unclear. These tumor cell vaccines presumably activate T cells independently, but elegant work by Huang *et al*. (1996) challenges this theory. By using a murine model of colon cancer, investigators found that although some degree of direct T-cell activation could occur through B7.1-modified tumor cells, the anti-tumor T cell response to B7.1$^+$ tumor cell vaccines was primarily generated through professional APCs presenting tumor cell antigens derived from the more readily lysed B7.1$^+$-modified tumor cells. In other words, in this model, B7.1 expression by a tumor does not transform the tumor cell into an efficient APC able to activate T cells independently. Rather, T-cell costimulation by the tumor cell might simply enhance the target cell killing by tumor-specific T cells. These authors contend that it is the uptake of antigen from these lysed tumors cells by professional APCs and subsequent efficient presentation of tumor antigen to tumor-specific T cells that result in the long-term tumor immunity seen in these murine tumor models.

2. *Costimulatory Molecules: CD40L*

Normal B cells can function as APCs. However, their neoplastic counterparts fail to efficiently present antigens to allow for an efficient T-cell response. This failure, as noted previously, is due in part to the lack of costimulatory molecules expressed by the tumor. The CD40 receptor, expressed on APCs, including dendritic cells, macrophages, and B cells, binds CD40L, transiently expressed on activated CD4$^+$ T cells, resulting in APC activation, with upregulation of B7.1 and B7.2 allowing for efficient

activation of both T cells and NK cells (Kuwashima *et al*., 2001). Most neoplastic B cells fail to express costimulatory ligands, but many do express CD40 (Dilloo *et al*., 1997; Schultze and Johnson, 1999).

The potential role of CD40 activation in immunotherapy of B-cell malignancies was first described by Schultze *et al*. (1995) in the context of B-cell follicular lymphoma (FL) cells. In these studies, the investigators activated patient-derived FL cells by coculture on NIH 3T3 mouse fibroblasts genetically modified to express the human CD40L gene. Tumor cells activated in this manner upregulated costimulatory molecules B7.1 and B7.2, MHC class I and II molecules, and adhesion molecules ICAM I and LFA-3, thereby mimicking an APC phenotype. Mixed lymphocyte cultures with allogeneic T cells from healthy donors resulted in T-cell proliferation consistent with an acquired APC function by the tumor cells. Furthermore, alloreactive T cells primed in this manner could subsequently proliferate when cocultured with unstimulated tumor cells from the same donor.

In a subsequent study, Dilloo *et al*. (1997) demonstrated that coculture of the $CD40^+$ A20 murine lymphoma cell line with fibroblasts expressing CD40L enhanced expression of B7.1, as well as MHC class I and II molecules. Furthermore, vaccination of mice bearing established syngeneic A20 lymphoma tumor cells with irradiated A20 cells in conjunction with irradiated fibroblasts genetically engineered to express CD40L, IL-2, or both resulted in a delayed tumor progression. Optimal antitumor response was noted in mice immunized with A20 tumor cells in conjunction with fibroblasts expressing both CD40L and IL-2. As predicted, no antitumor response could be generated against a syngeneic $CD40^-$ myeloblastic cell line (WEHI-3) by using this vaccination strategy.

Kato *et al*. (1998) demonstrated that transduction of patient-derived chronic lymphocytic leukemia (CLL) tumor cells with murine CD40L resulted in an upregulation of B7.1, B7.2, ICAM-I, and LFA-3. Significantly, CD40L-transduced tumor cells were subsequently able to transactivate untransduced CLL cells *in vitro* to upregulate expression of B7.1 and ICAM-I as well. Transduced CLL cells were furthermore able to activate allogeneic as well as autologous T cells. In the autologous setting, stimulated T cells derived from mixed lymphocyte reactions subsequently demonstrated cytotoxic activity against unmodified autologous CLL cells.

In light of these promising preclinical results, investigators subsequently tested this approach in a clinical trial, treating 11 patients with progressive-intermediate and high-risk CLL in a standard Phase I dose-escalation trial (Wierda *et al*., 2000). Patients were given a single infusion of genetically modified autologous CLL cells ranging in doses from 3×10^8 to 3×10^9 CD40L-modified CLL cells. Percent transduction with CD40L of infused CLL cells in all but the first two patients enrolled in this study ranged from 34% to 72%. The investigators demonstrated increased *in vivo* expression of B7.1, B7.2, ICAM-1, and CD95 on untransduced CLL cells for several

days after infusion of the modified tumor cells, consistent with transactivation by the modified cells. Furthermore, treatment resulted in enhanced serum levels of IL-12 and IFN-γ 48 h after tumor cell infusion. Absolute increases in T cells were noted for 1–4 weeks after treatment. Further analysis of these T cells by ELISPOT and mixed lymphocyte cultures found that this increase correlated well with an absolute increase in tumor-specific T cells. Significantly, these responses correlated to a relatively sustained (>3 months) decrease in peripheral CLL cell count as well as lymph node size. Overall, this treatment was well tolerated. Several further clinical trials are underway to both optimize and confirm the efficacy of this therapeutic approach.

B. Genetic Enhancement of Immunogenicity: Tumor Cell Cytokine Secretion

The efficacy of systemic cytokines to stimulate tumor-specific immune effector cells in solid tumor malignancies has been established in selected solid tumors such as melanoma and renal cell carcinoma. However, these therapies are limited by the side effects of systemic cytokine therapy. The potential to reduce systemic cytokine exposure by the transfer of cytokine genes *ex vivo* into the tumor cells themselves allows for therapeutic concentrations of cytokines at the tumor site without the high systemic levels of cytokine associated with dose-limiting toxicities. Enhancing tumor immunogenicity through the transfer of various cytokine genes into tumor cells for immunization is therefore an area of intense investigation.

1. Interleukin-2

IL-2, a cytokine secreted by activated T cells, stimulates the activation and proliferation of T cells, NK cells, B cells, and monocytes and macrophages. Based on promising data derived from clinical studies demonstrating an improved outcome in patients with AML after systemic IL-2 treatment (Meloni *et al*., 1994; Soiffer *et al*., 1994), early studies in leukemic tumor cell gene modification involved retroviral transduction of human leukemic tumor cell lines with the IL-2 gene. IL-2 secretion by the tumor resulted in a decreased *in vivo* tumorigenic potential in nude mice thought to be mediated by host monocytes and macrophages (Cignetti *et al*., 1994). Furthermore, *in vitro* studies using mixed lymphocyte-tumor cell cultures have shown that in both the autologous and allogeneic setting, IL-2 secretion by tumor cells elicits an initial MHC-independent NK cell response followed by an MHC-restricted $CD8^+$ cytotoxic T-cell response (Cignetti *et al*., 2000). Alternatively, expression of IL-2 by modified syngeneic bone marrow stem cells enhanced the antileukemic effect in irradiated mice after stem cell transplant and subsequent tumor cell challenge (Tam and Klingemann, 1999).

Genetic modification with IL-2 of potentially antitumor NK T cells (cytokine-induced killer cells) has been tested in a Phase I clinical trial (Schmidt-Wolf *et al*., 1999). In this study by Schmidt-Wolf and coworkers, autologous NK T cells were isolated from patients with renal cell carcinoma, colorectal cancer, and lymphoma. After *ex vivo* electroporation of the IL-2 gene, modified NK T cells were infused back into the patients. Therapy resulted in an increase in peripheral blood T cells, and, significantly, one patient with lymphoma achieved a complete response. A clinical vaccine trial in ALL that uses tumor cells transduced to coexpress an allogeneic HLA class I antigen and IL-2 is reportedly ongoing (Borgmann *et al*., 1998). Results from this trial have not been published to date.

2. *Interleukin-12*

IL-12, a heterodimeric cytokine produced by APCs, induces cytokine production, including IFN-γ, by immune effector cells. Furthermore, IL-12 enhances the proliferation as well as cytotoxic activity of T and NK cells (Trinchieri, 1994). Recombinant IL-12 has been used to treat a variety of malignancies in murine tumor models. Unfortunately, IL-12 is quite toxic to mice at therapeutic levels (Car *et al*., 1995; Coughlin *et al*., 1997). In light of the immunostimulatory properties of IL-12, investigators have studied whether its expression by tumor cells could enhance the host cellular immune response while limiting systemic side effects. Again, this approach, like that of B7.1 and CD40L transduction, attempts to enhance immunogenicity by conferring APC-like properties on the tumor cell. Early studies in murine tumor models demonstrated the feasibility of this strategy. For example, Tahara *et al*. (1995) induced a long-lasting immunity to MCA207 murine sarcoma cell line by immunization with live MCA207 tumor cells transduced to express IL-12 both before and after inoculation with the wild-type tumor. Immunity was dependent on NK cells, IFN-γ secretion, and $CD4^+$ and $CD8^+$ T cells.

In vitro, coculture of human AML or ALL tumor cells with allogeneic or autologous peripheral blood mononuclear cells (PBMCs) in the context of IL-12 results in NK-mediated lytic activity against the tumor cells (Stine *et al*., 1998). This IL-12 effect is synergistic with IL-2, even if tumor cells are resistant to coculture with PBMCs in the context of IL-2 alone. *In vivo*, murine AML cells transduced to express IL-12 elicit a potent antitumor effect both on subsequent rechallenge with wild-type tumor and on preestablished wild-type leukemia (Dunussi-Joannopoulos *et al*., 1999). This effect was mediated through the induction of IFN-γ secretion, resulting in tumor cell expression of MHC and costimulatory molecules, and was associated with an increase in tumor-specific $CD8^+$ cytotoxic T cells. More recently, Saudemont *et al*. (2002) reported similar results in a DA1-3b murine model of AML. In this model, immunization with irradiated DA1-3b tumor cells modified to express IL-12 was able to eradicate

preestablished wild-type leukemia. This effect was associated with activation of NK cells and IFN-γ expression by $CD4^+$ and $CD8^+$ T cells. In contrast to the earlier study, the IL-12 effect was $CD4^+$, not $CD8^+$ T-cell dependent. In another study, transplantation of 32Dc13 murine myeloid progenitor cells transduced to express IL-12 (32DIL-12) was associated with enhanced IFN-γ expression by mouse spleen cells (Xu *et al.*, 2001). Furthermore, these mice became resistant to challenge with transformed 32Dp210 leukemic cells through NK-cell-mediated tumor lysis.

Genetic modification of murine B-cell lymphoma A20 cells to express IL-12 resulted in tumor rejection when injected in syngeneic mice (Pizzoferrato *et al.*, 1997). Vaccinated mice were subsequently protected against rechallenge with the parental A20 cell line. Furthermore, additional modification of A20 cells with B7.1 enhanced the ability of naïve mice to reject unmodified A20 cells coinjected at the same site with the modified A20/B7.1/IL-12 tumor cells. Tumor cell growth was delayed when the unmodified A20 cells were injected at other sites. Antitumor immunity in this model was mediated by both $CD4^+$ and $CD8^+$ T cells.

3. Granulocyte-Macrophage Colony-Stimulating Factor

In an early landmark study, Dranoff *et al.* (1993) compared the ability of various cytokines to induce immunity when secreted by modified tumor cells. Investigators retrovirally transduced the otherwise nonimmunogenic B16 murine melanoma cell line with a panel of seven different cytokine genes: IL-2, IL-4, IL-5, IL-6, IFN-γ, TNFα and granulocyte-macrophage colony-stimulating factor (GMCSF). Only immunization with irradiated tumor cells expressing GMCSF resulted in a potent, long-lasting immunity to subsequent challenge with the wild-type B16 tumor. The immune response in this setting was T cell dependent.

GMCSF, a cytokine produced by activated T cells, macrophages, endothelial cells, and fibroblasts, stimulates the bone marrow to produce neutrophils and monocytes, but, more significantly, recruits and enhances the ability of dendritic cells and macrophages to function as APCs (Dranoff, 2002; Morrissey *et al.*, 1987). In theory, secretion of GMCSF by genetically modified tumor cells therefore enhances the recruitment of professional APCs to the tumor. APCs subsequently express tumor antigen in the context of MHC class I and II molecules as well as costimulatory ligands, including B7.1. Subsequent interaction with tumor-specific T cells results in an optimal activation and proliferation of these effector cells, thereby generating long-lasting tumor immunity.

The efficacy of GMCSF tumor cell vaccines in a murine model of AML was described by Dunussi-Joannopoulos *et al.* (1998). In these studies, mice immunized with murine AML cells transduced with GMCSF failed to develop leukemia. GMCSF induced immunity in this model was not T cell

dependent. Furthermore, immunization of mice 2 weeks after wild-type tumor cell inoculation with irradiated $GMCSF^+$ AML tumor cells resulted in an 80% cure rate, whereas all mice immunized with irradiated AML $B7.1^+$ cells developed lethal leukemia. Significantly, wild-type tumor challenge 4 months after rescue with the irradiated $GMCSF^+$ AML vaccine resulted in a 67% tumor rejection rate, demonstrating persistent antitumor immunity. Subsequently, several other murine models of acute leukemia have shown that the immunogenicity of $GMCSF^+$ tumor vaccines can be further enhanced by additional genetic modification of tumor cells with B7.1 or CD40L or both (Nakazaki *et al*., 1998; Stripecke *et al*., 1999; Vereecque *et al*., 2000).

Vaccination with irradiated GMCSF-transduced lymphoma cells results in the eradication of preestablished murine A20 lymphomas, whereas vaccination with A20 cells expressing either IL-2 or B7.1 has no effect on survival (Levitsky *et al*., 1996). Antitumor immunity in this setting is dependent on both $CD4^+$ and $CD8^+$ T cells. Furthermore, investigators have demonstrated the generation of idiotype-specific T cells in mice immunized with the $GMCSF^+$ A20 tumor cell line.

4. *Interferon-α*

Treatment with systemic interferon (IFN)-α has been used for several hematologic malignancies, including CML, hairy cell leukemia, and lymphoma. Although patients with CML respond variably to IFN-α therapy, treatment is hampered by side effects when administered systemically. To address this therapeutic limitation, investigators have engineered CML tumor cells to express IFN-α. Salesse *et al*. (1998) successfully transduced both the K562 CML tumor cell line and primary patient-derived $CD34^+$ CML tumor cells with the IFN-2 gene. Transduced tumor cells demonstrated an increased HLA class I expression and decreased *in vitro* proliferation. This effect was further enhanced by concomitant transduction with the IFN-γ gene (Salesse *et al*., 2000). The relevance of these data to the *in vivo* setting is currently unknown.

V. Immunization Against Hematologic Malignancies: Dendritic Cell Vaccines

Many current vaccination strategies for hematologic malignancies involve optimizing efficient TA presentation by the tumor cell to effector T cells, but an alternative approach is to engineer APCs capable of directly presenting TAs to the host immune system. It has long been realized that dendritic cells (DCs) pulsed with tumor-associated peptides when cocultured with peripheral blood T cells specifically enhance the activation and proliferation of tumor-targeted T cells. Nonetheless, this approach has limitations

(De Veerman *et al.*, 1999; Szabolcs *et al.*, 1997). First, because of the rapid turnover of the peptide–MHC complex, T-cell binding is limited to a relatively short period of time and therefore might not allow for efficient T-cell activation. Second, this approach requires identification of target peptides capable of binding various MHC haplotypes, both for class I and class II molecules, if activation of both $CD4^+$ and $CD8^+$ T cells is desired. Third, the number of TA-specific T-cell clones activated is limited to the peptides selected. Finally, this approach is exceedingly sensitive to concentration of the peptide in the DC culture: if it is too low, no T-cell activation occurs; if it is too high, less efficient, low-binding T-cell clones might be selected (Mutis *et al.*, 2002). Because DCs can be genetically modified by a variety of approaches, including retroviral transduction (Szabolcs *et al.*, 1997), these limitations might be overcome by the introduction and expression of the TA cDNA. Introduction of a whole TA into the DC allows for the sustained expression of TA peptides on class I and potentially class II MHC molecules. This approach bypasses the necessity of identifying appropriate MHC haplotype-specific peptides and likely allows for an increased number of both class I and class II peptides to be presented, resulting in a more polyclonal $CD4^+$ and $CD8^+$ antitumor response. Significantly, immunization of mice with TA-modified DCs results in the generation of TA-specific T-cell immunity to subsequent challenge with tumor cells bearing the TA (De Veerman *et al.*, 1999).

The potential for genetically modified DCs to generate allogeneic tumor-specific T cells for adoptive cell therapy in the post-stem cell transplant setting was demonstrated by Mutis *et al.* (2002). The graft-versus-leukemia (GVL) effect seen after allogeneic stem cell transplant for leukemia is mediated, in part, through donor T cells specific for mismatched minor histocompatibility antigens (mHAgs). Expression of two such antigens, HA-1 and HA-2, is restricted to hematopoietic cells. Adoptive transfer of donor T cells targeting these antigens could therefore induce a GVL effect, avoiding the undesirable graft-versus-host disease commonly seen in the allotransplant setting. Donor HA-1-specific T cells can be generated through coculture of donor T cells on DCs pulsed with HLA class I haplotype-specific HA-1 peptides. This is a long and cumbersome process. To address this limitation, investigators retrovirally transduced $HA\text{-}1^-$ donor DCs with HA-1 cDNA to allow for a sustained expression of HA-1 peptide–MHC complexes. Stimulation of donor T cells on genetically modified DCs resulted in the generation of HA-1-specific T cells within 14–21 days. Significantly, T cells generated in this manner were capable of lysing $HA\text{-}1^+$ leukemia cells *in vitro*. The generation of T cells specific to EBV antigens by using DCs genetically modified to express LMP2A or LMP2B for the potential treatment of EBV+ Hodgkin's lymphoma has been reported as well (Gahn *et al.*, 2001).

To date, in hematologic malignancies, TA genetically modified DCs have not been tested in the clinical setting. Targeting foreign antigens such

as HA-1, HA-2, LMP2A, and LMP2B is essentially tumor specific and therefore should prove to be safe. However, the successful application of genetically modified DCs as vaccines in the autologous setting will require the careful selection of suitable TA targets in order to avoid inducing potentially deleterious autoimmune disease.

VI. Adoptive T-Cell Therapy Directed Against Hematologic Malignancies

The *ex vivo* generation and subsequent infusion of autologous tumor-specific T cells into the tumor-bearing host, referred to as adoptive transfer of immunity, is another potential immunotherapeutic approach to treating malignancies. Originally, tumor-infiltrating T cells were isolated from resected tumor tissue. These tumor-specific T-cell populations could subsequently be expanded through the *ex vivo* antibody-mediated cross-linking of the TCR, resulting in a nonspecific signal 1 to the T cell, in the context of supraphysiologic concentrations of IL-2. Alternatively, tumor-specific T cells can be first cloned and subsequently expanded. However, several significant problems exist with this paradigm. The need to culture and expand tumor-reactive cells stems from their paucity in the cancer patient. The difficulties in isolating and subsequently expanding functional tumor-specific T cells are major obstacles (Melief *et al.*, 2000). Furthermore, generation of T-cell lines or clones is a cumbersome, time-consuming process. These limitations could, in theory, be overcome by the *ex vivo* genetic modification of patient T cells to recognize antigens present on the tumor. To date, two approaches have been used to genetically generate tumor-specific T cells: (1) through the transduction of TCR α/β chain genes specific to tumor cell antigens, and (2) through the transduction of tumor-specific artificial antigen receptors (Sadelain *et al.*, 2003).

A. T-Cell Receptor Gene Transfer into Autologous T Cells

Because the specificity of any given T cell is mediated through the α- and β-chain heterodimer of the TCR complex, the most obvious approach to redirecting T-cell specificity would be to insert into patient T cells the TCR genes cloned from a tumor-specific T cell restricted to the patient's HLA. The successful redirecting of T-cell specificity by transfer of the TCR α and β chains was first demonstrated in transgenic mice (Dembic *et al.*, 1986). Thereafter, several groups successfully transduced functional α/β chain heterodimers into human Jurkat leukemia T cells (Calogero *et al.*, 2000; Cole *et al.*, 1995). Successful targeting of primary human T cells to a tumor antigen was first reported in human peripheral blood T cells modified with

TCR α and β chains to recognize MART-1, an antigen expressed by most human melanomas (Clay *et al.*, 1999). Other tumor antigens, including MDM2, the EBV protein LMP2, and the melanoma tumor antigen gp100, have also been targeted in this manner (Morgan *et al.*, 2003; Orentas *et al.*, 2001; Stanislawski *et al.*, 2001).

T-cell immunotherapy through α/β chain transduction, as described here, has several potential limitations imparted by the MHC restriction of antigen recognition. First, because TCR engagement requires the expression of a specific MHC–peptide complex by the tumor cell, immune escape might be readily achieved through downregulation or loss of MHC expression, or by the lack of processing of the antigen into the targeted peptide, even if the antigen is still expressed. Second, MHC restriction dictates that a different receptor be cloned for the same antigen in a multiplicity of genetic backgrounds. In other words, a wide variety of different HLA-restricted antigen-specific T-cell clones would need to be isolated—not a trivial matter in itself—and HLA typing of all prospective patients would need to be obtained for such therapy to become universally applicable. Third, the successful generation of tumor-specific T cells through TCR gene transduction is restricted to either HLA class I or class II peptides. At least two receptors would have to be transferred if one were to engineer both $CD4^+$ and $CD8^+$ tumor-reactive T cells. Fourth, genetic modification in this manner can target T cells to protein antigens, but has no applicability to carbohydrate and glycolipid antigens that are often overexpressed on tumor cells. Finally, although TCR transduction has so far resulted in redirecting T-cell specificity as anticipated, there is the risk that hybrid TCRs could form between the endogenous and transduced α/β chains, resulting in generation of unintended specificity to other self-antigens. This latter concern can be addressed by designing TCR chains with restricted pairing abilities (Chung *et al.*, 1994; Willemsen *et al.*, 2000).

B. Chimeric Antigen Receptors

An alternative approach to generating tumor-specific T cells is through the introduction of artificial T-cell receptors, which we refer to as chimeric antigen receptors (CARs). CARs are proteins containing a TA-specific binding region fused to an intracellular domain capable of delivering an activating (signal 1) signal to the T cell. These engineered receptors are most commonly constructed by the fusion of a tumor-specific TA-binding domain, usually a single-chain fragment (scFv) antibody, although physiologic receptors or their ligands have been used as well (Altenschmidt *et al.*, 1996; Irving and Weiss, 1991; Muniappan *et al.*, 2000; Romeo and Seed, 1991), to either the TCR ζ chain-signaling domain or the FcϵRI γ chain-signaling domain. Tumor-specific scFvs are constructed by cloning the heavy- and light-chain variable regions of a TA-specific monoclonal antibody, most commonly of

murine origin, separated by a short peptide linker into a single polypeptide. The resulting protein maintains antigenic specificity. Once cloned, the gene encoding an scFv is readily fused to either the TCR ζ chain or the FcϵRI γ chain, creating a functional CAR. Introduction of the CAR gene into T cells results in the surface expression of the CAR, thereby altering the specificity of the transduced T cell to recognize a given TA.

Recognition by CARs of target antigens in an MHC-independent manner offers in principle several significant advantages over physiologic T-cell antigen recognition. First, receptor specificity is readily generated in CARs, because murine monoclonal antibodies specific to many cell surface tumor antigens already exist or are easily produced. Second, once generated, the same CAR construct can be used to treat patients independent of HLA phenotype. Third, because these artificial receptors are functional in both $CD4^+$ and $CD8^+$ T cells, transduction of patient T cells with CARs will generate both T-helper and T-cytotoxic tumor-specific cells, which might result in a more potent and sustained antitumor T-cell response. Fourth, CARs can recognize not only protein antigens but also carbohydrate (Mezzanzanica *et al*., 1998) and glycolipid antigens (Krause *et al*., 1998; Yun *et al*., 2000), allowing for the targeting of T cells to an expanded range of antigens. Finally, the risk of receptor chain reassortment associated with α/β chain transduction is not present.

Eshhar *et al*. (1993) were the first to redirect the specificity of a CAR, using a single-chain variable fragment (scFv) as the antigen recognition motif. Using a hapten-specific scFv fused to either the extracellular or the intracellular domain of the CD3 ζ chain or the FcεRI γ chain, these authors showed specific signaling by both receptors in a mouse CTL hybridoma cell line as measured by IL-2 secretion and specific lysis of hapten-conjugated A20 mouse lymphoma cells. In a similar manner, the same investigators successfully targeted mouse CTL hybridoma cells to erb-B2, a member of the epidermal growth factor receptor (EGFR) family of proteins commonly overexpressed in breast and other cancer cells (Stancovski *et al*., 1993). Since these initial reports, the construction of multiple CARs directed against a wide variety of tumor antigens has been published. Successful redirected cytolysis has been achieved in T-cell hybridomas, primary murine and human T cells, and patient-derived primary T cells. Furthermore, *in vivo* efficacy of CAR-modified T cells has been demonstrated in mouse tumor models by using modified T cells targeting erb-B2 (Altenschmidt *et al*., 1997; Moritz *et al*., 1994), the colorectal carcinoma antigen CEA (Darcy *et al*., 2000; Haynes *et al*., 2001), the adenocarcinoma antigen TAG-72 (McGuinness *et al*., 1999), and the ovarian carcinoma antigen FBP (Hwu *et al*., 1995; Wang *et al*., 1998).

Several hematologic malignancies have been targeted in this manner as well. Specifically, construction of CARs targeting CD19 (Brentjens *et al*., 2003; Cooper *et al*., 2003) and CD20 (Jensen *et al*., 1998) expressed on B-cell

malignancies, as well as CD30, targeting non-Hodgkin's lymphomas (Hombach *et al*., 1998), have been developed. In our laboratory, we have constructed a CD19-specific CAR termed 19zl. When expressed in T cells derived from healthy donors, 19zl mediates CD19-specific lysis of a wide panel of B-cell cancer cell lines, including Burkitts lymphoma, ALL, and large-cell lymphomas *in vitro*. Modified T cells are readily expanded *ex vivo* to clinically significant numbers through coculture on mouse NIH 3T3 fibroblasts engineered to express both CD19 and B7.1 in the context of IL-15. T cells expanded in this manner successfully eradicated established systemic Raji Burkitt lymphoma cells in 50–75% of SCID-Beige mice. Tumor eradication was mediated by $CD8^+$ T cells and dependent on *in vivo* costimulation with B7.1. Furthermore, T cells derived from patients with high-risk CLL could be successfully transduced with the 19zl CAR and readily expanded. Significantly, patient-derived modified T cells were able to subsequently lyse autologous CLL cells *in vitro*. In light of this promising data, we are currently in the process of initiating a clinical trial using 19zl-transduced autologous T cells in the treatment of patients with B-cell malignancies. Several clinical trials using adoptive transfer of T cells engineered to express either CD19- or CD20-specific CARs have been initiated elsewhere (Cooper *et al*., 2003). Results from these trials have not been published to date.

The lack of costimulatory molecules on most tumor cells is a potential limitation to the success of CAR-mediated adoptive T-cell therapies. Our data using 19zl-transduced T cells confirmed the necessity of T-cell costimulation *in vivo* (Brentjens *et al*., 2003). Treatment of mice bearing an established pre-B cell ALL cell line (NALM6) that does not express either B7.1 or B7.2 with 19zl-transduced T cells resulted in an overall survival benefit when compared to controls but no long-term survival. However, when modified to express B7.1, 40% of mice with established NALM6/B7.1 tumors were cured after treatment with 19zl-transduced T cells. To overcome the need for tumor-cell-mediated costimulation, we and others have further modified the CAR to include the cytoplasmic signaling domain of the CD28 receptor proximal to the CD3-chain signaling domain (Haynes *et al*., 2002; Hombach *et al*., 2001; Maher *et al*., 2002). T cells transduced with CARs containing the CD28 domain exhibit B7.1-independent costimulation *in vitro* and enhanced antitumor activity *in vivo*. Whether these constructs enhance T-cell survival and proliferation in the clinical setting remains to be established.

VII. Conclusion

The development of efficient techniques for gene transfer has proven to be an invaluable asset to tumor immunologists in their efforts to harness the immune system to treat cancer. Because most hematologic malignancies

remain incurable despite standard chemotherapeutic approaches, the potential of novel immunotherapeutic strategies holds great promise for improved survival of these diseases in the future. Genetic approaches to engineer better tumor cells, dendritic cells, and tumor-specific T cells have to date generated a great deal of promising preclinical data. However, most of these approaches have yet to be tested in a clinical setting. Although further preclinical studies will be essential to the advancement of the field, only through the establishment of scientifically sound clinical trials will we ultimately be able to judge their therapeutic benefit.

References

Altenschmidt, U., Kahl, R., Moritz, D., Schnierle, B. S., Gerstmayer, B., Wels, W., and Groner, B. (1996). Cytolysis of tumor cells expressing the Neu/erbB-2, erbB-3, and erbB-4 receptors by genetically targeted naive T lymphocytes. *Clin. Cancer Res.* **2,** 1001–1008.

Altenschmidt, U., Klundt, E., and Groner, B. (1997). Adoptive transfer of *in vitro*-targeted, activated T lymphocytes results in total tumor regression. *J. Immunol.* **159,** 5509–5515.

Blay, J. Y., Burdin, N., Rousset, F., Lenoir, G., Biron, P., Philip, T., Banchereau, J., and Favrot, M. C. (1993). Serum interleukin-10 in non-Hodgkin's lymphoma: A prognostic factor. *Blood* **82,** 2169–2174.

Borgmann, A., von Stackelberg, A., Baumgarten, E., Uchanska-Ziegler, B., Ziegler, A., Wittig, B., and Henze, G. (1998). Immunotherapy of acute lymphoblastic leukemia by vaccination with autologous leukemic cells transfected with a cDNA expression plasmid coding for an allogeneic HLA class I antigen combined with interleukin-2 treatment. *J. Mol. Med.* **76,** 215–221.

Boyer, M. W., Vallera, D. A., Taylor, P. A., Gray, G. S., Katsanis, E., Gorden, K., Orchard, P. J., and Blazar, B. R. (1997). The role of B7 constimulation by murine acute myeloid leukemia in the generation and function of a CD8+ T-cell line with potent *in vivo* graft-versus-leukemia properties. *Blood* **89,** 3477–3485.

Brentjens, R. J., Latouche, J. B., Santos, E., Marti, F., Gong, M. C., Lyddane, C., King, P. D., Larson, S., Weiss, M., Riviere, I., and Sadelain, M. (2003). Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. *Nat. Med.* **9,** 279–286.

Calogero, A., Hospers, G. A., Kruse, K. M., Schrier, P. I., Mulder, N. H., Hooijberg, E., and de Leij, L. F. (2000). Retargeting of a T cell line by anti MAGE-3/HLA-A2 alpha beta TCR gene transfer. *Anticancer Res.* **20,** 1793–1799.

Car, B. D., Eng, V. M., Schnyder, B., LeHir, M., Shakhov, A. N., Woerly, G., Huang, S., Aguet, M., Anderson, T. D., and Ryffel, B. (1995). Role of interferon-gamma in interleukin 12-induced pathology in mice. *Am. J. Pathol.* **147,** 1693–1707.

Chung, S., Wucherpfennig, K. W., Friedman, S. M., Hafler, D. A., and Strominger, J. L. (1994). Functional three-domain single-chain T-cell receptors. *Proc. Natl. Acad. Sci. USA* **91,** 12654–12658.

Cignetti, A., Guarini, A., Carbone, A., Forni, M., Cronin, K., Forni, G., Gansbacher, B., and Foa, R. (1994). Transduction of the IL2 gene into human acute leukemia cells: Induction of tumor rejection without modifying cell proliferation and IL2 receptor expression. *J. Natl. Cancer Inst.* **86,** 785–791.

Cignetti, A., Guarini, A., Gillio Tos, A., Reato, G., and Foa, R. (2000). Interleukin-2 gene-transduced human leukemic cells induce major histocompatibility complex-restricted

and -unrestricted anti-leukemic effectors in mixed lymphocyte-tumor cultures. *Cancer Gene Ther.* **7,** 167–176.

Clay, T. M., Custer, M. C., Sachs, J., Hwu, P., Rosenberg, S. A., and Nishimura, M. I. (1999). Efficient transfer of a tumor antigen-reactive TCR to human peripheral blood lymphocytes confers anti-tumor reactivity. *J. Immunol.* **163,** 507–513.

Cole, D. J., Weil, D. P., Shilyansky, J., Custer, M., Kawakami, Y., Rosenberg, S., and Nishimura, M. I. (1995). Characterization of the functional specificity of a cloned T-cell receptor heterodimer recognizing the MART-1 melanoma antigen. *Cancer Res.* **55,** 748–752.

Collins, R. H., Jr., Shpilberg, O., Drobyski, W. R., Porter, D. L., Giralt, S., Champlin, R., Goodman, S. A., Wolff, S. N., Hu, W., Verfaillie, C., List, A., Dalton, W., Ognoskie, N., Chetrit, A., Antin, J. H., and Nemunaitis, J. (1997). Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J. Clin. Oncol.* **15,** 433–444.

Cooper, L. J., Topp, M. S., Serrano, L. M., Gonzalez, S., Chang, W. C., Naranjo, A., Wright, C., Popplewell, L., Raubitschek, A., Forman, S. J., and Jensen, M. C. (2003). T-cell clones can be rendered specific for CD19: Toward the selective augmentation of the graft-versus-B-lineage leukemia effect. *Blood* **101,** 1637–1644.

Costello, R. T., Rey, J., Fauriat, C., Gastaut, J. A., and Olive, D. (2003). New approaches in the immunotherapy of haematological malignancies. *Eur. J. Haematol.* **70,** 333–345.

Coughlin, C. M., Wysocka, M., Trinchieri, G., and Lee, W. M. (1997). The effect of interleukin 12 desensitization on the antitumor efficacy of recombinant interleukin 12. *Cancer Res.* **57,** 2460–2467.

Czuczman, M. S., Grillo-Lopez, A. J., White, C. A., Saleh, M., Gordon, L., LoBuglio, A. F., Jonas, C., Klippenstein, D., Dallaire, B., and Varns, C. (1999). Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. *J. Clin. Oncol.* **17,** 268–276.

Darcy, P. K., Haynes, N. M., Snook, M. B., Trapani, J. A., Cerruti, L., Jane, S. M., and Smyth, M. J. (2000). Redirected perforin-dependent lysis of colon carcinoma by *ex vivo* genetically engineered CTL. *J. Immunol.* **164,** 3705–3712.

De Veerman, M., Heirman, C., Van Meirvenne, S., Devos, S., Corthals, J., Moser, M., and Thielemans, K. (1999). Retrovirally transduced bone marrow-derived dendritic cells require CD4+ T cell help to elicit protective and therapeutic antitumor immunity. *J. Immunol.* **162,** 144–151.

Dembic, Z., Haas, W., Weiss, S., McCubrey, J., Kiefer, H., von Boehmer, H., and Steinmetz, M. (1986). Transfer of specificity by murine alpha and beta T-cell receptor genes. *Nature* **320,** 232–238.

Dilloo, D., Brown, M., Roskrow, M., Zhong, W., Holladay, M., Holden, W., and Brenner, M. (1997). CD40 ligand induces an antileukemia immune response *in vivo*. *Blood* **90,** 1927–1933.

Dranoff, G. (2002). GM-CSF-based cancer vaccines. *Immunol. Rev.* **188,** 147–154.

Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K., Jackson, V., Hamada, H., Pardoll, D., and Mulligan, R. C. (1993). Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc. Natl. Acad. Sci. USA* **90,** 3539–3543.

Dunn, G. P., Bruce, A. T., Ikeda, H., Old, L. J., and Schreiber, R. D. (2002). Cancer immunoediting: From immunosurveillance to tumor escape. *Nat. Immunol.* **3,** 991–998.

Dunussi-Joannopoulos, K., Dranoff, G., Weinstein, H. J., Ferrara, J. L., Bierer, B. E., and Croop, J. M. (1998). Gene immunotherapy in murine acute myeloid leukemia: Granulocyte-macrophage colony-stimulating factor tumor cell vaccines elicit more potent antitumor immunity compared with B7 family and other cytokine vaccines. *Blood* **91,** 222–230.

Dunussi-Joannopoulos, K., Runyon, K., Erickson, J., Schaub, R. G., Hawley, R. G., and Leonard, J. P. (1999). Vaccines with interleukin-12-transduced acute myeloid leukemia cells elicit very potent therapeutic and long-lasting protective immunity. *Blood* **94,** 4263–4273.

Dunussi-Joannopoulos, K., Weinstein, H. J., Nickerson, P. W., Strom, T. B., Burakoff, S. J., Croop, J. M., and Arceci, R. J. (1996). Irradiated B7-1 transduced primary acute myelogenous leukemia, (AML) cells can be used as therapeutic vaccines in murine AML. *Blood* **87,** 2938–2946.

Eshhar, Z., Waks, T., Gross, G., and Schindler, D. G. (1993). Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc. Natl. Acad. Sci. USA* **90,** 720–724.

Gahn, B., Siller-Lopez, F., Pirooz, A. D., Yvon, E., Gottschalk, S., Longnecker, R., Brenner, M. K., Heslop, H. E., Aguilar-Cordova, E., and Rooney, C. M. (2001). Adenoviral gene transfer into dendritic cells efficiently amplifies the immune response to LMP2A antigen: A potential treatment strategy for Epstein-Barr virus-positive Hodgkin's lymphoma. *Int. J. Cancer* **93,** 706–713.

Garrido, F., Ruiz-Cabello, F., Cabrera, T., Perez-Villar, J. J., Lopez-Botet, M., Duggan-Keen, M., and Stern, P. L. (1997). Implications for immunosurveillance of altered HLA class I phenotypes in human tumours. *Immunol. Today* **18,** 89–95.

Grillo-Lopez, A. J., White, C. A., Varns, C., Shen, D., Wei, A., McClure, A., and Dallaire, B. K. (1999). Overview of the clinical development of rituximab: First monoclonal antibody approved for the treatment of lymphoma. *Semin Oncol.* **26,** 66–73.

Hainsworth, J. D., Burris, H. A., 3rd, Morrissey, L. H., Litchy, S., Scullin, D. C., Jr., Bearden, J. D., 3rd, Richards, P., and Greco, F. A. (2000). Rituximab monoclonal antibody as initial systemic therapy for patients with low-grade non-Hodgkin lymphoma. *Blood* **95,** 3052–3056.

Haynes, N. M., Snook, M. B., Trapani, J. A., Cerruti, L., Jane, S. M., Smyth, M. J., and Darcy, P. K. (2001). Redirecting mouse CTL against colon carcinoma: Superior signaling efficacy of single-chain variable domain chimeras containing TCR-zeta vs Fc epsilon RI-gamma. *J. Immunol.* **166,** 182–187.

Haynes, N. M., Trapani, J. A., Teng, M. W., Jackson, J. T., Cerruti, L., Jane, S. M., Kershaw, M. H., Smyth, M. J., and Darcy, P. K. (2002). Single-chain antigen recognition receptors that costimulate potent rejection of established experimental tumors. *Blood* **100,** 3155–3163.

Hirano, N., Takahashi, T., Ohtake, S., Hirashima, K., Emi, N., Saito, K., Hirano, M., Shinohara, K., Takeuchi, M., Taketazu, F., Tsunoda, S., Ogura, M., Omine, M., Saito, T., Yazaki, Y., Ueda, R., and Hirai, H. (1996). Expression of costimulatory molecules in human leukemias. *Leukemia* **10,** 1168–1176.

Hirst, W. J., Buggins, A., Darling, D., Gaken, J., Farzaneh, F., and Mufti, G. J. (1997). Enhanced immune costimulatory activity of primary acute myeloid leukaemia blasts after retrovirus-mediated gene transfer of B7.1. *Gene Ther.* **4,** 691–699.

Hombach, A., Heuser, C., Sircar, R., Tillmann, T., Diehl, V., Pohl, C., and Abken, H. (1998). An anti-CD30 chimeric receptor that mediates CD3-zeta-independent T-cell activation against Hodgkin's lymphoma cells in the presence of soluble CD30. *Cancer Res.* **58,** 1116–1119.

Hombach, A., Wieczarkowiecz, A., Marquardt, T., Heuser, C., Usai, L., Pohl, C., Seliger, B., and Abken, H. (2001). Tumor-specific T cell activation by recombinant immunoreceptors: CD3 zeta signaling and CD28 costimulation are simultaneously required for efficient IL-2 secretion and can be integrated into one combined CD28/CD3 zeta signaling receptor molecule. *J. Immunol.* **167,** 6123–6131.

Hsu, F. J., Benike, C., Fagnoni, F., Liles, T. M., Czerwinski, D., Taidi, B., Engleman, E. G., and Levy, R. (1996). Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nat. Med.* **2,** 52–58.

Hsu, F. J., Caspar, C. B., Czerwinski, D., Kwak, L. W., Liles, T. M., Syrengelas, A., Taidi-Laskowski, B., and Levy, R. (1997). Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma—long-term results of a clinical trial. *Blood* **89,** 3129–3135.

Huang, A. Y., Bruce, A. T., Pardoll, D. M., and Levitsky, H. I. (1996). Does B7-1 expression confer antigen-presenting cell capacity to tumors *in vivo*? *J. Exp. Med.* **183,** 769–776.

Hwu, P., Yang, J. C., Cowherd, R., Treisman, J., Shafer, G. E., Eshhar, Z., and Rosenberg, S. A. (1995). *In vivo* antitumor activity of T cells redirected with chimeric antibody/T-cell receptor genes. *Cancer Res.* **55,** 3369–3373.

Irving, B. A., and Weiss, A. (1991). The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. *Cell* **64,** 891–901.

Jenkins, M. K., Taylor, P. S., Norton, S. D., and Urdahl, K. B. (1991). CD28 delivers a costimulatory signal involved in antigen-specific IL-2 production by human T cells. *J. Immunol.* **147,** 2461–2466.

Jensen, M., Tan, G., Forman, S., Wu, A. M., and Raubitschek, A. (1998). CD20 is a molecular target for scFvFc:zeta receptor redirected T cells: Implications for cellular immunotherapy of CD20+ malignancy. *Biol. Blood Marrow Transplant.* **4,** 75–83.

Kato, K., Cantwell, M. J., Sharma, S., and Kipps, T. J. (1998). Gene transfer of CD40-ligand induces autologous immune recognition of chronic lymphocytic leukemia B cells. *J. Clin. Invest.* **101,** 1133–1141.

Kolb, H. J., Mittermuller, J., Clemm, C., Holler, E., Ledderose, G., Brehm, G., Heim, M., and Wilmanns, W. (1990). Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* **76,** 2462–2465.

Koya, R. C., Kasahara, N., Pullarkat, V., Levine, A. M., and Stripecke, R. (2002). Transduction of acute myeloid leukemia cells with third generation self-inactivating lentiviral vectors expressing CD80 and GM-CSF: Effects on proliferation, differentiation, and stimulation of allogeneic and autologous anti-leukemia immune responses. *Leukemia* **16,** 1645–1654.

Krause, A., Guo, H. F., Latouche, J. B., Tan, C., Cheung, N. K., and Sadelain, M. (1998). Antigen-dependent CD28 signaling selectively enhances survival and proliferation in genetically modified activated human primary T lymphocytes. *J. Exp. Med.* **188,** 619–626.

Kuwashima, N., Kageyama, S., Eto, Y., and Urashima, M. (2001). CD40 ligand immunotherapy in cancer: An efficient approach. *Leuk. Lymph.* **42,** 1367–1377.

Laport, G. F., and Larson, R. A. (1997). Treatment of adult acute lymphoblastic leukemia. *Semin. Oncol.* **24,** 70–82.

Leroy, P., Slos, P., Homann, H., Erbs, P., Poitevin, Y., Regulier, E., Colonna, F. Q., Devauchelle, P., Roth, C., Pavirani, A., and Mehtali, M. (1998). Cancer immunotherapy by direct *in vivo* transfer of immunomodulatory genes. *Res. Immunol.* **149,** 681–684.

Letterio, J. J., and Roberts, A. B. (1998). Regulation of immune responses by TGF-beta. *Annu. Rev. Immunol.* **16,** 137–161.

Levitsky, H. I., Montgomery, J., Ahmadzadeh, M., Staveley-O'Carroll, K., Guarnieri, F., Longo, D. L., and Kwak, L. W. (1996). Immunization with granulocyte-macrophage colony-stimulating factor-transduced, but not B7-1-transduced, lymphoma cells primes idiotype-specific T cells and generates potent systemic antitumor immunity. *J. Immunol.* **156,** 3858–3865.

Maher, J., Brentjens, R. J., Gunset, G., Riviere, I., and Sadelain, M. (2002). Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta/CD28 receptor. *Nat. Biotechnol.* **20,** 70–75.

Mascarenhas, L., Stripecke, R., Case, S. S., Xu, D., Weinberg, K. I., and Kohn, D. B. (1998). Gene delivery to human B-precursor acute lymphoblastic leukemia cells. *Blood* **92,** 3537–3545.

Matsumoto, K., and Anasetti, C. (1999). The role of T cell costimulation by CD80 in the initiation and maintenance of the immune response to human leukemia. *Leuk. Lymph.* **35,** 427–435.

Matulonis, U., Dosiou, C., Freeman, G., Lamont, C., Mauch, P., Nadler, L. M., and Griffin, J. D. (1996). B7-1 is superior to B7-2 costimulation in the induction and maintenance of T cell-mediated antileukemia immunity. Further evidence that B7-1 and B7-2 are functionally distinct. *J. Immunol.* **156,** 1126–1131.

Matulonis, U. A., Dosiou, C., Lamont, C., Freeman, G. J., Mauch, P., Nadler, L. M., and Griffin, J. D. (1995). Role of B7-1 in mediating an immune response to myeloid leukemia cells. *Blood* **85,** 2507–2515.

Mayer, R. J., Davis, R. B., Schiffer, C. A., Berg, D. T., Powell, B. L., Schulman, P., Omura, G. A., Moore, J. O., McIntyre, O. R., and Frei, E., 3rd. (1994). Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. *N. Engl. J. Med.* **331,** 896–903.

McGuinness, R. P., Ge, Y., Patel, S. D., Kashmiri, S. V., Lee, H. S., Hand, P. H., Schlom, J., Finer, M. H., and McArthur, J. G. (1999). Anti-tumor activity of human T cells expressing the CC49-zeta chimeric immune receptor. *Hum. Gene Ther.* **10,** 165–173.

Melief, C. J., Toes, R. E., Medema, J. P., van der Burg, S. H., Ossendorp, F., and Offringa, R. (2000). Strategies for immunotherapy of cancer. *Adv. Immunol.* **75,** 235–282.

Meloni, G., Foa, R., Vignetti, M., Guarini, A., Fenu, S., Tosti, S., Tos, A. G., and Mandelli, F. (1994). Interleukin-2 may induce prolonged remissions in advanced acute myelogenous leukemia. *Blood* **84,** 2158–2163.

Mezzanzanica, D., Canevari, S., Mazzoni, A., Figini, M., Colnaghi, M. I., Waks, T., Schindler, D. G., and Eshhar, Z. (1998). Transfer of chimeric receptor gene made of variable regions of tumor-specific antibody confers anticarbohydrate specificity on T cells. *Cancer Gene Ther.* **5,** 401–407.

Morgan, R. A., Dudley, M. E., Yu, Y. Y., Zheng, Z., Robbins, P. F., Theoret, M. R., Wunderlich, J. R., Hughes, M. S., Restifo, N. P., and Rosenberg, S. A. (2003). High efficiency TCR gene transfer into primary human lymphocytes affords avid recognition of melanoma tumor antigen glycoprotein 100 and does not alter the recognition of autologous melanoma antigens. *J. Immunol.* **171,** 3287–3295.

Moritz, D., Wels, W., Mattern, J., and Groner, B. (1994). Cytotoxic T lymphocytes with a grafted recognition specificity for ERBB2-expressing tumor cells. *Proc. Natl. Acad. Sci. USA* **91,** 4318–4322.

Morrissey, P. J., Bressler, L., Park, L. S., Alpert, A., and Gillis, S. (1987). Granulocyte-macrophage colony-stimulating factor augments the primary antibody response by enhancing the function of antigen-presenting cells. *J. Immunol.* **139,** 1113–1119.

Muniappan, A., Banapour, B., Lebkowski, J., and Talib, S. (2000). Ligand-mediated cytolysis of tumor cells: Use of heregulin-zeta chimeras to redirect cytotoxic T lymphocytes. *Cancer Gene Ther.* **7,** 128–134.

Mutis, T., Ghoreschi, K., Schrama, E., Kamp, J., Heemskerk, M., Falkenburg, J. H., Wilke, M., and Goulmy, E. (2002). Efficient induction of minor histocompatibility antigen HA-1-specific cytotoxic T-cells using dendritic cells retrovirally transduced with HA-1-coding cDNA. *Biol. Blood Marrow Transplant.* **8,** 412–419.

Mutis, T., Schrama, E., Melief, C. J., and Goulmy, E. (1998). CD80-transfected acute myeloid leukemia cells induce primary allogeneic T-cell responses directed at patient specific minor histocompatibility antigens and leukemia-associated antigens. *Blood* **92,** 1677–1684.

Nakazaki, Y., Tani, K., Lin, Z. T., Sumimoto, H., Hibino, H., Tanabe, T., Wu, M. S., Izawa, K., Hase, H., Takahashi, S., Tojo, A., Azuma, M., Hamada, H., Mori, S., and Asano, S.

(1998). Vaccine effect of granulocyte-macrophage colony-stimulating factor or CD80 gene-transduced murine hematopoietic tumor cells and their cooperative enhancement of antitumor immunity. *Gene Ther.* **5,** 1355–1362.

Naldini, L., Blomer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F. H., Verma, I. M., and Trono, D. (1996). *In vivo* gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science* **272,** 263–267.

Nelson, E. L., Li, X., Hsu, F. J., Kwak, L. W., Levy, R., Clayberger, C., and Krensky, A. M. (1996). Tumor-specific, cytotoxic T-lymphocyte response after idiotype vaccination for B-cell, non-Hodgkin's lymphoma. *Blood* **88,** 580–589.

Orentas, R. J., Roskopf, S. J., Nolan, G. P., and Nishimura, M. I. (2001). Retroviral transduction of a T cell receptor specific for an Epstein-Barr virus-encoded peptide. *Clin. Immunol.* **98,** 220–228.

Papadopoulos, E. B., Ladanyi, M., Emanuel, D., Mackinnon, S., Boulad, F., Carabasi, M. H., Castro-Malaspina, H., Childs, B. H., Gillio, A. P., Small, T. N. *et al.* (1994). Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. *N. Engl. J. Med.* **330,** 1185–1191.

Pizzoferrato, E., Chu, N. R., Hawley, T. S., Lieu, F. H., Barber, B. H., Hawley, R. G., Watts, T. H., and Berinstein, N. L. (1997). Enhanced immunogenicity of B cell lymphoma genetically engineered to express both B7-1 and interleukin-12. *Hum. Gene Ther.* **8,** 2217–2228.

Roddie, P. H., Paterson, T., and Turner, M. L. (2000). Gene transfer to primary acute myeloid leukaemia blasts and myeloid leukaemia cell lines. *Cytokines Cell Mol. Ther.* **6,** 127–134.

Romeo, C., and Seed, B. (1991). Cellular immunity to HIV activated by CD4 fused to T cell or Fc receptor polypeptides. *Cell* **64,** 1037–1046.

Sadelain, M., Riviere, I., and Brentjens, R. (2003). Targeting tumours with genetically enhanced T lymphocytes. *Nat. Rev. Cancer* **3,** 35–45.

Salesse, S., Lagarde, V., Ged, C., de Verneuil, H., Reiffers, J., and Mahon, F. X. (2000). Retroviral coexpression of IFN-alpha and IFN-gamma genes and inhibitory effects in chronic myeloid leukemia cells. *J. Interferon Cytokine Res.* **20,** 577–587.

Salesse, S., Moreau-Gaudry, F., Pigeonnier-Lagarde, V., Mazurier, F., Chahine, H., Ged, C., de Verneuil, H., Reiffers, J., and Mahon, F. X. (1998). Retroviral vector-mediated transfer of the interferon-alpha gene in chronic myeloid leukemia cells. *Cancer Gene Ther.* **5,** 390–400.

Saudemont, A., Buffenoir, G., Denys, A., Desreumaux, P., Jouy, N., Hetuin, D., Bauters, F., Fenaux, P., and Quesnel, B. (2002). Gene transfer of CD154 and IL12 cDNA induces an anti-leukemic immunity in a murine model of acute leukemia. *Leukemia* **16,** 1637–1644.

Schmidt-Wolf, I. G., Finke, S., Trojaneck, B., Denkena, A., Lefterova, P., Schwella, N., Heuft, H. G., Prange, G., Korte, M., Takeya, M., Dorbic, T., Neubauer, A., Wittig, B., and Huhn, D. (1999). Phase I clinical study applying autologous immunological effector cells transfected with the interleukin-2 gene in patients with metastatic renal cancer, colorectal cancer and lymphoma. *Br. J. Cancer* **81,** 1009–1016.

Schultze, J., and Johnson, P. (1999). A stimulating new target for cancer immunotherapy. *Lancet* **354,** 1225–1227.

Schultze, J., Nadler, L. M., and Gribben, J. G. (1996). B7-mediated costimulation and the immune response. *Blood Rev.* **10,** 111–127.

Schultze, J. L. (1997). Vaccination as immunotherapy for B cell lymphoma. *Hematol. Oncol.* **15,** 129–139.

Schultze, J. L., Cardoso, A. A., Freeman, G. J., Seamon, M. J., Daley, J., Pinkus, G. S., Gribben, J. G., and Nadler, L. M. (1995). Follicular lymphomas can be induced to present alloantigen efficiently: A conceptual model to improve their tumor immunogenicity. *Proc. Natl. Acad. Sci. USA* **92,** 8200–8204.

Smyth, M. J., Hayakawa, Y., Takeda, K., and Yagita, H. (2002). New aspects of natural-killer-cell surveillance and therapy of cancer. *Nat. Rev. Cancer* **2,** 850–861.

Soiffer, R. J., Murray, C., Gonin, R., and Ritz, J. (1994). Effect of low-dose interleukin-2 on disease relapse after T-cell-depleted allogeneic bone marrow transplantation. *Blood* **84,** 964–971.

Sotomayor, E. M., Borrello, I., and Levitsky, H. I. (1996). Tolerance and cancer: A critical issue in tumor immunology. *Crit. Rev. Oncol.* **7,** 433–456.

Stancovski, I., Schindler, D. G., Waks, T., Yarden, Y., Sela, M., and Eshhar, Z. (1993). Targeting of T lymphocytes to Neu/HER2-expressing cells using chimeric single chain Fv receptors. *J. Immunol.* **151,** 6577–6582.

Stanislawski, T., Voss, R. H., Lotz, C., Sadovnikova, E., Willemsen, R. A., Kuball, J., Ruppert, T., Bolhuis, R. L., Melief, C. J., Huber, C., Stauss, H. J., and Theobald, M. (2001). Circumventing tolerance to a human MDM2-derived tumor antigen by TCR gene transfer. *Nat. Immunol.* **2,** 962–970.

Staveley-O'Carroll, K., Sotomayor, E., Montgomery, J., Borrello, I., Hwang, L., Fein, S., Pardoll, D., and Levitsky, H. (1998). Induction of antigen-specific T cell anergy: An early event in the course of tumor progression. *Proc. Natl. Acad. Sci. USA* **95,** 1178–1183.

Stine, K. C., Warren, B. A., and Becton, D. L. (1998). Interleukin-12 (IL-12) enhances lysis of non-lymphoid leukemia cell lines in vitro. *Leukemia* **12,** 1204–1209.

Stripecke, R., Cardoso, A. A., Pepper, K. A., Skelton, D. C., Yu, X. J., Mascarenhas, L., Weinberg, K. I., Nadler, L. M., and Kohn, D. B. (2000). Lentiviral vectors for efficient delivery of CD80 and granulcyte-macrophage colony-stimulating factor in human acute lymphoblastic leukemia and acute myeloid leukemia cells to induce antileukemic immune responses. *Blood* **96,** 1317–1326.

Stripecke, R., Skelton, D. C., Pattengale, P. K., Shimada, H., and Kohn, D. B. (1999). Combination of CD80 and granulocyte-macrophage colony-stimulating factor coexpression by a leukemia cell vaccine: Preclinical studies in a murine model recapitulating Philadelphia, chromosome-positive acute lymphoblastic leukemia. *Hum. Gene Ther.* **10,** 2109–2122.

Szabolcs, P., Gallardo, H. F., Ciocon, D. H., Sadelain, M., and Young, J. W. (1997). Retrovirally transduced human dendritic cells express a normal phenotype and potent T-cell stimulatory capacity. *Blood* **90,** 2160–2167.

Tahara, H., Zitvogel, L., Storkus, W. J., Zeh, H. J., 3rd, McKinney, T. G., Schreiber, R. D., Gubler, U., Robbins, P. D., and Lotze, M. T. (1995). Effective eradication of established murine tumors with IL-12 gene therapy using a polycistronic retroviral vector. *J. Immunol.* **154,** 6466–6474.

Tam, Y. K., and Klingemann, H. G. (1999). Antileukemic effect of interleukin-2-transduced murine bone marrow after autologous transplantation. *Biol. Blood Marrow Transplant.* **5,** 231–242.

Tolba, K. A., Bowers, W. J., Hilchey, S. P., Halterman, M. W., Howard, D. F., Giuliano, R. E., Federoff, H. J., and Rosenblatt, J. D. (2001). Development of herpes simplex virus-1 amplicon-based immunotherapy for chronic lymphocytic leukemia. *Blood* **98,** 287–295.

Trinchieri, G. (1994). Interleukin-12: A cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. *Blood* **84,** 4008–4027.

Vasmel, W. L., Sijts, E. J., Leupers, C. J., Matthews, E. A., and Melief, C. J. (1989). Primary virus-induced lymphomas evade T cell immunity by failure to express viral antigens. *J. Exp. Med.* **169,** 1233–1254.

Vereecque, R., Buffenoir, G., Preudhomme, C., Hetuin, D., Bauters, F., Fenaux, P., and Quesnel, B. (2000). Gene transfer of GM-CSF, CD80 and CD154 cDNA enhances survival in a murine model of acute leukemia with persistence of a minimal residual disease. *Gene Ther.* **7,** 1312–1316.

Wang, G., Chopra, R. K., Royal, R. E., Yang, J. C., Rosenberg, S. A., and Hwu, P. (1998). A T cell-independent antitumor response in mice with bone marrow cells retrovirally transduced with an antibody/Fc-gamma chain chimeric receptor gene recognizing a human ovarian cancer antigen. *Nat. Med.* **4,** 168–172.

Wendtner, C. M., Kofler, D. M., Theiss, H. D., Kurzeder, C., Buhmann, R., Schweighofer, C., Perabo, L., Danhauser-Riedl, S., Baumert, J., Hiddemann, W., Hallek, M., and Buning, H. (2002). Efficient gene transfer of CD40 ligand into primary B-CLL cells using recombinant adeno-associated virus (rAAV) vectors. *Blood* **100,** 1655–1661.

Wierda, W. G., Cantwell, M. J., Woods, S. J., Rassenti, L. Z., Prussak, C. E., and Kipps, T. J. (2000). CD40-ligand (CD154) gene therapy for chronic lymphocytic leukemia. *Blood* **96,** 2917–2924.

Willemsen, R. A., Weijtens, M. E., Ronteltap, C., Eshhar, Z., Gratama, J. W., Chames, P., and Bolhuis, R. L. (2000). Grafting primary human T lymphocytes with cancer-specific chimeric single chain and two chain TCR. *Gene Ther.* **7,** 1369–1377.

Xu, Y. X., Gao, X., Janakiraman, N., Chapman, R. A., and Gautam, S. C. (2001). IL-12 gene therapy of leukemia with hematopoietic progenitor cells without the toxicity of systemic IL-12 treatment. *Clin. Immunol.* **98,** 180–189.

Yun, C. O., Nolan, K. F., Beecham, E. J., Reisfeld, R. A., and Junghans, R. P. (2000). Targeting of T lymphocytes to melanoma cells through chimeric anti-GD3 immunoglobulin T-cell receptors. *Neoplasia* **2,** 449–459.

Index

Contents of Previous Volumes

Volume 40

Volume 41

Volume 42

Volume 43

Volume 44

Volume 45

Volume 46

Volume 47

Hormones and Signaling
Edited by Bert W. O'Malley

Volume 48

HIV: Molecular Biology and Pathogenesis: Viral Mechanisms
Edited by Kuan-Teh Jeang

Volume 49

HIV: Molecular Biology and Pathogenesis: Clinical Applications
Edited by Kuan-Teh Jeang

Volume 50