John R.W. Masters and Bernhard O. Palsson (Eds.)

Human Cell Culture

Vol. III

Cancer Cell Lines Part 3: Leukemias and Lymphomas

Kluwer Academic Publishers

HUMAN CELL CULTURE

Volume III: Cancer Cell Lines Part 3

Human Cell Culture

Volume 3

The titles published in this series are listed at the end of this volume.

Human Cell Culture

Volume III

Cancer Cell Lines

Part 3: Leukemias and Lymphomas

edited by

John R.W. Masters
University College London, UK

and

Bernhard O. Palsson
Department of Bioengineering, University of California San Diego, USA

KLUWER ACADEMIC PUBLISHERS
NEW YORK, BOSTON, DORDRECHT, LONDON, MOSCOW

eBook ISBN: 0-306-46877-8
Print ISBN: 0-792-36225-X

Created in the United States of America

Visit Kluwer Online at: http://kluweronline.com
and Kluwer's eBookstore at: http://ebooks.kluweronline.com

Contents

Chapter 1

Human Leukemia-Lymphoma Cell Lines: Historical Perspective, State of the Art and Future Prospects

Hans G. Drexler[1] and Jun Minowada[2]
[1] *DSMZ-German Collection of Microorganisms and Cell Cultures, Department of Human and Animal Cell Cultures, Braunschweig, Germany;*[2] *Fujisaki Cell Center, Hayashibara Biochemical Laboratories, Okayama, Japan. Tel: +49-531-2616-160; Fax: +49-531-2616-150; E-mail: hdr@dsmz.de*

1. BURKITT CELL LINES WERE THE FIRST HUMAN HEMATOPOIETIC CELL LINES

In 1951 at Johns Hopkins University (Baltimore, Maryland, USA), Gey et al. established the first continuously growing human cell line (HeLa) from uterine cervix carcinoma [1]. The HeLa cell line and most other human cell lines subsequently established from various solid tumors adhere to the culture vessel growing in monolayers. In 1963 at the University of Ibadan, Nigeria, Pulvertaft established the first continuous human hematopoietic cell lines, a series of cell lines derived from Nigerian patients with Burkitt lymphoma in a suspension-type cell culture: RAJI is the best known cell line of this panel [2] (Table 1). In suspension cultures, these cells are free-floating, singly or in clusters, in the nutrient medium.

Electron microscopic analysis of these and subsequent Burkitt lymphoma-derived cell lines led to the identification of herpes-type virus particles which were later designated Epstein-Barr virus (EBV) [4,5]. The first leukemia-derived cell line was thought to be RPMI 6410, established from an American patient with acute myeloid leukemia (AML) containing similar herpes-type virus (EBV) particles in the cells [3]; however, it was shown later that this cell line was derived from normal bystander B-cells immortalized spontaneously by EBV infection and not from the leukemia cells.

The etiological significance of EBV for lymphomagenesis was questioned by several findings. Several hundred lymphoblastoid cell lines (LCLs) were established from the peripheral blood of patients with leukemias, lymphomas, other malignant tumors, and even from many healthy individuals [7–9,60]. However, EBV was detected in every cell line irrespective of the blood

J.R.W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol. III, 1–18.

Table 1. Historical milestones in the establishment of hematopoietic cell lines (chronological by the year of publication)

Year of publication	Year est.	Cell line	Investigator	Remarks: Type of cell line, derivation from malignancy, unique features	Ref.
1964	1963	RAJI, JIJOYE, OGUN, KUDI	Pulvertaft	B-cell lines (EBV+) from African Burkitt lymphoma	2
1964	1964	RPMI 6410	Iwakata & Grace	'AML-derived' cell line (EBV+) → isolation of herpes-type virus	3
1965	1964	EB-1, -2, -3	Epstein et al.	B-cell lines (EBV+) from African Burkitt lymphoma → EBV isolation	4, 5
1965	1964	CCRF-CEM	Foley et al.	T-cell line (EBV−) from T-ALL, E-rosette-negative T-cells	6
1967	1966	RPMI 7206 + 18 B-LCLs	Moore et al.	EBV+ B-LCL from normal blood donors → EBV immortalizes B-LCL	7, 8
1967	1966	RPMI 8226	Moore et al.	Plasma cell line (EBV−) from multiple myeloma	9, 10
1972	1971	MOLT-1, -2, -3, -4	Minowada et al.	T-cell lines (EBV−) from T-ALL → E-rosetting of T-cells	11
1973	1970	K-562	Lozzio & Lozzio	Myeloid-erythroid cell line from Ph+ CML-blast crisis → t(9;22) *BCR-ABL* (M-bcr) fusion gene	12–14
1974	1972	U-698-M	Nilsson & Sundström	B-cell line (EBV−) from B-cell lymphoma	15
1974	1973	SU-DHL-1	Epstein & Kaplan	ALCL cell line from malignant histiocytosis → t(2;5) *NPM-ALK* fusion gene	16
1975	1973	BJA-B	Menezes et al.	B-cell line (EBV−) from African Burkitt lymphoma	17
1976	1973	SU-AmB-1, -2	Epstein et al.	B-cell lines (EBV−) from American Burkitt lymphoma → t(8;14) *MYC-IGH* fusion gene	18
1976	1974	U-937	Sundström & Nilsson	Monocytic cell line from histiocytic lymphoma	19
1977	1974	REH	Rosenfeld et al.	Precursor B-cell line from cALL → t(12;21) *ETV6/TEL-AML1* fusion gene	20
1977	1975	JBL	Miyoshi et al.	B-cell line (EBV−) from Japanese Burkitt lymphoma	21
1977	1975	NALM-1	Minowada et al.	Precursor B-cell line from Ph+ CML-blast crisis	22–24
1977	1976	BALM-1, -2	Minowada et al.	B-cell lines (EBV+) from B-ALL	25
1977	1976	JURKAT/JM	Schneider et al.	T-cell line from T-cell NHL → IL-2 producer	26,27
1977	1976	HL-60	Collins et al.	Promyelocytic cell line from AML M2	28

Continued on next page

Table 1. (continued)

Year of publication	Year est.	Cell line	Investigator	Remarks: Type of cell line, derivation from malignancy, unique features	Ref.
1978	1977	KG-1	Koeffler & Golde	Myelocytic cell line from AML	29
1979	1976	NALM-6 to -15	Minowada et al.	Precursor B-cell lines from pre B-ALL	30
1979	1977	SKW-3	Hirano et al.	T-cell line from T-CLL	31
1979	1978	L-428	Schaadt et al.	(Non-T non-B type) Hodgkin cell line from nodular sclerosis Hodgkin's disease	32,33
1980	(?)	H9/HUT 78	Gazdar et al.	T-cell line from Sézary syndrome → HIV isolation	34,35
1980	1978	CTCL-2	Poiesz et al.	T-cell line (HTLV–1+) from Sézary syndrome → HTLV–1 isolation	36,37
1980	1978	MT-1	Miyoshi et al.	T-cell line (HTLV–1+) from ATL → immortalizing T-cells by co-culture	38
1980	1978	THP-1	Tsuchiya et al.	Monocytic cell line from AML M5	39
1981	1979	JOK-1	Andersson et al.	B-cell line from HCL	40
1982	1979	697	Findley et al.	Precursor B-cell line from ALL → t(1;19) *E2A-PBX1* fusion gene	41
1982	1980	HEL	Martin & Papayannopoulou	Erythroid cell line from AML M6	42
1985	(?)	RS4;11	Stong et al.	Precursor B-cell line from ALL → t(4;11) *MLL-AF4* fusion gene	43
1985	1981	KU-812	Kishi et al.	Basophilic cell line from CML-blast crisis	44
1985	1983	MEG-01	Ogura et al.	Megakaryocytic cell line from CML-blast crisis	45
1985	1983	YT	Yodoi et al.	T-/NK cell line from thymoma → NK activity	46
1985	1984	EoL-1	Saito et al.	Eosinophilic cell line from eosinophilic leukemia	47
1986	1982	HDLM-1, -2, -3	Lok et al.	(T-type) Hodgkin cell line from nodular sclerosis Hodgkin's disease	48
1987	1983	TOM-1	Okabe et al.	Precursor B-cell line from Ph+ ALL → t(9;22) *BCR-ABL* (m-bcr) fusion gene	49

Continued on next page

Table 1. (continued)

Year publication	Year of est.	Cell line	Investigator	Remarks: Type of cell line, derivation from malignancy, unique features	Ref.
1988	(?)	HMC-1	Butterfield et al.	Mast cell line from mast cell leukemia	50
1988	1987	M-07e	Avanzi et al.	Megakaryocytic cell line from AML M7 → constitutively growth factor-dependent	51
1989	1987	TF-1	Kitamura et al.	Erythroid cell line from AML M6 → constitutively growth factor-dependent	52
1991	1988	ME-1	Yanagisawa et al.	Monocytic cell line from AML M4eo → inv(16) *CBFB-MYH11* fusion gene	53
1991	1989	Kasumi-1	Asou et al.	Myelocytic cell line from AML M2 → t(8;21) *AML1-ETO* fusion gene	54
1991	1989	NB4	Lanotte et al.	Promyelocytic cell line from AML M3 → t(15;17) *PML-RARA* fusion gene	55
1991	1990	DoHH2	Kluin-Nelemans et al.	B-cell line from B-cell NHL → t(14;18) *IGH-BCL2* fusion gene	56
1994	1991	MDS92	Tohyama et al.	Myelocytic cell line from MDS (RARS/RAEB)	57
1995	1992	BC-1	Cesarman et al.	B-cell line from AIDS-PEL → HHV-8 isolation	58

Modified from reference [59]. This selective and arbitrary list summarizes historical milestones in the establishment of human leukemia-lymphoma cell lines and is not intended to be comprehensive. Since the first publication in 1964 more than 1000 individual cell lines have been described. It is intended to list the first cell lines for the various types and subtypes of diseases and for the different categories of cell lineages, and the first cell lines with unique or specific features (e.g. chromosomal translocations leading to fusion genes).

AIDS – acquired immunodeficiency syndrome; ALCL – anaplastic large cell lymphoma; ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia (M2, myeloblastic; M3, promyelocytic; M4eo, myelomonocytic with eosinophils; M5, monocytic; M6, erythroid; M7, megakaryocytic); AML1-ETO – fusion gene in t(8;21)(q22;q22); ATL – adult T-cell leukemia; B-cell – B-lymphocyte; BCR-ABL – fusion gene in t(9;22)(q34;q11); cALL – common ALL; CBFB-MYH11 – fusion gene in inv(16)(p13q22); CLL – chronic lymphocytic leukemia; CML – chronic myelocytic leukemia; E2A-PBX1 – fusion gene in t(1;19)(q23;p13); EBV – Epstein-Barr virus; E-rosetting – formation of rosettes with sheep erythrocytes (T-cell marker); ETV6/TEL-AML1 – fusion gene in t(12;21)(p13;q22); HCL – hairy cell leukemia; HHV-8 – human herpesvirus type-8; HIV – human immunodeficiency virus; HTLV-1 – human T-leukemia virus-1; IGH-BCL2 = fusion gene in t(14;18)(q32;q21); IL-2 – interleukin-2; LCL – lymphoblastoid (normal B-) cell line; M-bcr/m-bcr – major/minor breakpoint cluster region in t(9;22)(q34;q11); MDS – myelodysplastic syndromes; MLL-AF4 = fusion gene in t(4;11)(q21;q23); MYC-IGH – fusion gene in t(8;14)(q24;q32); NHL – non-Hodgkin's lymphoma; NK – natural killer; NPM-ALK – fusion gene in t(2;5)(p23;q35); PEL – primary effusion lymphoma; Ph – Philadelphia chromosome; PML-RARA – fusion gene in t(15;17)(q22;q11); pre B – precursor B-lymphocyte; RARS/RAEB – refractory anemia with ring sideroblasts/excess of blasts; T-cell – T-lymphocyte.

donor's health status. Furthermore, Moore and his colleagues established the first proven human myeloma cell line, RPMI 8226, which was ironically free from EBV infection [10,61,62], Most of the Burkitt lymphoma cell lines derived from North American [18,63] and Japanese patients [21] lacked EBV genomes. Moreover, the African Burkitt lymphoma cell line BJA-B was found to be free from EBV infection [17]. Thus the mere presence of EBV does not prove the neoplastic nature of the infected cells. Further studies established that EBV infection is capable of immortalizing certain B-cell subsets in normal human leukocyte cultures, hence the designation 'EBV+ B-lymphoblastoid cell lines (B-LCL)'; this term was adopted to define this type of non-malignant lymphoid cells [8].

Cytogenetic analysis of Burkitt cell lines led to the demonstration of specific chromosome translocations, t(8;14) or t(2;8) involving either heavy- or light-chain immunoglobulin genes and the c-myc oncogene [64–67]. Thus, the availability of these early hematopoietic cell lines stimulated the initial and subsequent research in diverse areas of biomedical sciences.

2. THE FIRST T LYMPHOCYTE CELL LINES

In 1971, the hematopoietic cell lines MOLT-1, -2, -3, and -4 were established from the peripheral blood of a patient in relapse from acute lymphoblastic leukemia (ALL) by Minowada et al. [11]. These cells lacked surface and cytoplasmic immunoglobulins (as signs of B-cell lineage commitment) and EBV infection. The most distinctive characteristic of the MOLT-1, -2, -3 and -4 cells was their rosette-forming ability with sheep, goat, horse and pig erythrocytes. This new immunological test referred to as the 'E-rosette test' was then found to be a specific normal human T-cell membrane marker, now termed CD2 [11].

Prior to the establishment of the MOLT 1–4 cell lines, Foley et al. had established a cell line, CCRF-CEM, from the peripheral blood of a patient with ALL, but at that time they were not able to prove its commitment to the T-cell lineage which was later shown by immunophenotyping [6].

3. ESTABLISHMENT OF CELL LINES FROM ALL HEMATOPOIETIC CELL LINEAGES

In the 1970s several still widely used and hence extremely important leukemia-lymphoma cell lines were established; just to name a few: K-562 as the first myeloid cell line and as a paradigm for a pluripotential cell line [12]; HL-60, KG-1, U-937 and THP-1 as myelocytic and monocytic model

cell lines with differentiation potential [19,28,29,39]; REH, NALM-1 and NALM-6 as precursor B-cell lines derived from patients with common ALL, pre B-ALL and lymphoid blast crisis of CML [20,25,30].

Table 1 shows the chronological steps in the establishment of the first cell lines representing each of the respective subtypes of human leukemias and lymphomas and several other cell lines that were instrumental in the detection of significant new scientific information, e.g. for the isolation of viruses, cloning of chromosomal translocation breakpoints and their new fusion genes, etc. In recent years, a number of excellent reviews have summarized and presented in great detail specific groups or types/subtypes of human leukemia-lymphoma cell lines [68–100].

The development of several public cell line banks with the ensuing availability of large panels of human leukemia-lymphoma cell lines has tremendously enhanced research in this area. A primary function of cell line banks is to provide authenticated, clean and well-characterized cell material [101,102]. The most extensive public, non-profit collection of leukemia-lymphoma cell lines has been established at the DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany; website: <www.dsmz.de>). Other cell banks such as ATCC-American Type Culture Collection (Manassas, Virginia, USA; website: <www.atcc.org>), JCRB-Japanese Collection of Research Bioresources (Tokyo, Japan; website: <http://cellbank.nihs.go.jp/defaulte.htm>) and RIKEN (Tsukuba, Ibaraki, Japan; website: <www.rtc.riken.go.jp>) also hold a limited number of the most often used and best-known leukemia-lymphoma cell lines.

4. COMMON CHARACTERISTICS OF LEUKEMIA-LYMPHOMA CELL LINES

Leukemia-lymphoma cell lines demonstrate the following three common characteristics:

- monoclonal origin
- differentiation arrest at a discrete stage during maturation in each lineage
- sustained proliferation of the cultured cells.

Until the availability of first unpurified and later recombinant growth factors in the 1980s, cell line proliferation was operationally ‘growth factor-independent’, i.e. no external cytokines were added and cells grew autonomously; however, it must be assumed that fetal bovine serum and human serum which are commonly employed as medium supplements contain certain growth-enhancing molecules. Furthermore, the autocrine stimulation of some cell lines by elaboration of known (and possibly so far unknown) growth factors has been shown [103,104]. Constitutively growth factor-dependent

cell lines represent a new category of leukemia cell lines which were first developed deliberately in the late 1980s [99,105].

The analysis of the wide range of diverse leukemia-lymphoma cell lines has allowed for the recognition of a number of facts about human hematopoietic malignancies:

- There is no specific common marker for leukemias and lymphomas in a strict sense, although operationally there are markers and/or marker profiles specific for certain subtypes of leukemias and lymphomas, such as the expression of the leukemia subtype-specific hybrid or fusion gene products.
- Multiple marker profiles of cultured leukemia-lymphoma cell lines are very similar if not identical to the marker profiles of corresponding fresh leukemia and lymphoma cells.
- There is considerable heterogeneity or near individuality in the marker profiles of cultured leukemia-lymphoma cell lines; this appears to reflect differentiation arrest of the malignant cells at various stages of normal hematopoietic cell differentiation in a general sense.
- The availability and utilization of these leukemia-lymphoma cell lines have greatly facilitated the steady progress that has been made in molecular biological and molecular genetic studies of human hematopoietic malignancies. This has been particularly evident with regard to the possible association of unique cytogenetic markers with immunoglobulin and T-cell receptor genes and a vast array of cellular oncogenes [75,106]; these findings have been extended readily to fresh leukemias and lymphomas.
- Cell lines are extremely useful for a nearly unlimited variety of practical purposes; including screening of monoclonal antibodies, pharmaceutical drugs and hormones in order to evaluate their antigenic specificity and differential effects in a variety of subtypes of leukemias and lymphomas [107]; selection of subclones based on specific features such as drug-resistance or additional chromosomal or molecular aberrations [108]; cloning of translocation breakpoints and analysis of the incidence of fusion genes, point mutations and deletions [75,109]; use of cytokine-dependent cell lines for the establishment of bioassay systems [110]; examination of expression of cytokine receptors and proliferative response to cytokines [111,112]; studies on virus susceptibility and propagation [77].

5. CATEGORIZATION AND CLASSIFICATION OF LEUKEMIA-LYMPHOMA CELL LINES

There are various possibilities for categorizing human leukemia-lymphoma cell lines, for instance according to the diagnosis of the patient or according to immunophenotypes, specific cytogenetic alterations, functional characteristics or other features of the cultured cells. The most often used categorization of these cell lines is based on the physiological spectrum of the normal hematopoietic cell lineages: firstly, lymphoid versus myeloid and secondly T-cell, B-cell, NK cell versus myelocytic, monocytic, megakaryocytic and erythrocytic (in addition to specific subtypes such as myeloblastic, promyelocytic, eosinophilic, basophilic for the myelocytic cell lineage; precursor B-cell, mature B-cell, plasma cell for the B-cell lineage; and immature and mature T-cells) (Table 2). It is still a matter of debate whether natural killer (NK) cells represent a third lymphoid lineage [119] or whether they are a branch of the T-cell lineage.

The most useful technique for assigning a given cell line to one of the major cell lineages is undoubtedly immunophenotyping. The more extensive and complete the immunoprofile, the more precise is the categorization and classification of the cell lineage-derivation and status of arrested differentiation along this cell axis. Other techniques may add highly valuable information in cases of uncertainty of cell lineage assignment (see below).

Commonly this assignment of any given leukemia-lymphoma cell line to a cell lineage and stage of arrested differentiation, based on its immunological and other phenotypes, does not present any problems. Exceptions to this rule are the Hodgkin's disease (HD) and anaplastic large cell lymphoma (ALCL)-derived cell lines. Although the lymphoid nature of Hodgkin-Reed-Sternberg cells (the presumed neoplastic cells in HD) appears to be established [83,118] and thus HD-derived cell lines may be assigned to lymphoid T- or B-cell categories, their uniqueness and the fact that such cell lines display very unusual and often asynchronous marker profiles which are not found in normal cells, justifies a separate category for HD cell lines and the equally unusual ALCL lines. Continuous human dendritic cell lines have not as yet been described; such presumably unparalleled cell lines with specific profiles will require their own category.

6. CHARACTERIZATION OF LEUKEMIA-LYMPHOMA CELL LINES

Cell lines originating from different cell lineages are, for the most part, impossible to distinguish by morphology alone. Since leukemia cell lines

Table 2. Categorization of leukemia-lymphoma cell lines

Main type	Physiological hematopoietic cell lineage	Subtype of neoplastic cell line	Prototype of cell line[a]
Lymphoid	B-cell	Precursor B-cell line	REH
		B-cell line	U-698-M
		Plasma cell line	RPMI-8226
	T-cell	Immature T-cell line	CCRF-CEM
		Mature T-cell line	SKW-3
	NK cell	NK cell line	YT
Myeloid	Myelocytic	Myelocytic cell line	HL-60
		Promyelocytic cell line	NB4
		Eosinophilic cell line	EoL-1
		Basophilic cell line	KU-812
	Monocytic	Monocytic cell line	U-937
	Erythrocytic	Erythrocytic cell line[b]	K-562
	Megakaryocytic	Megakaryocytic cell line[b]	MEG-01
Dendritic	Lymphoid? myelomonocytic?	Dendritic cell line[c]	–[c]
Hodgkin/ALCL	Lymphoid? other?	Hodgkin cell line[d]	L-428
	T-cell/null cell	ALCL cell line	SU-DHL-1

Modified from references [113,114].

[a] The best known cell lines for each of these categories are often the 'oldest' cell lines (nearly all available from cell line banks DSMZ or ATCC); see Table 1 for the historical perspective.

[b] It is often difficult to assign cell lines to either the erythrocytic or megakaryocytic cell lineage as most of these cell lines express features of both lineages, e.g. (hemo)globin, specific transcription factors, surface antigens, differentiation potential, etc.; thus, it may be preferable to use the term 'erythrocytic-megakaryocytic cell line'.

[c] At present, no continuous human dendritic cell line has been published; see references [115–117] for reviews on this controversial cell type.

[d] See references [69,118] for reviews on this controversial cell type.

commonly grow as single or clustered cells in suspension or only loosely adherent to the flask, single cell populations can be easily prepared and the cells can thus be characterized and classified. Table 3 lists a variety of parameters useful for the description of the cells and a panel of possible tests applicable for the phenotypic and functional characterization of most cell lines. This necessary multiparameter examination of the cellular phenotype provides important information on the likely cell of origin, the variable stringency of maturation arrest, and the predominantly normal pattern of gene expression. The list is not intended to cover comprehensively all possible informative parameters, as with new techniques becoming available and research areas extending to new avenues, other or entirely new features might be of interest

to scientists. Thus, only some of the features of the phenotypic profiles of cell lines which are most often studied are highlighted. Immunophenotypic analysis and cytogenetic karyotyping appear to be the most important and informative examinations (Table 3). It is also important to indicate when in the life of a cell line particular data were generated and also whether alterations in the phenotypic features of the cells might occur during prolonged culture.

While the extent of the analytical characterization of leukemia cell lines is variable, a minimum data set is obligatory and essential for the identification, description and culture of a cell line; these data include the clinical and cell culture description of the cell line (the example of the human leukemia cell line HL-60 is given in Table 4). Clearly, the origin of an established cell line must be documented sufficiently.

7. ESTABLISHMENT AND DESCRIPTION OF NEW LEUKEMIA-LYMPHOMA CELL LINES

We discerned six cardinal requirements for the description and publication of new leukemia-lymphoma cell lines (Table 5).

7.1. Immortality

First, a cell line should be grown in continuous culture for at least 6 months, even better for more than a year. Upon addition of growth factors, primary neoplastic cells or normal cells can be kept in culture for several months before proliferation ceases; these cultures cannot be regarded as 'continuous cell lines'. Continuous cell lines have been defined as cultures that apparently are capable of an unlimited number of population doublings (immortalization); it should be recognized that an immortalized cell is not necessarily one that is neoplastically or malignantly transformed [121].

7.2. Verification of Neoplastic Origin

A cell line established from a patient with leukemia is not necessarily a 'leukemia cell line'. For instance, it appears to be 10- to 100-fold easier to establish an EBV-transformed B-lymphoblastoid cell line (EBV+ B-LCL) from a patient with leukemia than a neoplastic leukemia cell line [122]. Thus, the neoplastic nature of the cell line should be demonstrated by functional assays or by the detection of clonal cytogenetic abnormalities. With regard to the latter point, it is of note that among 596 well- or partly characterized leukemia-lymphoma cell lines (excluding sister cell lines, subclones, EBV+

Table 3. Analytical characterization of leukemia-lymphoma cell lines

Parameter	Details and examples
Most important data	
Clinical data	• Patient's data (see Table 4)
In vitro culture	• Growth kinetics, proliferative characteristics (see Table 4)
Immunophenotyping	• Surface marker antigens (fluorescence microscopy, flow cytometry)
	• Intracytoplasmic and nuclear antigens (immunoenzymatic staining)
Cytogenetics:	• Structural and numerical abnormalities
	• Specific chromosomal markers
Further characterization	
Morphology	• In-situ (flask, plate) under inverted microscope
	• Light microscopy (May-Grünwald-Giemsa staining)
	• Electron microscopy (transmission, scanning)
Cytochemistry	• Acid phosphatase, (α-naphthyl acetate esterase, others)
Genotyping	• Southern blot analysis of T-cell receptor (TCR) and immunoglobulin (Ig) heavy and light chain gene rearrangements
	• Northern blot analysis of expression of TCR and Ig transcripts
Cytokines	• Production of cytokines
	• Expression of cytokine receptors
	• Response to cytokines, dependency on cytokines
Functional aspects/ specific features	• Phagocytosis
	• Antigen presentation
	• Immunoglobulin production and secretion
	• (Hemo)globin synthesis
	• Capacity for (spontaneous or induced) differentiation
	• Positivity for EBV or HTLV-1 or other viruses
	• Heterotransplantability into mice or other animals
	• Colony formation in agar/methylcellulose – clonogenicity
	• Production/secretion of specific proteins
	• Natural killer cell activity
	• Oncogene expression
	• Transcription factor expression
	• Unique point mutations
Date of analysis	• Age of cell line at time of analysis
	• Possible changes in the specific marker profile over prolonged culture

Adapted from references [113,114,120].

Table 4. Sets of clinical and cell culture data required for leukemia-lymphoma cell lines

Parameter	Example
Identification	
Name of cell line	HL-60
Cell phenotype	Myelocytic cell
Clinical data	
Original disease of patient	Initially AML M3, later corrected to AML M2
Disease status	At diagnosis
Patient data (age, race, sex)	35-year-old Caucasian woman
Source of material	Peripheral blood
Year of establishment	1976
Cell culture data	
Culture medium	90% RPMI 1640 + 10% FBS
Subcultivation routine	Maintain at 0.1–0.5 × 10^6cells/ml, split ratio 1:5 to 1:10 every 2–3 days
Minimum cell density	0.5–1.0 × 10^5 cells/ml
Maximum cell density	1.0–1.5 × 10^6 cells/ml
Doubling time	24–36 hours
Cell storage conditions	70% RPMI 1640 + 20% FBS + 10% DMSO
In situ morphology	Round, single cells in suspension
Mycoplasma contamination	None – checked with PCR
EBV status	Negative – checked by PCR

Modified from references [113,114,120].

B-LCLs, Burkitt and ATL cell lines) for which karyotypes have been published in the literature, only six cell lines (1%) showed a normal karyotype without any structural or numerical aberrations (five precursor B-/mature B-cell lines and one immature T-cell line). Colony formation in methylcellulose or agar or heterotransplantability into immunosuppressed mice are often regarded as signs suggestive of neoplasticity.

7.3. Authentication

The origin of a new cell line must be proven by authentication, i.e. it must be shown that the cultured cells are indeed derived from the presumed patient's tumor. It has been estimated that 10–20% of human leukemia-lymphoma cell lines are misidentified or cross-contaminated, thus 'false cell lines'. The method of choice for identity control is forensic-type DNA fingerprinting [123]. Microsatellite analysis does not appear to be sufficient as the loci seem

Table 5. Cardinal requirements for new leukemia-lymphoma cell lines

- Immortality
- Verification of neoplastic origin
- Authentication
- Scientific significance
- Characterization
- Availability

Modified from references [113,114].

to be unstable; immunophenotyping will not suffice either as cell lines of the same category will often have similar if not identical immunoprofiles. Unique cytogenetic marker chromosomes or molecular biological analyses (e.g. identical clonal gene rearrangement patterns on Southern blots) might also provide unequivocal evidence for the derivation of the cell line from the patient.

7.4. Scientific Significance and Characterization

For the sake of the necessities important for scientific publications, namely novelty and scientific significance, the new cell line should have features not yet detected in previously established cell lines. A thorough characterization of the cells (see above and Table 3) will often detect unique characteristics of cell lines proving their scientific importance.

7.5. Availability

The availability of cell lines to other qualified investigators upon request is of utmost importance. Some journals have adopted the policy that any readily renewable resources, including cell lines published in the journal, shall be made available to all qualified investigators in the field, if not already obtainable from commercial sources. The policy stems from the long-standing scientific principle that authenticity requires reproducibility. While cell lines are proprietary and unique, suitable material transfer agreements can be drawn up between the provider and requester [124]. By providing authenticated and unique biological material, cell line banks play a major role in leukemia-lymphoma research [101,102].

8. SUCCESS RATE OF ESTABLISHING LEUKEMIA-LYMPHOMA CELL LINES

The deliberate establishment of new leukemia-lymphoma cell lines remains by and large an unpredictable random process (except for EBV+ Burkitt lymphoma and HTLV-1 + ATL cell lines). Few systematic attempts to develop continuous new cell lines have been reported, thus a reasonable estimate of the success is in the range of 1–10% for myeloid cell lines and 10–20% for lymphoid cell lines [120], It appears somewhat easier to establish precursor B-cell lines where success rates of up to 66% have been reported, however only when applying special techniques and culture conditions (reviewed in ref. [95]). It should be noted that some of these latter cell lines have extremely long doubling times (10–14 days) which clearly limits their usefulness [125]. Furthermore, a method for establishing T-cell lines from pediatric T-ALL cases with a very high success rate was described [126]. The reproducibility of this method in other hands and the long-term growth (immortalization) of these cultures is not known and remains to be established.

Difficulties in establishing continuous human leukemia-lymphoma cell lines may also originate from the inappropriate selection of nutrients and growth factors for these cells. Thus, a suitable microenvironment for hematopoietic cells, whether they are malignant or normal, cannot yet be created; it should be remembered that normal cells cannot be cultured long-term without EBV or HTLV-1 infection. In a strict sense, they are also no longer considered normal cells. A much higher percentage of leukemia samples can be grown in vivo in immunodeficient mice (athymic, SCID, NOD/SCID, etc.) after xenotransplantation than in vitro [127]; these murine microenvironments are, however, not likely to be entirely representative of microenvironments in human organs [120]. Many types of neoplastic cells may not be capable of indefinite proliferation. The success rate for establishing a hematopoietic cell line appears to be higher for more immature than for more mature cells, and for lymphoid (T- and B-cell precursor) than for myeloid (including monocytic, erythrocytic and megakaryocytic) cell lines. Specimens from patients at relapse and specimens obtained from ascites, pleural effusion and the leukemic phase of lymphoma may represent more suitable material for cell culture attempts.

9. FUTURE PROSPECTS

We estimate that more than 1000 leukemia-lymphoma cell lines have been established. However, only a relatively small percentage of these cell lines has been sufficiently well described and characterized; in many cases it is

not known whether these cell lines are truly continuously proliferating and whether these cell lines do still exist. Efforts should be undertaken so that publications of new cell lines fulfil the requirements listed in Table 5. Furthermore, the awareness of scientists of the benefits of institutionalized cell culture collections should be heightened; otherwise, substantial numbers of unique and potentially important cell lines might be lost. Despite more than 35 years of hematopoietic cell culture, it is not understood why certain cells start to multiply indefinitely in culture and others do not. This enigma shows that our present in vitro culture conditions by no means accurately reflect the physiological in vivo microenvironment. Much further work is required to achieve significant improvements in the efficiency of immortalization. The use of certain cytokines might permit the long-term culture of many leukemia cells giving these cells the possibility to adapt to in vitro conditions or to give the few cells that a priori are amenable to in vitro growth the necessary time to multiply to sufficient cell numbers; subsequently, in some cases, continuous cell lines might evolve. Future technical innovations, e.g. transfection with oncogenes such as the anti-apoptotic gene bcl-2 or with a mutant p53 gene, might also improve the success rate enhancing the frequency of immortalization (although such cell lines are unique, they may not reflect the pathophysiology of the real leukemia in patients).

The availability of large numbers of continuous leukemia- lymphoma cell lines has facilitated clinical and immunobiological studies of hematopoietic malignancies. As with all in vitro studies, one should be cautious before extrapolating the data gathered from such studies to the in vivo situation. Thus, it is of paramount importance that the findings generated by cell line studies should be substantiated by studies with fresh leukemia cells. Despite this limitation, it is obvious that continuous leukemia-lymphoma cell lines have played major roles in the advancement of leukemia research. Undoubtedly, the acquisition of new information about human hematopoietic malignancies will be greatly furthered by continued research utilizing leukemia-lymphoma cell lines, optimally combined with studies on primary material.

References

1. Gey GO, Coffman WD, Kubicek MT. *Cancer Res* 12: 264–265, 1952.
2. Pulvertaft RJV. *Lancet* i: 238–240, 1964.
3. Iwakata S, Grace JT Jr. *NY State J Med* 64: 2279–2282, 1964.
4. Epstein MA, Barr YM. *J Natl Cancer Inst* 34: 231–240, 1965.
5. Epstein MA, Achong BG, Barr YM. *Lancet* i: 702–703, 1964.
6. Foley GE, Lazarus H, Farber S et al. *Cancer* 18: 522–529, 1965.
7. Moore GE, Gerner RE, Franklin HA. *J Am Med Assoc* 199: 519–524, 1967.
8. Moore GE, Minowada J. *In Vitro* 4: 100–114, 1969.

9. Belpomme D, Minowada J, Moore GE. *Cancer* 30: 282–287, 1972.
10. Matsuoka Y, Moore GE, Yagi Y et al. *Proc Soc Exp Biol Med* 125: 1246-1250, 1967.
11. Minowada J, Ohnuma T, Moore GE. *J Natl Cancer Inst* 49: 891–895, 1972.
12. Lozzio CB, Lozzio BB. *Blood* 45: 321–334, 1975.
13. Lozzio B, Lozzio CB. *Leukemia Res* 3: 363–370, 1979.
14. Andersson L, Nilsson K, Gahmberg CG. *Int J Cancer* 23: 143–147, 1979.
15. Nilsson K, Sundström C. *Int J Cancer* 13: 808–823, 1974.
16. Epstein AL, Kaplan HS. *Cancer* 34: 1851–1872, 1974.
17. Menezes J, Leibold W, Klein G et al. *Biomedicine* 22: 276–284, 1975.
18. Epstein AL, Henle W, Henle G et al. *Proc Natl Acad Sci USA* 73: 228–232, 1976.
19. Sundström C, Nilsson K. *Int J Cancer* 17: 565–577, 1976.
20. Rosenfeld C, Goutner A, Choquet C et al. *Nature* 267: 841–843, 1977.
21. Miyoshi I, Hiraki S, Kubonishi I et al. *Cancer* 40: 2999–3003, 1977.
22. Minowada J, Tsubota T, Greaves MF et al. *J Natl Cancer Inst* 59: 83–87, 1977.
23. Minowada J, Koshiba H, Janossy G et al. *Leukemia Res* 3: 261–266, 1979.
24. LeBien TW, HozierJ, Minowada J et al. *New Engl J Med* 301: 144–147, 1979.
25. Minowada J, Oshimura M, Tsubota T et al. *Cancer Res* 37: 3096–3099, 1977.
26. Schneider U, Schwenk HU, Bornkamm G. *Int J Cancer* 19: 621–626, 1977.
27. Gillis S, Watson J. *J Exp Med* 152: 1709–1719, 1980.
28. Collins SJ, Gallo RC, Gallagher RE. *Nature* 270: 347–349, 1977.
29. Koeffler HP, Golde DW. Science 200: 1153–1154, 1978.
30. Hurwitz R, Hozier J, LeBien T et al. *Int J Cancer* 23: 174–180, 1979.
31. Hirano T, Kishimoto T, Kuritani T et al. *J Immunol* 123: 1133–1140, 1979.
32. Schaadt M, Fonatsch C, Kirchner H et al. *Blut* 38: 185–190, 1979.
33. Schaadt M, Diehl V, Stein H et al. *Int J Cancer* 26: 723–731, 1980.
34. Gazdar AF, Carney DN, Bunn PA et al. *Blood* 55: 409–417, 1980.
35. Popovic M, Sarngadharan MG, Read E et al. *Science* 224: 497–500, 1984.
36. Poiesz BJ, Ruscetti FW, Mier JW et al. *Proc Natl Acad Sci USA* 77: 6815–6819, 1980.
37. Poiesz BJ, Ruscetti FW, Gazdar AF et al. *Proc Natl Acad Sci USA* 77: 7415–7419, 1980.
38. Miyoshi I, Kubonishi I, Sumida M et al. *Gann* 71: 155–156, 1980.
39. Tsuchiya S, Yamabe M, Yamaguchi Y et al. *Int J Cancer* 26: 171–176, 1980.
40. Andersson L, Gahmberg CG, Jansson SE et al 1981 In: Knapp W (ed.): *Leukemia Markers.* Academic Press, London, 297–300.
41. Findley Jr HW, Cooper MD, Kim TH et al. *Blood* 60: 1305–1309, 1982.
42. Martin P, Papayannopoulou T. *Science* 216: 1233–1235, 1982.
43. Stong RC, Korsmeyer SL, Parkin JL et al. *Blood* 65: 21–31, 1985.
44. Kishi K. *Leukemia Res* 9: 381–390, 1985.
45. Ogura M, Morishima Y, Ohno R et al. *Blood* 66: 1384–1392, 1985.
46. Yodoi J, Teshigawara K, Nikaido T et al. *J Immunol* 134: 1623–1630, 1985.
47. Saito H, Bourinbaiar A, Ginsburg M et al. *Blood* 66: 1233–1240, 1985.
48. Drexler HG, Gaedicke G, Lok MS et al. *Leukemia Res* 10: 487–500, 1986.
49. Okabe M, Matsushima S, Morioka M et al. *Blood* 69: 990–998, 1987.
50. Butterfield JH, Weiler D, Dewald G et al. *Leukemia Res* 12: 345–355, 1988.
51. Avanzi GC, Lista P, Giovinazzo B et al. *Brit J Haematol* 69: 359–366, 1988.
52. Kitamura T, Tange T, Terasawa T et al. *J Cell Physiol* 140: 323–334, 1989.
53. Yanagisawa K, Horiuchi T, Fujita S. *Blood* 78: 451–457, 1991.
54. Asou H, Tashiro S, Hamamoto K et al. *Blood* 77: 2031–2036, 1991.
55. Lanotte M, Martin-Thouvenin V, Najman S et al. *Blood* 77: 1080–1086, 1991.

56. Kluin-Nelemans HC, Limpens J, Meerabux J et al. *Leukemia* 5: 221–224, 1991.
57. Tohyama K, Tsutani H, Ueda T et al. *Brit J Haematol* 87: 235–242, 1994.
58. Cesarman E, Moore PS, Rao PH et al. *Blood* 86: 2708–2714, 1995.
59. Drexler HG, Minowada J. *Leukemia Lymphoma* 31: 305–316, 1998.
60. Prempree T, Marz T. *Nature* 216: 810–811, 1966.
61. Moore GE, Kitamura H. *NY State J Med* 68: 2054–2060, 1968.
62. Minowada J, Nonoyama M, Moore GE et al. *Cancer Res* 34: 1898–1903, 1974.
63. Pagano J, Huang CH, Levine P. *New Engl J Med* 289: 1395–1399, 1973.
64. Manolov G, Manolova Y. *Nature* 237: 33–34, 1972.
65. Zech L, Haglund U, Nilsson K et al. *Int J Cancer* 17: 47–56, 1976.
66. Kaiser-McCaw B, Epstein AL, Kaplan HS et al. *Int J Cancer* 19: 482–486, 1977.
67. Dalla-Favera R, Bregni M, Erikson J et al. *Proc Natl Acad Sci USA* 79: 7824–7827, 1982.
68. Collins SJ. *Blood* 70: 1233–1244, 1987.
69. Diehl V, von Kalle C, Fonatsch C et al. *Semin Oncol* 17: 660–672, 1990.
70. Drexler HG, Amlot PL, Minowada J. *Leukemia* 1: 629–637, 1987.
71. Drexler HG, Jones DB, Diehl V et al. *Hematol Oncol* 7: 95–113, 1989.
72. Drexler HG, Minowada J. *Human Cell* 5: 42–53, 1992.
73. Drexler HG. *Leukemia Lymphoma* 9: 1–26, 1993.
74. Drexler HG. *Leukemia Res* 18: 919–927, 1994.
75. Drexler HG, MacLeod RAF, Borkhardt A et al. *Leukemia* 9: 480–500, 1995.
76. Drexler HG, Quentmeier H, MacLeod RAF et al. *Leukemia Res* 19: 681–691, 1995.
77. Drexler HG, Uphoff CC, Gaidano G et al. *Leukemia* 12: 1507–1517, 1998.
78. Drexler HG, MacLeod RAF, Uphoff CC. *Leukemia Res* 23: 207–215, 1999.
79. Ferrero D, Rovera G. *Clin Haematol* 13: 461–487, 1984.
80. Haluska FG, Brufsky AM, Canellos GP. *Blood* 84: 1005–1019, 1994.
81. Hassan HT, Freund M. *Leukemia Res* 19: 589–594, 1995.
82. Hassan HT, Drexler HG. *Leukemia Lymphoma* 20: 1–15, 1995.
83. Herbst H, Stein H, Niedobitek G. *Crit Reviews Oncogenesis* 4: 191–239, 1993.
84. Hoffman R. *Blood* 74: 1196–1212, 1989.
85. Hozumi M. *CRC Crit Reviews Oncol/Hematol* 3: 235–277, 1985.
86. von Kalle C, Diehl V. *Int Review Exp Pathol* 33: 185–203, 1992.
87. Keating A. *Baillière's Clin Haematol* 1: 1021–1029, 1987.
88. Koeffler HP, Golde DW. *Blood* 56: 344–350, 1980.
89. Koeffler HP. *Blood* 62: 709–721, 1983.
90. Koeffler HP. *Semin Hematol* 23: 223–236, 1986.
91. Lübbert M, Koeffler HP. *Blood* Reviews 2: 121–133, 1988.
92. Lübbert M, Koeffler HP. *Cancer Reviews* 10: 33–62, 1988.
93. Lübbert M, Herrmann F, Koeffler HP. *Blood* 77: 909–924, 1991.
94. Matsuo Y, Minowada J. *Human Cell* 1: 263–274, 1988.
95. Matsuo Y, Drexler HG. *Leukemia Res* 22: 567–579, 1998.
96. Minowada J. *Cancer Reviews* 10: 1–18, 1988.
97. Nilsson K. *Human Cell* 5: 25–41, 1992.
98. Nilsson K. 1994 In: Dammacco F, Barlogie B (eds.): *Challenges in Modern Medicine,* Vol 4 – *Multiple Myeloma and Related Disorders.* Ares-Serono Symposia Publications, 83–92.
99. Oval J, Taetle R. *Blood Reviews* 4: 270–279, 1990.
100. Schaadt M, von Kalle C, Tesch H et al. *Cancer Reviews* 10: 108–122, 1988.
101. Hay RJ, Reid YA, McClintock PR et al. *J Cell Biochem* 24: 107–130, 1996.

102. Drexler HG, Dirks W, MacLeod RAF, Quentmeier H, Steube K, Uphoff CC (eds.): *DSMZ Catalogue of Human and Animal Cell Cultures* (7th Edition), Braunschweig, 1999.
103. Freedman MH, Grunberger T, Correa P et al. *Blood* 81: 3068–3075, 1993.
104. Pietsch T. *Nouv Rev Fr Hematol* 35: 285–286, 1993.
105. Drexler HG, Zaborski M, Quentmeier H. *Leukemia* 11: 701–708, 1997.
106. Drexler HG, Borkhardt A, Janssen JWG. *Leukemia Lymphoma* 19: 359–380, 1995.
107. Chabner BA. *J Natl Cancer Inst* 82: 1083–1085, 1990.
108. Nielsen D, Skovsgaard T. *Biochim Biophys Acta* 1139: 169–183, 1992.
109. Drexler HG. *Leukemia* 12: 845–859, 1998.
110. Mire-Sluis AR, Page L, Thorpe R. *J Immunol Methods* 187: 191–199, 1995.
111. Drexler HG. *Leukemia* 10: 588–599, 1996.
112. Drexler HG, Quentmeier H. *Leukemia* 10: 1405–1421, 1996.
113. Drexler HG, Matsuo Y, Minowada J. *Human Cell* 11: 51–60, 1998.
114. Drexler HG, Matsuo Y. *Leukemia* 13: 835–842, 1999.
115. Hart DNJ. *Blood* 90: 3245–3287, 1997.
116. Banchereau J, Steinman RM. *Nature* 392: 243–252, 1998.
117. Robinson SP, Saraya K, Reid CDL. *Leukemia Lymphoma* 29: 477–490, 1998.
118. Drexler HG. *Leukemia Lymphoma* 8: 283–313, 1992.
119. Spits H, Lanier LL, Phillips JH. *Blood* 85: 2654–2670, 1995.
120. Drexler HG, Gignac SM, Minowada J. 1994 Hematopoietic cell lines. In: Hay RJ, Park JG, Gazdar A (eds.): *Atlas of Human Tumor Cell Lines.* Academic Press, Orlando, 213–250.
121. Schaeffer WI. *In Vitro Cell Dev Biol* 26: 97–101, 1990.
122. Nilsson K, Pontén J. *Int J Cancer* 15: 321–341, 1975.
123. Häne B, Tümmler M, Jäger K et al. *Leukemia* 6: 1129–1133, 1992.
124. Kaushansky K. *Blood* 91: 1–2, 1998.
125. Zhang LQ, Downie PA, Goodell WR et al. *Leukemia* 7: 1865–1874, 1993.
126. Smith SD, McFall P, Morgan R et al. *Blood* 73: 2182–2187, 1989.
127. Uckun FM. *Blood* 88: 1135–1146. 1996.

Chapter 2

B-Cell Precursor Cell Lines

Stefan Faderl and Zeev Estrov
Department of Bioimmunotherapy, Box 302, M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA. Tel: +1-713-794-1675; Fax; +1-713-745-2374; E-mail: zestrov@notes.mdacc.tmc.edu

1. INTRODUCTION

Lymphopoiesis is a complex process. It originates from a pool of uncommitted stem cells capable of self-renewal and, at the same time, giving rise to early progenitors which develop along an orderly path of differentiation through precursor cell stages of increasing maturation committed to produce lymphocytes. The concept of a lymphoid stem cell has long been a matter of debate [1]. However, experiments in cell cultures and with lethally irradiated mice where fetal bone marrow cells expressing high levels of CD34 were able to reconstitute human B and T cells, point toward a population of CD34- and TdT (terminal deoxynucleotide transferase)-expressing pluripotent hematopoietic cells that can serve as lymphoid progenitors [2].

B-cell ontogeny occurs in two phases. Antigen-dependent expansion and differentiation occurs in spleen and lymph nodes and is mediated by the interaction of surface immunoglobulin (sIg)-positive B cells with accessory signals from T cells, macrophages, natural killer (NK) cells, and other antigen-presenting cells such as dendritic cells. Antigen-independent maturation occurs in the bone marrow and precedes these events. This phase of B cell development is characterized by rearrangement of the Ig heavy and light chain genes and sequential expression of their transcription products in the cytoplasm and on the surface of B cells. It eventually results in the production of sIg-expressing B lymphocytes. In this sense, B-cell precursors (BCP) represent stages in B cell maturation prior to expression of sIg, and various stages within BCP are distinguished by the status of their Ig rearrangements and antigen expression (CD markers).

The Ig heavy and light chain loci are organized into multiple genes that result in the transcription of the variable (V) and constant (C) regions of the Ig proteins. Additional genes code for the joining (J) and diversity (D)

J.R.W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol. III, 19–59.

regions, the latter in the heavy chain locus only, that encode the hypervariable region of the V segments [3]. The genes are separated by noncoding introns of varying lengths. For the B lymphocyte to produce and excrete functional Ig proteins, an orderly progression of gene rearrangements takes place that starts at the μ heavy chain locus and from there progresses to the κ and λ light chain loci. Initial steps involve joining of D_H and J_H regions followed by $V_H \rightarrow DJ_H$ joining by splicing out intervening intron sequences. Crucial enzymes catalyzing these steps are TdT which adds nontemplate-directed nucleotides in between the joining regions [4], and proteins encoded by the genes *RAG* (recombination activating gene)-*1* and *RAG-2* [5]. A B-cell at this stage is called a pro-B cell, i.e., it is capable of completing a functional rearrangement of the Ig gene locus. Once cytoplasmic $Ig\mu$ is expressed, the BCP is termed a pre-B cell and rearrangement of the light chain loci begins [1]. Until expression of a functional μ/κ or, in the case of nonfunctional κ rearrangements, μ/λ sIg, a surrogate light chain (SLC) molecule composed of the covalently linked $\lambda 5$ and the noncovalently linked V_{pre-B} protein is attached to $Ig\mu$.

At every stage along the developmental path of lymphopoiesis, expansion of a monoclonal population of cells with a phenotype of immature cells manifests most frequently as acute lymphoblastic leukemia (ALL). Leukemic lymphoblasts share many of the phenotypic, immunological, and genetic properties of their normal B-cell counterparts. ALL is the most common form of childhood neoplasia. Only 20% of ALL occur in patients older than 17 years of age [6]. ALL can be classified according to morphology, histocytochemistry, immunophenotype and, increasingly, molecular abnormalities and cytogenetics [7]. The French–American–British (FAB) Cooperative Group distinguishes three morphological groups according to cell size, nuclear/cytoplasmic (N/C) ratio, presence or absence of nucleoli, and cytoplasmic vacuolation [8]. L1 morphology, most frequent in childhood ALL, is thus characterized by a population of small, homogenous cells with a relatively high N/C ratio and indistinct nucleoli. L2 cells are more heterogenous in size, have a lower N/C ratio and express several, often large nucleoli. L3 morphology is almost exclusively a feature of mature B-cell leukemia (Burkitts leukemia) associated with characteristic chromosomal translocations.

A more widespread system used for the classification of ALL is based on expression of distinct surface and cytoplasmic markers detected by immunophenotyping. The European Group for the Immunological Classification of Leukemias (EGIL) has established guidelines for the immunophenotypic diagnosis of ALL [9]. B lineage ALL is defined by the expression of at least two of the three early B cell markers CD19, CD22, and CD79a. According to the degree of B lymphoid differentiation of the leukemic cells, BCP ALL is

further defined as pro-B-ALL (B-I) without further expression of other B-cell antigens, common ALL (B-II) if CD10-positive, and pre-B-ALL if cIgμcan be detected (B-III). The expression of sIg or cytoplasmic/surface light chains qualifies the leukemia as a mature B-cell ALL and is not the subject of this chapter.

The first BCP ALL cell line, REH, was established in 1974 from the leukemic cells of a girl with ALL in relapse [10]. Since then, more than 150 BCP ALL cell lines have been described, although only a handful are well characterized [11]. The establishment of lymphoid leukemic cell lines is still a challenge and is successful in at most 10% of cases [11]. BCP ALL cell lines have been established mainly from children with ALL, most frequently in relapse or with resistant disease. Some are derived from patients with chronic myelogenous leukemia (CML) in lymphoid blast crisis and some have been classified as acute undifferentiated leukemias (AUL). Many cell lines harbor typical leukemic cytogenetic abnormalities such as translocations t(1;19) and t(9;22) with their respective fusion gene products. These karyotype aberrations are associated with poor clinical outcome and therefore cell lines with these changes enable the study of these neoplasms. It should also be noted, however, that many cell lines have additional cytogenetic aberrations, some of which occur in vitro. Many cell lines are found to respond to cytokines by either proliferation or inhibition of proliferation, and identification of cytokine receptors on the surface of some of the cell lines has further facilitated the study of cytokine interactions and signal transduction pathways in BCP ALL. The growing repertoire of BCP cell lines creates more opportunities to analyze the unique biological properties of the vast heterogeneity of human BCP ALL.

2. CLINICAL CHARACTERIZATION

More than 80 BCP cell lines are summarized in Table 1, according to the degree of B-lymphoid differentiation (B-I, B-II, and B-III). In nine of the cell lines (G2, IARC-318, JM, Km-3, LILA-1, LK-63, Tree92, Z-119, and Z-181), expression of cytoplasmic Ig is unknown or not clearly identified to warrant categorization at either B-II or B-III stage of differentiation. Tree92 is unusual as it is derived from a patient with mature B-cell ALL (ALL L3) and shows faint expression of surface Igs, otherwise displaying markers of a BCP line. The majority of cell lines were derived from patients with ALL (78/85, 92%). Two cell lines were derived from patients with lymphoid blast crisis of CML (CML-BC). Three patients were classified as acute undifferentiated leukemia (AUL) and one as lymphoblastic lymphoma (LL). No information was given as to the diagnosis of the patient in one case. The sex

distribution is 42 from male patient and 40 from females. No information was provided in three cases. Not surprisingly, and in keeping with the distribution of incidence rates according to age in ALL, the majority of BCP cell lines were derived from children of 15 years of age or less (51/78, 65%). Twenty cell lines (26%) were established from samples of adults with ALL. In seven cases, the age of the patient is unknown. Among the 51 children, 10 (20%) were infants less than one year of age.

FAB morphology has largely lost its role in classification of ALL and its prognostic significance, except for ALL L3. Nevertheless, in cases where information as to the FAB type was given, the majority of patients with ALL displayed ALL FAB type L1 (14/21, 67%). FAB type L3 is usually associated with mature B-cell ALL and not BCP ALL. However, cell line Tree92, although consistent with BCP characteristics, was derived from a patient with L3 morphology. Most cell lines were developed from patients at relapse or with resistant disease (36/85, 31%). Twenty-three samples (27%) were established from diagnostic specimens. Two patients were in lymphoid blast crisis of CML. Information is lacking in 25 cases (29%). Nearly equal numbers of cell lines were derived from peripheral blood (30/85, 35%) or bone marrow (36/85, 42%). One line was developed from both peripheral blood and bone marrrow sources, and one line from peritoneal fluid (PF). No source was provided in 17 (20%) cases. The majority of cell lines were established in the 1980s (21/47, 45%). Equal numbers, where a reliable date could be ascertained, were developed in the 1970s and 1990s (13/47, 28%, each). The preferred choice of medium is RPMI-1640 supplemented with either FCS or FBS.

Sister cell lines were established from several patients. In the case of NALM-19, NALM-20, NALM-24/-25, and NALM-29, EBV-positive non-leukemic B lymphoid cell lines were developed. In other cases, longitudinal lines were established from the same patient at first, second, or further relapse (NALM-6 to NALM-13, NALM-21/-22/-23, NALM-30/-31/-32, SUP-B19, SUP-B28, SUP-B31, UoC-B5, UoC-B6, KH-3A and KH-3B, PC-53A).

3. IMMUNOPHENOTYPICAL CHARACTERIZATION

Precursor B-cell development (Table 2) is characterized by sequential expression of cytoplasmic and surface marker antigens that allow classification into three broad categories. Guidelines for the classification of acute lymphoblastic leukemias were proposed by the European Group for the Immunological Classification of Leukemias [9]. Accordingly, BCP cell lines can be divided into pro-B lines (B-I), common-B lines (B-II), and pre-B lines (B-III). Ten of the 87 (11%) cell lines described here belong to the pro-B cell

Table 1. Precursor B-cell lines: clinical characteristics

Cell line	Patient age/sex	Diagnosis	Treatment status	Specimen site	Year est.	Culture medium	Ref.
Pro-B cell lines (B-I)							
A-1	?/M	ALL	Relapse	PB		α-MEM + 10% FBS	12,13
B1	14/M	ALL	Relapse	BM	1990	α-MEM + 10% FBS	14
HBL-3	9/F	ALL	Diagnosis	BM	1985	RPMI-1640 + 20% FBS	15
JKB-1	16/F	ALL	Relapse	BM	1992	RPMI-1640 + 10% FCS	16
KH-4	11/M	ALL	Resistant	BM	1982	RPMI-1640 + 20% FBS	17
KOCL-51	<1/M	ALL (L1)					18
NALM-19[a]	26/M	AUL	Diagnosis	PB	1988		19
RS4;11	32/F	ALL (L2)	Relapse	BM	1983	RPMI-1640 + 5% FCS	20
SEM	5/F	ALL	Relapse	PB		IMDM + 10% FCS	21
TOM-1	54/F	ALL	Resistant	BM	1983	RPMI-1640 + 10% FBS	22

Continued on next page

Table 1. (continued)

Cell line	Patient age/sex	Diagnosis	Treatment status	Specimen site	Year est.	Culture medium	Ref.
Common-B cell lines (B-II)							
BV173	45/M	CML-BC	Blast crisis	PB	1980	RPMI-1640 + 10% FCS	23
EU-1	16/M	ALL	Relapse	BM	NA	RPMI-1640 + 10% FCS	24,25
HAL-01	17/F	ALL (L2)		PB	1990	RPMI-1640 + 25% FCS	26
HOON	9/M	ALL			1982	RPMI-1640 + 4% FCS	27
HYON	11/M	ALL			1982	RPMI-1640 + 4% FCS	27
MHH-CALL-2	15/F	ALL	Diagnosis	PB		[b]	28
MHH-CALL-3	11/F	ALL	Diagnosis	BM		[b]	28
KH-3[c]	14/F	ALL	Relapse	BM	1983	RPMI-1640 + 20% FBS	17
Kid-92	28/M	ALL	Resistant	PB	1992		29
KOPN-1	<1/F	ALL (L1)					30
KOPN-K	5/M	ALL			1985		11
LC4-1[d]	13/F	ALL (L1)		PB/BM		IMD medium + 15% FCS	31
MIELIKI	1/F	ALL (L1)	Diagnosis	BM		RPMI-1640 + 10% FCS	32
MR-87	4/M	ALL	Diagnosis	BM		RPMI-1640 + 10% FCS	33
NALL-1	14/M	ALL	Relapse	PB	1976	RPMI-1640 + 20% HCS	34
NALM-16	12/F	ALL	Relapse	PB	1977	RPMI-1640 + 10% FCS	35,36
NALM-20[e,f]	62/M	AUL	Diagnosis	PB	1989	RPMI-1640 + 10% FCS	37
NALM-24/-25[g]	42/F	ALL	Diagnosis	PB	1990	RPMI-1640 + 10% FCS	38
NALM-27/-28	38/M	ALL	Diagnosis	PB	1995		11

Continued on next page

Table 1. (continued)

Cell line	Patient age/sex	Diagnosis	Treatment status	Specimen site	Year est.	Culture medium	Ref.
Common-B cell lines (B-II) (continued)							
NALM-29[h,i]	46/M	ALL	Diagnosis	PB	1995		11
NALM-33/-34	72/M	ALL	Diagnosis	PB	1987		11
OM9;22	19/F	ALL[j]	Relapse	BM	1987	RPMI-1640 + 20% FCS	39
PC-53	33/F	ALL (L2)[k]	Relapse	BM	1985	IMDM + 10% FCS	40
RCH-ACV	8/F	ALL	Relapse	BM		RPMI-1640 + 10% FCS	41
REH	15/F	ALL	Relapse	PB	1975	RPMI-1640	10
SUP-B2	5/M	ALL	Relapse			Modified McCoy 5A medium+15% FCS	42
SUP-B7	2/F	ALL (L1)[l]	Diagnosis	BM	1983	NA	43
SUP-B26[m]	5/M	ALL	Relapse	BM		Modified McCoy 5A medium + 15% FCS	42
TC78	7/M	ALL (L2)	Relapse	BM		RPMI-1640 + 10% FCS	44
UoC-B1	15/F	ALL	Relapse	BM		Modified McCoy 5A medium+15% FCS	42
UoC-B4[n]	3/F	ALL	Relapse	PF		Modified McCoy 5A medium + 15% FCS	42
UoC-B7	5/M	ALL	Relapse	BM		Modified McCoy 5A medium + 15% FCS	42
UoC-B8	3/M	ALL	Diagnosis	BM		Modified McCoy 5A medium + 15% FCS	42
UoC-B9	9/F	ALL	Diagnosis	BM		Modified McCoy 5A medium + 15% FCS	42
UoC-B10	26/M	ALL	Diagnosis	BM		Modified McCoy 5A medium+15% FCS	42

Continued on next page

Table 1. (continued)

Cell line	Patient age/sex	Diagnosis	Treatment status	Specimen site	Year est.	Culture medium	Ref.
Pre-B cell lines (B-III)							
207	10/M	ALL	Relapse	BM	1980	Modified McCoy 5A medium + 20% FCS	45
697	12/M	ALL	Relapse	BM	1979	Modified McCoy 5A medium+20% FCS	45
BLIN-1	11/M	ALL	Diagnosis	BM	1988	RPMI-1640+25% FBS+10%v/v L-BCGF	46
HPB-NULL	47/M	ALL		PB	1978		11
INC	68/F	ALL			1985		29
KLM-2	M	ALL		PB			47
KM3							48
KMO-90	12/F	ALL	Diagnosis	BM	1990	RPMI-1640 + 10% FCS	49
KOCL-33	<1/F	ALL (L1)					50
KOCL-44	<1/F	ALL (L1)					50
KOCL-45	<1/M	ALL (L1)					50
KOCL-50	<1/F	ALL (L1)					50
KOCL-58	<1/M	ALL (L1)					50
KOPB-26	1/F	ALL (L1)					50
KOPN-8	<1/F	ALL		PB	1977		11
LAZ-221	24/F	ALL	Diagnosis	PB	1977	Medium 199 + 5% FBS	51
NALM-1[o]	3/F	CML-BC	Blast crisis	PB	1975	RPMI-1640 + 10% FCS	52
NALM-6[p]	19/M	ALL	Relapse	PB	1976	RPMI-1640 + 10% FCS	53

Continued on next page

Table 1. (continued)

Cell line	Patient age/sex	Diagnosis	Treatment status	Specimen site	Year est.	Culture medium	Ref.
Pre-B cell lines (B-III) (continued)							
NALM-17/-18	9/M	ALL		PB	1978		11
NALM-26	24/M	ALL	Diagnosis	PB	1992		54
P30/OHKUBO	11/F	ALL (L2)	Relapse	BM	1980		55
PER-278	10/M	ALL (L1)	Diagnosis	BM	1987		56
PRE-ALP	6/F	ALL (L1)	Diagnosis	BM		RPMI-1640 + 10% FCS	57
SMS-SB	16/F	LL	Relapse	PB	1977	RPMI-1640 + 15% FBS	58
SUP-B15	9/M	ALL	Relapse	BM			59
SUP-B16[q]	10/F	ALL	Relapse	BM		Modified McCoy 5A medium + 15% FCS	42
SUP-B24[r]	3/M	ALL	Diagnosis	BM		Modified McCoy 5A medium + 15% FCS	42
SUP-B27	15/M	ALL	Relapse	BM		Modified McCoy 5A medium + 15% FCS	42
Tahr-87	27/M	AUL			1980		60
TS-2	3/F	ALL (L1)	Relapse	PB	1994	RPMI-1640 + 10% FBS	61
UoC-B3[s]	14/F	ALL	Diagnosis	BM		Modified McCoy 5A medium + 15% FCS	42

Continued on next page

Table 1. (continued)

Cell line	Patient age/sex	Diagnosis	Treatment status	Specimen site	Year est.	Culture medium	Ref.
CyIgM status unknown (B-II/III)							
G2	?/F	ALL	Relapse	PB		α-MEM + 10% FBS	13
IARC-318	ALL						28
JM	14/M	ALL	Relapse	PB	1977 (?)	RPMI-1640 + 10% FCS	62
Km-3	12/M	ALL	Relapse	PB	1977 (?)	RPMI-1640 + 10% FCS	62
LILA-1		ALL		PB		RPMI-1640 + 10% FCS	63
LK-63	F	ALL		PB		RPMI-1640 + 10% FCS	63
Tree92	9/M	ALL (L3)			1992		29
Z-119	25/F	ALL (L2)	Relapse	BM	1990	RPMI-1640 + 10% FCS	64
Z-181	32/M	ALL	Relapse	BM	1991	RPMI-1640 + 10% FCS	64

BM – bone marrow, PB – peripheral blood, PF – peritoneal fluid.

[a] B239 and B240 (EBV-positive B-LCL) were established from the same leukemic sample. [b] Cell lines were grown in basal growth media (RPMI 1640, Dulbecco's MEM, α-MEM, Iscove's MDM, McCoy's 5A, Gibco BRL) supplemented with 5–20% FBS. [c] Two cell lines (KH-3A and KH-3B) were established from the same patient. KH-3A at first, and KH-3B at second relapse. Two more clones (KH-3A-2 and KH-3A-3) were established from cell line KH-3A [17]. [d] subclone of LC4 cells with most favorable growth characteristics. [e] B250 (EBV-positive, non-leukemic B-LCL) was established from the same leukemic sample. [f] NALM-21/-22/-23 were established from the same patient as NALM-20, however at relapse. [g] B262 (EBV-positive, non-leukemic B-LCL) was established from the same leukemic sample. [h] B391 (EBV-positive B-LCL) was established from the same leukemic sample. [i] NALM-30/-31/-32 were established from the same patient as NALM-27/-28, however at relapse. [j] cell lines post-BMT. [k] PC-53A was established from the same patient later during her terminal relapse. [l] Cell line was predominantly FAB L2 morphology. [m] SUP-B28 was established from the same patient at second relapse. [n] UoC-B6 was established from the same patient at second relapse. [o] initial in vitro testing of NALM-1 cells after establishment of the cell line did not reveal cytoplasmic Ig. However, in further experiments in 1978, the authors detected cytoplasmic fluorescence with affinity purified anti-μ [66]. [p] NALM-6 is one of 8 leukemia cell lines (NALM-6 to NALM-13) which were derived from the same patient at relapse. The data here refer to NALM-6 which was further characterized in Minnesota as NALM-6-M1. [q] SUP-B19 was established from the same patient at second relapse. [r] SUP-B31 was established from the same patient at first relapse. [s] UoC-B5 was established from the same patient at first relapse.

category. However, except for two lines (NALM-19, SEM), expression of CD19 in association with absence of expression of CD10, cytoplasmic Ig, and surface membrane Ig, have been the basis for classification into category B-I. Expression of CD79a has been very sporadically tested. Common B-lines are distinguished by positivity for CD10 (common acute lymphoblastic leukemia antigen, CALLA) in the absence of cytoplasmic or surface immunoglobulin markers. Thirty-eight of the 87 cell lines (44%) meet these criteria. Finally, pre-B lines, i.e., cell lines with expression of cytoplasmic Ig with or without coexpression of CD10, account for 32 of 87 lines (37%). In a number of cases (9/87, 10%), the status of expression of cytoplasmic Ig is unclear. Coexpression of myeloid markers on otherwise ALL cells has been described in up to 20% of cases of ALL. Coexpression of at least one myeloid marker (CD13 or CD33) has been described in 18 of 50 cell lines tested (36%), with expression of two myeloid markers in another 6 cell lines (12%). Coexpression of T cell markers (CD5 and/or CD7) was found in only a minority of cell lines tested (7/70, 10%).

4. CYTOKINE-RELATED CHARACTERIZATION

Cytokine receptor expression, production of cytokines, and response to cytokines in terms of proliferation and differentiation are summarized in Table 3. Rarely are cell lines systematically screened with regard to response to cytokines. In most cases, cytokines are chosen according to personal interest, creating a patchy picture of influence of cytokines and biological response modifiers in the literature. Frequently tested cytokines are the interleukins (IL) IL-1, IL-3, IL-4, IL-6, and IL-7, the interferons, TNF-α, and hematopoietic growth factors such as G-CSF and GM-CSF. IL-1α and 1-β were found to stimulate proliferation of B1 cells [14]. IL-4 was mainly inhibitory for the proliferation of SEM, KM3, MIELIKI, and REH cells [21,32,67]. IL-7 caused variable responses. Growth inhibition in B1, 697, NALM-6, and MIELIKI cells [11,32,42,68–71] was partly due to upregulation of apoptotic pathways mediated by Fas-ligand/APO-1 signaling cascades. Promotion of cell proliferation has been observed in JKB-1, PRE-ALP, NALM-20, NALM-21/-22/-23, NALM-24/-25, and OM9;22 cells [16,21,39,57]. Receptors for cytokines and production of cytokines have been described for a few cell lines (Table 3). Growth-factor dependent cell lines are infrequent among BCP lines.

Table 2. Precursor B-cell lines: immunophenotypical characterization

Cell line	HLA-DR	CD 19	CD 22	CD 79a	CD 10	CyIgM	SmIg	CD 20	CD 5	CD 7	CD 13	CD 33	CD 34	CD 85	CD 19	CD 127	CD 135	Ref.
Pro-B cell lines (B-I)																		
A-1	+	+			−	−	−	−										12,13
B1	+	+			−	−	−	−	−	−	−	+						14
HBL-3	+	+			−	−	−	−	−	−	−	−						15
JKB-1	+	+	−		−	−	−	−	−	−	−	−	−					16
KH-4	+				−	−	−	−	−									17
KOCL-51	+	+			−	−	−		−	−	+							50
NALM-19	+	+	+	+	−	−	−	−	+	−	+	−	−	+	+	+	+	11
RS4;11	+	+			−	−	−	−										20
SEM	+	+	+		−	−	−	−		−	+	+	−					21
TOM-1	+	+			−[a]	−	−											22

Continued on next page

Table 2. (continued)

Cell line	HLA-DR	CD 19	CD 22	CD 79a	CD 10	CyIgM	SmIg	CD 20	CD 5	CD 7	CD 13	CD 33	CD 34	CD 85	CD 19	CD 127	CD 135	Ref.
Common-B cell lines (B-II)																		
BV173	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	−	+	11
EU-1	+	+			+	−	−				+[b]	+[b]						24,25
HAL-01	+	+	+	+	+	−	−	−	−	−	+	−	−	+	+	−	+	26
HOON	+				+	−	−	−										27
HYON	+				+	−	−	−										27
KH-3	+				+	−	−	−	−									17
Kid-92	+	+	+	+	+	−	−	−	−	−	+	−	−	+	+	+	+	11
KOPN-1	+	+			+	−	−		−	−	−	+						50
KOPN-K	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−	+	11
LC4-1	+	+			+	−	−	+	−	−	−	−						31
MHH-CALL-2	+	+			+	−	−											28
MHH-CALL-3	+	+			+	−	−											28
MIELIKI	+	+	+		+	−	−	+	−	−	−[c]	−[c]						32
MR-87	+	+			+	−	−	−	−	−	+	+	−					33
NALL-1	+	+	+	+	+	−	−	+	+	−	−	+	−	−	+	+	+	11
NALM-16	+	+	+	+	+	−	−	+	+	−	−	−	−	−	+	+	+	11
NALM-20	+	+	+	+	+	−	−	−	−	−	+	−	+	+	+	+	+	11,37
NALM-21/-22/-23	+	+	+	+	+	−	−	−	−	−	+	−	+	−	+	+	+	11,37

Continued on next page

Table 2. (continued)

Cell line	HLA-DR	CD 19	CD 22	CD 79a	CD 10	CyIgM	SmIg	CD 20	CD 5	CD 7	CD 13	CD 33	CD 34	CD 85	CD 19	CD 127	CD 135	Ref.
Common-B cell lines (B-II) (continued)																		
NALM-24/-25	+	+	+	+	+	−	−	−	−	−	+	−	+	+	+	+	+	11,38
NALM-27/-28	+	+	+	+	+	−	−	−	−	−	+	−	+	+	−	−	+	11
NALM-29	+	+	+	+	+	−	−	−	−	−	+	−	−	+	+	+	+	11
NALM-30/-31/-32	+	+	+	+	+	−	−	−	−	−	+	−	−	−	+	+	+	11
NALM-33/-34	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−	+	11
OM9;22	+	+	+	+	+	−	−	−	−	−	+	+	+	+	−	−	+	11,39
PC-53	+	+			+	−	−	−	−	−	−	−						40
RCH-ACV	+	+			+	−	−	−	−									41
REH	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	+	11
SUP-B2	+	+			+	−	−	−	−			−	−					42
SUP-B7	+	+			+	−	−		−	−			−					43
SUP-B26	+	+			+	−	−	−	−	−				−				42
TC78	+				+	−	−	−			−	−						44
UoC-B1[d]	+	+			+	−	−	−	−	−			−					42
UoC-B4[d]	+	+			+	−	−	−[e]	−	−			−					42
UoC-B7[d]	+	+			+	−	−	−	−	−			−					42
UoC-B8[d]	+	+			+	−	−	−	−	−			−					42
UoC-B9[c]	+	+			+	−	−	+	−	−			−					42
UoC-B10	+	+			+	−	−	+	−	−			−					42
UoC-B11	+	+			+	−	−		−	−			−					65

Continued on next page

Table 2. (continued)

Cell line	HLA-DR	CD 19	CD 22	CD 79a	CD 10	CyIgM	SmIg	CD 20	CD 5	CD 7	CD 13	CD 33	CD 34	CD 85	CD 19	CD 127	CD 135	Ref.
Pre-B cell lines (B-III)																		
207	+				+	+	+[f]		−									45
697	+				+	+	+[f]	−										45
BLIN-1	+	+			+	+	−	+/−	+									46
HPB-NULL	+	+	+	+	−	+	−	−	+	−	−	−	−	+	+	+	+	11
INC	+	+	+	+	+	+	−	+	−	−	+	−	−	+	+	+	+	11
KLM-2	+	+	+	+	+	+	−	+	−	−	−	−	−	+	+	−	−	11
KMO-90	+	+		+	+	+	−	−	−	−	−	−						49
KM3	+	+			+	+	−	−										67
KOCL-33	+	+			−	+	−		−	−	−	−						50
KOCL-44	+	+			+	+	−		−	−	−	+						50
KOCL-45	+	+			−	+	−		−	−	−	−						50
KOCL-50	+	+			−	+	−		−	+	−	+						50
KOCL-58	+	+			−	+	−		−	−	−	−						50
KOPB-26	+	+			−	+	−		−	−	−	−						50
KOPN-8	+	+	+	+	+	+	−	−	−	−	−	−	−	+	+	+	+	11
LAZ-221	+	+	+	+	+	+	−	+	+	−	−	−	−	+	+	+	+	11
NALM-1	+	+	+	+	+	+	−	+	−	−	−	−	+	+	+	+	+	11
NALM-6	+	+	−	+	+	+	−	−	−	−	−	−	−	+	+	+	−	11
NALM-17/-18	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	11
NALM-26	+	+	−	+	+	+	−	−	−	−	−	−	−	−	+	+	+	11

Continued on next page

Table 2. (continued)

Cell line	HLA-DR	CD 19	CD 22	CD 79a	CD 10	CyIgM	SmIg	CD 20	CD 5	CD 7	CD 13	CD 33	CD 34	CD 85	CD 19	CD 127	CD 135	Ref.
Pre-B cell lines (B-III) (continued)																		
P30/OHKUBO	+	+	+	+	+	+	−	−	−	−	−	−	−	+	+	+	+	11
PER-278	+	+			+	+	−	−		−								56
PRE-ALP	+	+			+	+	−	+					−					57
SMS-SB	+				−	+	−		−	−								58
SUP-B13	+	+			+	+	−		−	−			+/−					65
SUP-B15	+	+			+	+	−											59
SUP-B16	+	+			−	+	−	+	−	−			−					42
SUP-B24	+	+			+	+	−	−	−	−			−					42
SUP-B27	+	−			+	+	−	−	−	−			−					42
Tahr-87	+	+	+	+	+	+	−	−	−	−	+	−	+	+	+	+	+	11
TS-2	+	+	+		−	+	−	−				−						61
UoC-B3	+	+			+	+	−	−	−	−								42

Continued on next page

Table 2. (continued)

Cell line	HLA-DR	CD 19	CD 22	CD 79a	CD 10	CyIgM	SmIg	CD 20	CD 5	CD 7	CD 13	CD 33	CD 34	CD 85	CD 19	CD 127	CD 135	Ref.
CyIgM status unknown (B-II/III)																		
G2	+	+			−	?	−	−										13
IARC-318		+			+	?	−											
JM						?	−											62
KM-3						?	−											62
LILA-1	+	+			+	?	−	−										63
LK-63	−	+			+	?	−[g]	−										63
Tree 92		+	+		−	?	−[h]	−	−	−	−							29
Z-119	+	+	+		+	?	NA	+	−	−	+	+	+					64
Z-181	+	+	+		+	?	NA	+	−	−	+	−	+					64

[a] Up to three months after the beginning of culture cells expressed CD10, but lost positivity for CALLA thereafter. [b] Expression of myeloid markers CD13, CD14, CD33 only after treatment with DMSO for 3 days. [c] Cell line expressed CD15. [d] All cell lines are positive for expression of CD15. [e] CD20 expression is positive in cell line UoC-B6 from same patient at second relapse. [f] SIg staining faint by immunofluorescence. [g] Lk-63 has been described to express membrane IgM by the authors. The origin of the cell line of a typical CD10-expressing childhood ALL with a t(1;19) prompted classification as a pre-B-cell line. [h] Limited expression of sIg (μ, 19%; λ, 6%).

Table 3. Precursor B-cell Lines: cytokine-related characterization

Cell lines	Cytokine receptor expression	Cytokine production	Proliferation response to cytokines	Differentiation response to cytokines	Dependency on cytokines	Ref.
Pro-B cell lines (B-I)						
B1	IL-1, IL-6, IL-7, γIFN, TNFα	IL-1α and IL-1β	IL-1α and IL-1β stimulate cell growth. IL-6, IL-7, γIFN, and TNF are potent inhibitors of B1 cell growth		Autocrine stimulation of growth by IL-1	14
JKB-1			Increased proliferation with SCF and IL-3 or IL-7. SCF and IL-3/IL-7 act synergistically. Decreased proliferation by IL-6			16
RS4;11	Constitutive expression of CD95/APO-1					70
SEM	Expresses mRNA for IL-7 receptor		IL-7 enhances proliferation of SEM cells; IL-4, TNF-α, IFN-α, IFN-γ inhibit cell growth			21

Continued on next page

Table 3. (continued)

Cell lines	Cytokine receptor expression	Cytokine production	Proliferation response to cytokines	Differentiation response to cytokines	Dependency on cytokines	Ref.
Common-B cell lines (B-II)						
EU-1		TNF-α (mRNA, protein)	Resistant to inhibition by TNF-α			24
HAL-01			Proliferation of growth is suppressed by IL-3 in a dose-dependent fashion			26
HOON	Express endoglin as part of TGF-β receptor complex					72
KOPN-1			Growth inhibition by rhIFNα			68
MIELIKI	CDw124 and CDw127 are associated with high affinity binding for IL-4 and IL-7		IL-4 and IL-7 mediate growth suppression of cell line			32
MR-87	Expresses receptors for IL-7 and IL-4		No proliferative response to IL-7			39
NALM-16	Constitutive expression of CD95/APO-1 on cell surface					70

Continued on next page

Table 3. (continued)

Cell lines	Cytokine receptor expression	Cytokine production	Proliferation response to cytokines	Differentiation response to cytokines	Dependency on cytokines	Ref.
Common-B cell lines (B-II)						
NALM-20			Enhanced colony growth with IL-7			39
NALM-21/-22/-23			Enhanced colony growth with IL-7			39
NALM-24/-25			Enhanced colony growth with IL-7			39
OM9;22	Receptors for IL-7 and IL-4		Enhanced colony growth by IL-7 and decreased growth by IL-4	Treatment with IL-7 induces expression of CD20		39
REH			Decreased proliferation of cells with exposure to rIL-4; rIL-4 downregulates IL-3-induced proliferation; decreased cell proliferation by IFN-γ	Induction of marker maturation of leukemic cells by incubation with rIL-4; IFN-γ induces expression of class I MHC antigens, whereas TPA induces expression of class II MHC antigens		48,67,73

Continued on next page

Table 3. (continued)

Cell lines	Cytokine receptor expression	Cytokine production	Proliferation response to cytokines	Differentiation response to cytokines	Dependency on cytokines	Ref.
Pre-B cell lines (B-III)						
697	Constitutive expressionon of CD95/APO-1 cell surface		IL-7 enhances susceptibility of cells to CD95/APO-1 mediated apoptosis			70
BLIN-1	High affinity IL-3 receptors		Increased proliferation with L-BCGF, IL-3		Cells are dependent on L-BCGF for optimal growth	46
HPB-NULL	Expression of IL-1 receptors	IL-2	No proliferative response to rIL-1			69,71
INC		IL-2				71
uKM3			rIL-4 decreases proliferation of cells in culture; decreased cell proliferation by IFN-γ	Induction of surface marker maturation (increased expression of CD20, CD23)		48,67
LAZ-221		IL-2				71
NALM-1		IL-2				71

Continued on next page

Table 3. (continued)

Cell lines	Cytokine receptor expression	Cytokine production	Proliferation response to cytokines	Differentiation response to cytokines	Dependency on cytokines	Ref.
Pre-B cell lines (B-III) (continued)						
NALM-6	Express endoglin as part of TGF-β receptor complex; expression of IL-1 receptors; constitutive expression of CD95/APO-1 on cell surface	IL-2	Growth inhibition by rhIFNa; no proliferative response to stimulation with rIL-1; IL-7 enhances susceptibility of cells to CD95/APO-1 induced apoptosis			68–72
PRE-ALP			Incubation with IL-7 causes cell proliferation in dose-dependent manner	No induction of TPA, differentiation by IL-7, and other cytokines		57
CyIgM status unknown (B-II/III)						
Z-119	No binding site for GM-CSF	IL-1β, G-CSF, GM-CSF				64
Z-181	High-affinity receptors for GM-CSF; RT-PCR shows transcripts present for GM-CSF receptor	IL-1β, G-CSF, GM-CSF	Anti-GM-CSF antibodies signficantly suppress colony formation in a dose-dependent fashion			64

5. CYTOGENETIC AND MOLECULAR CHARACTERIZATION

As in vivo, cell lines in vitro are characterized by complex chromosomal aberrations, both structural and numerical [74]. Karyotypes of BCP cell lines that have been characterized and described are summarized in Table 4. Besides a wide array of modal numbers, three structural chromosomal aberrations are prominent, including translocations t(9;22), the Philadelphia chromosome, t(4;11), and t(1;19). Not surprisingly, and reflective of in vivo incidence rates, the Philadelphia translocation is the most common. Translocation t(9;22)(q34;q11) occurs in 12 of the cell lines included. In most cases, the *BCR-ABL* fusion product has been characterized. Next most frequent was translocation t(1;19) resulting in the characteristic *E2A-PBX1* fusion product. Interestingly, BCP cell line TS-2 is carrying a t(1;19) abnormality, but no evidence of the *E2A-PBX1* gene rearrangement was found by RT-PCR. Cell line KMO-90 is also harboring a point mutation at codon 177 of the p53 gene as identified by SSCP analysis [49]. Translocation t(4;11) was found in four cell lines.

Deletions of the *CDKN2* gene ($p16^{INK4a}/p14^{ARF}$) have been identified in some BCP cell lines [80]. In some cases, cell lines have been used for the cloning of novel fusion genes and the localization of genes at or near chromosomal breakpoints. The sequence of a putative tumor suppressor gene, i.e. *p16/p14* and *p15*, on chromosome 9p21 has been facilitated by analysis of various tumor cell lines [81,82], The localization of these genes, encoding cyclin-dependent kinase inhibitor proteins, has also been established in cell lines [83,84]. The sequence of the *MLL* gene spanning the breakpoint at chromosome 11q23 was determined by analysis of the BCP cell line RS4;11 [85]. The fusion of the *TEL* gene on chromosome 12 to the *AML1* gene on chromosome 21, as it occurs in up to 30% of childhood B-lineage ALL, has also been demonstrated in tumor cell lines [86]. Identification of the same cytogenetic and/or molecular profile between patient specimen and cell line provides a good means to establish authenticity of the cell line created.

6. FUNCTIONAL CHARACTERIZATION

Doubling times of BCP cell lines vary widely, ranging from less than 24 hours to one week. Most cell lines have been tested for EBV infection and found to be negative (Table 5). Not surprisingly, BCP cell lines are MPO-negative, although in some cases MPO expression at the mRNA level was demonstrated. The significance of this finding in vivo is unclear [99]. The

Table 4. Precursor B-cell lines: cytogenetic characterization

Cell lines	Karyotype	Characteristic translocation	Genetic rearrangements	Ref.
Pro-B cell lines (B-I)				
A-1	45, XY with multiple structural and numerical abnormalities			13
B1	45, XY, der(1)t(1;8)(p36;q13), −4, +6, −9, der(10)t(1;10)(q15;p15), der(11)t(4;11)(q21;q23)	t(4;11)(q21;q23)		14
HBL-3	46, XX, −3, −9, −9, +der(3) t(3;?), +der(9) t(9;?), +der(9) t(1;9)			15
JKB-1	46, XX, t(9;14)(p21;q32)			16
KH-4	46, XY; 45, XY, −E			17
KOCL-51	46, XY, del(11)(q23)		11q32	75
NALM-19	46, XY, −11, +der(11)t(11;?)(11pter-11q23?)/46, XY			19,37,38
RS4;11	46, XX, i (7q), t(4;11)(q21;q23)	t(4;11)(q21;q23)		20
SEM	46, XX, t(4;11)(q21;q23), del(7)(p15), del(13)(q12)	t(4;11)(q21;q23)		21

Continued on next page

Table 4. (continued)

Cell lines	Karyotype	Characteristic translocation	Genetic rearrangements	Ref.
Common-B cell lines (B-II)				
BV173	47(46–48)<2n>X/XY, +mar, der(22)t(9;22)(q34;q11)	t(9;22)(q34;q11)	*BCR-ABL* (b2/a2)	76
EU-1	45, X, t(8;21), del(3p), del(4q), del(5q), der(12), der(12), der(16), der(16)			24
HAL-01	46, XX, t(1;17)(p34;q21), t(17;19)(q21;p13)	t(17;19)(q21;p13)	*E2A-HLF*	26
KH-3	46, XX; 46, XX, +2q; 45, XX, −E; 47, XX, +13			17
Kid-92	t(4;11)(q21;q23)	t(4;11)(q21;q23)	*MLL-AF4*	29
KOPN-1	46, XX, t(11;19)(q23;p13)			50,75
LC4-1	46, XX			31
MIELIKI	45, XX, t(7;9)			32
MR-87	46, XY, 9p−, 17p?, t(9p?q+, 22q−)	t(9;22)	*BCR-ABL* (e1a2)	33
NALL-1	43, −X, −Y			11
NALM-16	27, X, +10, +14, +18, +21, 7p+			29
NALM-20	46, XY, +2, −8, t(9;22)(q34;q11)	t(9;22)(q34;q11)	*BCR-ABL*	37
NALM-21	49, XY, −3, +4, +5, +18, −19, +mar, t(9;22)(q34;q11) (2)	t(9;22)(q34;q11)	*BCR-ABL*	37
NALM-24	45, XX, −15, −20, +mar, t(9;22)(q34;q11), del(9)(p21)	t(9;22)(q34;q11)	*BCR-ABL*	38
NALM-27	46, XY, t(9;22;10)(q34;q11;q22)	t(9;22)(q34;q11)	*BCR-ABL* (b3/a2)	11
NALM-29	46, XY, del(6)(q15q21), t(9;22)(q34;q11)	t(9;22)(q34;q11)	*BCR-ABL*	11
NALM-30	46-47, XY, −14, −15, −20, +4mar, add(3)(q11), del(6)(q15q21), t(9;22;10)(q34;q11;q22)	t(9;22)(q34;q11)	*BCR-ABL*	11
NALM-33	48, XY, +1, +21, t(8;14)(?q24;?q32), del(15)t(15;20)(p12;p/q11)			11

Continued on next page

Table 4. (continued)

Cell lines	Karyotype	Characteristic translocation	Genetic rearrangements	Ref.
Common-B cell lines (B-II) (continued)				
OM9;22	45, X, −X, −4, −8, −15, −16, −17, +4mar, +der(1)t(1;?)(q25;?), del(3)(q26), t(9;22)(q34;q11)	t(9;22)(q34;q11)	*BCR-ABL* (e1/a2)	39
PC-53	45, XX, −1, −1, −3, −14, −17, der(1)(1pter-q32 or 44::1p31-pter), +M1, +M2, +M3			40
RCH-ACV	47, XX, +8, t(1;19)(q23;p13.3)	t(1;19)(q23;p13.3)		41
REH	46, X, −X, +16, del(3)(p21.3p24), der(4)(4pter-q32::?16q24.3-qter), t(5;12)(q31.2;p13), der(12)(12qter-q23::12p13-q23::4q32-qter), der(16)t(16;21)(q24.3;q22) (2)/der(21)t(12;21)(p13;q22)	t(12;21)(p13;q22)	*TEL/ETV6-AML1*	28
SUP-B2	44, XY, t(2;4)(q13;q25), t(3;7)(q25;p15), inv(5)(q13q33), del(6)(q23q27), t(11;17)(p11.2;q11.2), −14, del(17)(p11p13), dic(21;21)(q21;q21)/88			
SUP-B7	46, XX, del(3)(q26q28)			43
SUP-B26	48, XY, der(5)t(5;?9)(q33;q12), del(8)(q22), der(12)t(?5;12)(q33;p13), +21, +21			42
TC78	48, XY, 2q+, 5q+, 6q−, 7q−, 8q+, 9p−, 12p−, +mar(2)			44
UoC-B1	46, X, −X, −1, −6, t(7;11)(q22;q13), add(12)(q24), der(12)t(1;12)(q21;q13), −17, der(19) t(17;19)(q22;p13), +4 mar	(17;19)(q21/22;p13)	*E2A-HLF*	42
UoC-B4	47, XX, t(2;14)(p11;q32), del(6)(q15q22), t(7;15)(q32;q15 or q21), add(16)(p13), +21 (4)/47, XX, del(8)(p11p21 or p21p23)			42
UoC-B7	46, XY, del(4)(q21q31), del(7)(q32q36), del(9)(p21 or p21p24), del(17)(p11p13)			42
UoC-B8	46, XY(13)/46, XY, dup(1)(q21q42), add(21)(p11) (6)			42
UoC-B9	46, XX			42
UoC-B10	46, XY, dic(8;22)(q24;p11), del(11)(p12), del(11)(q13), +i (12p), t(13;13)(q10;q10), der(20)t(17;20)(q11;q13.3)			42

Continued on next page

Table 4. (continued)

Cell lines	Karyotype	Characteristic translocation	Genetic rearrangements	Ref.
Pre-B cell lines (B-III)				
207	46, XY			45
697	46, XY, t(7;19)(q11;q13)			45
BLIN-1	46, XY, −9, +der(9)t(8;9)(q?21.2;p2?2)			46
KLM-2	47, XY, −4, −6, +7, −8, −9, −9, −14, +16, −17, −18, +der(4)t(4;?)(pter-q3?1::?), +der(6)t(6;18)(6qter-6p21::18q1?q-18qter), +der(9)t(9;17)(9qter-9p2?1::17q21-17qter), +der(9)t(9;11?)(9pter-9q34::11?q1?3-11?qter), +der(14or8)t(14;8)(14qter-14p1?1::8q1?1-8q24), +der(14)t(14;8)(14pter-14q32::8q24-8qter), mar+			11
KMO-90*	48, XX, +8, +19, t(1;19)(q23;p13)	t(1;19)(q23;p13)	*E2A-PBX1*	49
KOCL-33	46, XX, t(11;19)(q23;p13)			50,75
KOCL-44	46, XX, t(11;19)(q23;p13)			50,75
KOCL-45	46, XY, t(4;11)			50
KOCL-50	46, XXX, t(7;11)			50
KOCL-58	46, X?, t(4;11)			50
KOPB-26	46, XX, t(11;?)			50
KOPN-8	45, XX, −1, −13, −14, t(11;19)(q23;p13), t(8;13)(q24;q22), +der(1)t(1;?)(?;?), +der(13)t(13;14)(p11;q11)	t(11;19)(q23;p13)	*ENL-MLL*	11

Continued on next page

Table 4. (continued)

Cell lines	Karyotype	Characteristic translocation	Genetic rearrangements	Ref.
Pre-B cell lines (B-III) (continued)				
LAZ-221	45, XX, −9, −12, +t(9q12q)			51
NALM-1	45(42-47)<2>X, −X, der(9)t(9;22)(q24;q12), dup(13)(q21), der(22)t(9;22)(q34;q12)	t(9;22)(q34;q12)	*BCR-ABL*	76,77
NALM-6	46(43-47)<2n>XY, t(5;12)(q33.2;q13.2)			53,76,78
NALM-26	45, X, −9, −19, +mar, dic(1;11)(p13;q25), +del(1)(p13), +del(1)(q21), i(9)(p10), add(14)(q24)			79
P30/OHKUBO	46(45), X(X), del(2)(p23), del(9)(p12-q31), t(11;12)(q25;q13), inv(12)(q13q24)			11
PER-278	46, XY, −9, −19, +der(9) t(1;9)(q23;p13), +der(19) t(1;19)(q23;p13)	t(1;19)(q23;p13)		56
PRE-ALP	45, XX, −2, t(1;19)(q23;p13.3)	t(1;19)(q23;p13.3)	*E2A-PBX1*	57
SMS-SB	47, XX, +mar			58
SUP-B16	46, XX, t(4;9)(p12;q12), del(9)(q12q22), del(13)(q12q14)			42
SUP-B24	47, XY, −2, der(3), −5, inv(7)(p13p22), −9, del(12)(p11.2p13), −15, der(16)(q12q24), −17, +mar			42
SUP-B27	47, XY, t(1;19)(q23;p13.3), +8	t(1;19)((q23p13.3)		42
TS-2	47, X, −X, t(1;19)(q23;13), add(3)(q22), del(5)(q13), +6, del(6)(q12q23), del(9)(q13q22), del(11)(p11), add(15)(q22), +mar1	t(1;19)(q23;p13)	No evidence of *E2A- PBX1* gene rearrangement by RT-PCR	61
UoC-B3	46, XX, der(9)t(9;?9)(p1?2;q22), der(19)t(1;19)(q23;p13.3)	t(1;19)(q23;p13.3)		

Continued on next page

Table 4. (continued)

Cell lines	Karyotype	Characteristic translocation	Genetic rearrangements	Ref.
CyIgM status unknown (B-II/III)				
G2	46, XX, der(5) t(5;?)(q15;?), der(9) t(9;?)(p13;?), −11, der(14) t(14;?)(q22;?), +M1			13
LILA-1	46, XX, −9, t(1;19)(q23;p13), +der(9)(1;9)t(q11;p11)	t(1;19)(q23;p13)		42,63
LK-63	45, X, −X (or Y), +8, −9, −17, t(1;19)(q23;p13), +der(9)t(9;17)(p11;p11)	t(1;19)(q23;p13)		63
Tree92	(8;14)((q24;q32)			29
Z-119	45X, −?X/Y, der(1) t(1;8)(q24;q13), t(7;12)(q11.2;q24.3), t(9;22)(q34;q11.2)	t(9;22)(q34;q11)	*BCR-ABL* (e1a2)	64
Z-181	45, X-?X/Y, t(6;9;22)(q25;q34;q11.2), del(7)(p11.2;p22), +8, der(9) t(6;9;22)	t(9;22)(q34;q11)	*BCR-ABL* (e1a2)	64

* Point mutation at codon 177 of the p53 gene identified by SSCP analysis.

Table 5. Precursor B-cell Lines: functional characterization

Cell lines	Doubling time (hrs)	EBV status	Cytochemistry	Inducibility of differentiation	Engraftment into mice	Other features	Ref.
Pro-B cell lines (B-I)							
A-1	48	Negative			Can engraft into SCID mice (BM and peripheral blood)		12, 13
B1	40–50	Negative	PAS+, AP+, NSE+, SBB−				14
HBL-3	48–72	Negative	TdT+, MPO−, AP−, esterase-		Failed to inoculate into nude mice		15
JKB-1	24	Negative	PAS−, MPO−, NBE−, CAE−	Induction of differentiation after incubation with irradiated bone marrow stromal cells[a]			16
KH-4	72	Negative	TdT+, AP+, MPO−, SBB−, NSE−	Differentiation into B-cell lineage by treatment with TPA			17
KOCL-51			MPO−				50
NALM-19			TdT+, MPO−				11
RS4;11	60–70	Negative	TdT+, MPO−, SBB−, AP+, NSE+/−	TPA induces monocyte-like phenotype		Induction of apoptosis and decrease in cell viability by cyclosporin A	20, 59
SEM	48	Negative	TdT+, MPO−, SBB-, NSE−, PAS−				21

Continued on next page

Table 5. (continued)

Cell lines	Doubling time (hrs)	EBV status	Cytochemistry	Inducibility of differentiation	Engraftment into mice	Other features	Ref.
Common-B cell lines (B-II)							
BV173	30–35	Negative	TdT+, MPO− (protein), +(mRNA), PAS− esterase−	No induction of differentiation by TPA, retinoic acid, or butyric acid			23, 87
EU-1		Negative	TdT+, MPO−, SBB−, esterase−	Can be induced to differentiate along myeloid pathway by DMSO			24, 25
HAL-01	32	Negative	TdT+, MPO− (protein), +(mRNA), SBB−, PAS−, esterase−		Cells are transplantable into nude mice (ascites tumor, multiorgan infiltration)		26
HOON	24		MPO−, SBB−, PAS−, NSE−, AP+			Good stimulatory capacity in MLR	27
HYON	36		MPO−, SBB−, PAS−, NSE−, AP+			Does not stimulate lymphocytes in MLR	27
KH-3	48	Negative	TdT−, AP+, MPO−, SBB−, NSE−, PAS−	Can be induced to differentiate into T or B cells after treatment with TPA			17

Continued on next page

Table 5. (continued)

Cell lines	Doubling time (hrs)	EBV status	Cytochemistry	Inducibility of differentiation	Engraftment into mice	Other features	Ref.
Common-B cell lines (B-II) (continued)							
Kid-92	36.5	Negative	TdT+, MPO− (protein), + (mRNA)			Growth inhibition by dexamethasone	29
KOPN-1			MPO−				50,68
KOPN-K			TdT+, MPO−				11
LC4-1		Negative	MPO−	Treatment with PMA induces loss of CD10 expression			31
MIELIKI	120–144		TdT+				32
MR-87	120–144	Negative	TdT+, MPO+, SBB+, PAS-, NSE-[b]				27
NALL-1	60–90	Negative	TdT+, MPO− (protein)[c], +(mRNA)	Differentiative response to TPA as reduction in expression of CD10 and decrease in proliferation		Stimulate allogeneic lymphocytes in one-way MLR reactions	34, 88
NALM-16	36.7	Negative	TdT+, MPO−	Differentiative response to TPA as reduction in expression of CD10 and decrease in proliferation		No growth inhibition by dexamethasone; expresses 140 kD isoform of NCAM protein on cell surface	29, 36, 88

Continued on next page

Table 5. (continued)

Cell lines	Doubling time (hrs)	EBV status	Cytochemistry	Inducibility of differentiation	Engraftment into mice	Other features	Ref.
Common-B cell lines (B-II) (continued)							
NALM-20	72		TdT+, MPO− (protein), +(mRNA)				37
NALM-21/-22/-23	72		TdT+, MPO− (protein), +(mRNA)				37
NALM-24/-25	72		TdT+, MPO− (protein), +(mRNA)				38
NALM-27/-28			TdT+, MPO− (protein)[c], +(mRNA)				11
NALM-29			TdT+, MPO− (protein), +(mRNA)				11
NALM-30/-31/-32			TdT+, MPO− (protein) [c], +(mRNA)				11
NALM-33/-34			TdT+, MPO−				11
OM9;22	80		TdT+, MPO− (protein), +(mRNA), PAS−, esterase−				39
PC-53	70–80	Negative	TdT+, MPO−, PAS+, SBB−, esterases−			Secretion of autostimulatory factors suggested	40
RCH-ACV	36	Negative					41

Continued on next page

Table 5. (continued)

Cell lines	Doubling time (hrs)	EBV status	Cytochemistry	Inducibility of differentiation	Engraftment into mice	Other features	Ref.
Common-B cell lines (B-II) (continued)							
REH	34.7	Negative	TdT+, MPO− (protein), +(mRNA)	Differentiative response to TPA as reduction in expression of CD10 and decrease in proliferation; maturation by TPA to intermediate stages of differentiation[d]		Resistant to growth inhibition by dexamethasone, induction of apoptosis and decrease in cell viability by cyclosporin A; LIF activity in culture supernatant[e]	29, 53, 86, 87, 89
SUP-B2		Negative	TdT+				72
SUP-B7	168	Negative	TdT+, PAS+, AP+, NSE−, CE−, SBB−				43
SUP-B26		Negative	TdT+				42
TC78		Negative	TdT+, PAS+, MPO−, esterase−		Tumorigenicity was established in NIH Swiss *nu/nu* mice		90
UoC-B1		Negative	TdT+				42
UoC-B4		Negative	TdT+				42
UoC-B7		Negative	TdT+				42
UoC-B8		Negative	TdT+			Growth inhibited by exogenous PGE_2	65
UoC-B9		Negative	TdT+				42
UoC-B10		Negative	TdT−				42

Continued on next page

Table 5. (continued)

Cell lines	Doubling time (hrs)	EBV status	Cytochemistry	Inducibility of differentiation	Engraftment into mice	Other features	Ref.
Pre-B cell lines (B-III)							
207	60–70	Negative	TdT+, MPO−, esterase−, AP+, PAS+				45
697	60–70	Positive[f]	TdT+, MPO−, esterase-, AP+, PAS+				45
BLIN-1	43[g]	Negative	TdT−, NSE−, MPO−, SBB−, PAS+	Differentiation into sIgκ-positive B cells under serum-free conditions			46
HPB-NULL			TdT−, MPO−				11
INC	44.4	Negative	TdT+, MPO− (protein), +(mRNA			Sensitive to growth inhibition by dexamethsone	29
KLM-2			TdT−, MPO−	Macrophage differentiation in the presence of TPA and CSF			91
KM-3				Differentiation to intermediate stage of B-cell differentiation by TPA[f]			62, 87
KMO-90	72	Negative	AP+, MPO−, esterase−, PAS−				49

Continued on next page

Table 5. (continued)

Cell lines	Doubling time (hrs)	EBV status	Cytochemistry	Inducibility of differentiation	Engraftment into mice	Other features	Ref.
Pre-B cell lines (B-III) (continued)							
KOCL-33			MPO−				50
KOCL-44			MPO−				50
KOCL-45			MPO−				50
KOCL-50			MPO−				50
KOCL-58			MPO−				50
KOPB-26			MPO−				50
LAZ-221	96	Negative	TdT+, MPO−				11
NALM-1	72–120	Negative	TdT+, MPO− (protein), +(mRNA), SBB−, PAS−, NSE			Cells continue to grow up to 3 weeks with good viability without additional medium supplement	52
NALM-6	40.2	Negative	TdT+, MPO−	Differentiation to intermediate stage of B-cell differentiation by TPA[f]	Engrafts into SCID mice	Sensitive to growth inhibition by dexamethasone; expresses CD95 and undergoes apoptosis after exposure to anti-CD95; induction of apoptosis and decrease in cell viability by cyclosporin A	59, 87 92–95

Continued on next page

Table 5. (continued)

Cell lines	Doubling time (hrs)	EBV status	Cytochemistry	Inducibility of differentiation	Engraftment into mice	Other features	Ref.
Pre-B cell lines (B-III) (continued)							
NALM-17/-18			TdT+, MPO− (protein), +(mRNA)				11
NALM-26			TdT+, MPO−				11
P30/OHKUBO			TdT+, MPO− (protein), +(mRNA)				11
PER-278	62–68	EBNA+	TdT+, PAS+, AP−, MPO−, NSE−				56
PRE-ALP	24	Negative	MPO−				57
SMS-SB	48	Negative	TdT−			Overexpression of *c-fos* protooncogene, addition of soluble CD23 rescues cells from apoptosis	96, 97
SUP-B15		Negative				Induction of apoptosis and decrease in cell viability by cyclosporin A	59
SUP-B16		Negative	TdT+				42
SUP-B24		Negative	TdT+				42
SUP-B27		Negative	TdT+				42
Tahr-87			TdT+, MPO+ (protein) and mRNA				60
TS-2			MPO−, PAS−, α-NBE−, naphthol ASD chloro-acetate esterase−				61
UoC-B3		Negative	TdT+				42

Continued on next page

Table 5. (continued)

Cell lines	Doubling time (hrs)	EBV status	Cytochemistry	Inducibility of differentiation	Engraftment into mice	Other features	Ref.
CyIgM status unknown (B-II/III)							
G2	72	Negative					13
JM		Negative	AP+, PAS−, NSE−, MPO−				62
KM-3		Negative	PAS+, AP−, NSE−, MPO−	Differentiative response to TPA as reduction in expression of CD10 and decrease in proliferation. Downregulation of PKC with TPA			62 88 98
LILA-1		Negative		TPA induces appearance of sCD20 antigen on all cells			63
LK-63	13	Negative		TPA induces appearance of sCD20 antigen on all cells			63
Tree92	35.4	Negative	TdT+				29
Z-119	20–30	Negative	TdT−, PAS+				64
Z-181	30–40	Negative	TdT+, MPO−, NASD−, NSE−, PAS−				64

[a] As evidenced by expression of common-B-cell antigens (CD10, CD20), cyIgM, light chain gene rearrangement, and disappearance of TdT. [b] 5% of cells were reactive to MPO cytochemically. More than 90% were positive for MPO when examined by EM. [c] MPO positive with monoclonal antibody staining. [d] assessed by isoenzyme expression profile [87]. [e] after 48-hour incubation in serum free medium (RPMI 1640); effect potentiated by pulsing with PHA [89]. [f] EBNA and capsid antigens. [g] doubling time under optimal growth conditions, up to 96 hours without L-BCGF.

most consistent cytochemical profile in BCP ALL, however, is MPO-, SBB-, esterase-, AP+/–, PAS+/–, TdT+.

Differentiation has been described for a variety of BCP cell lines (Table 5). Exposure, in most cases to TPA or PMA, results in maturation along the B-lineage differentiation pathway, as is evident by acquisition of surface or cytoplasmic differentiation markers as well as intracellular isoenzyme expression patterns (KH-4, RS4;11, REH, BLIN-1, KLM-2, KM-3, NALM-6, KH-3, LC4-1, NALL-1, NALM-16, KM-3, LILA-1, LK-63). Cell line EU-1 can be induced to differentiate along the myeloid pathway by DMSO [25,80]. Engraftment into mice and establishment of the leukemic potential of BCP lines in vivo has been successfully done with A-1, HAL-01, NALM-6 and TC-78 cells.

Differences have been observed between sublines grown in different laboratories. For example, in a comparison of HL-60 promyelocytic leukemia cells from different institutions, it was observed that the morphology, cell surface markers and DNA histograms were indistinguishable. However, when induced to differentiate with TPA, only one subline of HL-60 cells differentiated into mature granulocytes.

7. CONCLUSIONS

Many BCP cell lines have been described, but most are used only for a specific purpose, and few have been adequately characterized. Cell lines provide a useful laboratory tool, but scientists should be aware of their limitations and care should be taken in extrapolating data to in vivo situations.

References

1. LeBien TW, in Henderson ES, Lister AT, Greaves MF (eds.) 1996 *Leukemia*, W.B. Saunders, Philadelphia.
2. Gore SD, Kastan MB, Civin CL. *Blood* 77: 1681, 1991.
3. Abbas AK, Lichtman AH, Pober JS (eds.) 1997 *Cellular and Molecular Immunology*, W.B. Saunders, Philadelphia.
4. Komori T, Okada A, Stewart V, et al. *Science* 261: 1171, 1993.
5. Schatz DG, Oettinger MA, Schlissel MS. *Annu Rev Immunol* 10: 359, 1992.
6. NCI 1989
7. Faderl S, Kantarjian HM, Talpaz M, Estrov Z. *Blood* 91: 3995–4019, 1998.
8. Bennett JM, Catovsky D, Daniel MT, et al. *Br J Haematol* 47: 553–561, 1981.
9. EGIL. *Leukemia* 9: 1783–1786, 1995.
10. Rosenfeld C, Goutner A, Choquet C, et al. *Nature* 267: 841–843, 1977.
11. Matsuo Y, Drexler HG. *Leukemia Res* 22: 567–579, 1998.
12. Kamel-Reid S, Letarte M, Sirard C, et al. *Science* 246: 1597–1600, 1989.

13. Kamel-Reid S, Dick JE, Greaves A, et al. *Leukemia* 6: 8–17, 1991.
14. Cohen A, Grunberger T, Vanek W, et al. *Blood* 78: 94–102, 1991.
15. Abe 1999.
16. Urashima M, Hasegawa N, Kamijo M, et al. *Am J Hematol* 46: 112–119, 1994.
17. Nagasaka-Yabe M, Maeda S, Mabuchi O, et al. *Jpn J Cancer Res* 79: 59–68, 1988.
18. Yamamoto 1993.
19. Matsuo Y, Ariyasu T, Ohmoto E, et al. *Cell* 4: 257–260, 1991a.
20. Stong RC, Korsmeyer SJ, Parkin JL, et al. *Blood* 65: 21–31, 1985.
21. Greil J, Gramatzki M, Burger R, et al. *Br J Haematol* 86: 275–283, 1994.
22. Okabe M, Matsushima S, Morioka M, et al. *Blood* 69: 990–998, 1987.
23. Pegoraro L, Matera L, Ritz J, et al. *J Natl Cancer Inst* 70: 447–453, 1983.
24. Zhou M, Findley HW, Ma L, et al. *Blood* 77: 2002–2007, 1991.
25. Zhou M, Zaki SR, Ragab AH, et al. *Leukemia* 8: 659–663, 1994.
26. Ohyashiki K, Fujieda H, Miyauchi J, et al. *Leukemia* 5: 322–331, 1991.
27. Okamura J, Okano H, Ikuno Y, et al. *Leukemia Res* 8: 97–104, 1984.
28. Uphoff CC, MacLeod RAF, Denkmann SA, et al. *Leukemia* 11: 441–447, 1997.
29. Tani A, Tatsumi E, Nakamura F, et al. *Leukemia* 10: 1592–1603, 1996.
30. Nakazawa S, Kaneko K, Sasaki Y, et al. *Jpn J Clin Hematol* 20 (Suppl 1): 189, 1978.
31. Yoshimura T, Mayumi M, Yorifuji T, et al. *Am J Hematol* 26: 47–54, 1987.
32. Renard N, Duvert V, Matthews DJ, et al. *Leukemia* 9: 1219–1226, 1995.
33. Okamura J, Yamada S, Ishii E, et al. *Blood* 72: 1261–1268, 1988.
34. Miyoshi I, Hiraki S, Tsubota T, et al. *Nature* 267: 843–844, 1977.
35. Kohno S, Minowada J, Sandberg AA. *J Natl Cancer Inst* 64: 485–493, 1980.
36. Patel K, Frost G, Rossell R, et al. *Leukemia Res* 16: 307–315, 1992.
37. Matsuo Y, Ariyasu T, Ohmoto E, et al. *Human Cell* 4: 335–338, 1991b.
38. Matsuo Y, Ariyasu T, Adachi T, et al. *Human Cell* 4: 339–342, 1991c .
39. Ohyashiki K, Miyauchi J, Ohyashiki JH, et al. *Leukemia* 7: 1034–1040, 1993.
40. Tarella C, Pregno P, Gallo E, et al. *Leukemia Res* 14: 177–184, 1990.
41. Jack I, Seshadri R, Garson P, et al. *Cancer Genet Cytogenet* 19: 261–269, 1986.
42. Zhang LQ, Downie PA, Goodell WR, et al. *Leukemia* 7: 1865–1874, 1993.
43. Smith SD, Morgan R, Galili N, et al. *Cancer Res* 47: 1652–1656, 1987.
44. Jenski LJ, Lampkin BC, Goh TS, et al. *Leukemia Res* 12: 1497–1506, 1985.
45. Findley HW, Cooper MD, Kim TH, et al. *Blood* 60: 1305–1309, 1982.
46. Wörmann B, Anderson JM, Liberty JA, et al. *J Immunol* 142: 110–117, 1989.
47. Fu SM, Hurley JN. *Proc Natl Acad Sci USA* 76: 6637–6640, 1979.
48. Blomhoff HK, Davies C, Ruud E, et al. *Scan J Immunol* 22: 611–617, 1985.
49. Sotomatsu M, Hayashi Y, Kawamura M, et al. *Leukemia* 10: 1615–1620, 1993.
50. Iida S, Saito M, Okazaki T, et al. *Leukemia Res* 16: 1155–1163, 1992.
51. Lazarus H, Barell EF, Krishan A, et al. *Cancer Res* 38: 1362–1367, 1978.
52. Minowada J, Tsubota T, Greaves MF, Walters TR. *J Natl Cancer Inst* 59: 83–87, 1977.
53. Hurwitz R, Hozier J, LeBien T, et al. *Int J Cancer* 23: 174–180, 1979.
54. Matsuo Y, Adachi T, Tsubota T. *Human Cell* 7: 221–226, 1994.
55. Hirose M, Minato K, Tobinai K, et al. *Jpn J Cancer Res* 73: 600–605, 1982.
56. Kees UR, Lukeis R, Ford J, et al. *Cancer Genet Cytogenet* 46: 201–208, 1990.
57. Pandrau D, Frances V, Martinez-Valdez H, et al. *Leukemia* 7: 635–642, 1993.
58. Smith RG, Dev VG, Shannon WA. *J Immunol* 126: 596–602, 1981.
59. Ito C, Ribeiro RC, Behm FG, et al. *Blood* 91: 1001–1007, 1998.
60. Yamaguchi N, Tatsumi E, Yoneda N, et al. *Blood* 76: 338a, 1990.
61. Yoshinari M, Imaizumi M, Eguchi M, et al. *Cancer Genet Cytogenet* 10: 95–102, 1998.

62. Schneider U, Schwenk H-U, Bornkam G, et al. *Int J Cancer* 19: 621–626, 1977.
63. Salvaris E, Novotny JR, Welch K, et al. *Leukemia Res* 16: 655–663, 1992.
64. Estrov Z, Talpaz M, Zipf TF, et al. *J Cell Physiol* 166: 618–630, 1996.
65. Giordano L, Moldwin RL, Downie PA, et al. *Leukemia Res* 21: 925–932, 1997.
66. Minowada J, Koshiba H, Janossy G, et al. *Leukemia Res* 3: 261–266, 1979.
67. Ouaaz F, Mentz F, Mossalayi MD, et al. *Eur J Haematol* 51: 276–281, 1993.
68. Shibata T, Shimada Y, Shimoyama M. *Jpn J Clin Oncol* 15: 67–75, 1985.
69. Uckun FM, Myers DE, Fauci AS, et al. *Blood* 74: 761–776, 1989.
70. Levy Y, Benlagha K, Buzyn A, et al. *Clin Exp Immunol 11*0: 329–335, 1997.
71. Holan V, Minowada J. *Neoplasma* 40: 3–8, 1993.
72. Zhang H, Shaw ARE, Mak A, Letarte M. *J Immunol* 156: 565–573, 1996.
73. Wang CY, A1-Katib A, Lane CL, et al. *J Exp Med* 158: 1757, 1983.
74. Drexler HG, MacLeod RAF, Janssen JWG. *Leukemia* 9: 480–500, 1995.
75. Iida S, Seto M, Yamamoto K, et al. *Jpn J Cancer Res* 84: 532–537, 1993.
76. Drexler HG, Dirks W, MacLeod RAF, et al. (eds.) 1997 *DSMZ Catalogue of Human and Animal Cell Lines*, Braunschweig, Germany: DSMZ.
77. Sonta S-I, Minowada J, Tsubota T, Sandberg AA. *J Natl Cancer Inst* 59: 833–835, 1977.
78. Wlodarska I, Aventin A, Ingléd-Esteve J, et al. *Blood* 89: 1716–1722, 1997.
79. Matsuo Y, Nakamura S, Ariyasu T, et al. *Leukemia* 10: 700–706, 1996.
80. Zhou M, Gu L, James CD, et al. *Leukemia* 9: 1159–1161, 1995.
81. Olopade OI, Jenkins RB, Ransom DT, et al. *Cancer Res* 52: 2523, 1992.
82. Liu Q, Neuhausen S, McClure M, et al. *Oncogene* 10: 1061, 1995.
83. Kamb A, Gruis NA, Weaver-Feldhaus J, et al. *Science* 264: 436, 1994.
84. Nobori T, Miura K, Wu DJ, et al. *Nature* 368: 753, 1994.
85. Ziemin-van der Poel S, McCabe NR, Gill HJ, et al. *Proc Natl Acad Sci USA* 88: 10735, 1991.
86. Romana SP, Mauchauffé M, Le Coniat M, et al. *Blood* 85: 3662, 1995.
87. Drexler HG, Gaedicke G, Minowada J. *Exp Hematol* 14: 732–737, 1986.
88. Sakagami H, Ozer H, Minowada J, et al. *Leukemia Res* 8: 187–195, 1984.
89. Rocklin RE, Blidy A, Butler J, Minowada J. *J Immunol* 127: 534–539, 1981.
90. Jerkins 1985.
91. Hiraoka A, Kubota K, Preisler H, Minowada J. *Blood* 59: 997–1000, 1982.
92. Dirks W, Schöne S, Uphoff C, et al. *Br J Haematol* 96: 584–593, 1997.
93. Arguello F, Sterry JA, Zhao YZ, et al. *Blood* 87: 4325–4332, 1996.
94. Onur EK, Reaman GH, Crankshaw DL, et al. *Leukemia Lymphoma* 28: 509–514, 1998.
95. Filshie R, Gottlieb D, Bradstock K. *Br J Haematol* 102: 1292–1300, 1998.
96. Tsai L-H, Nanu L, Smith RG, Ozanne B. *Oncogene* 6: 81–88, 1991.
97. White LJ, Ozanne BW, Graber P, et al. *Blood* 90: 234–243, 1997.
98. Ase K, Berry N, Kikkawa U, et al. *FEBS* 236: 396–400, 1988.
99. Serrano J, Román J, Jiménez A, et al. *Leukemia* 13: 175–180, 1999.

Chapter 3

B-Lymphoid Cell Lines

Ramzi M. Mohammad, Nathan Wall and Ayad Al-Katib
Department of Hematology and Oncology, Wayne State University and Karmanos Cancer Institute, Lande Research Building Room 317, 550E Canfield, Detroit, MI 48201, USA. Tel: +1-313-577-7919; Fax: +1-313-577-7925; E-mail: mohammad@karmanos.org

1. INTRODUCTION

Human B-cell tumors include a group of heterogeneous diseases with varying natural histories and responsiveness to therapy. Classic examples of B-cell tumors are Burkitt's lymphoma (BL), chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). These tumors express the conventional B-cell markers, that is, surface and/or cytoplasmic immunoglobulins. However, malignant transformation can affect precursors of the mature B-lymphocytes as exemplified by the non-T cell acute lymphoblastic leukemia (ALL). Such cases demonstrate immunoglobulin gene rearrangements and react with monoclonal antibodies to B-cell antigens. B-cell tumors, therefore, represent a spectrum of disorders extending from the immature stem cell to the most mature plasma cell of the B-lineage.

Unlike the granulocytic series where each stage of differentiation has characteristic light microscopic features, morphology is not a reliable indicator of the B-cell differentiation stage. Monoclonal antibodies to B-cell differentiation antigens have been developed, some of which are not only lineage-specific but also stage-restricted. The gain or loss of such markers in response to exogenous agents can provide an objective measure of a change in the differentiation state of the B-cells. Based on this assumption, a number of hypothetical models for B-cell differentiation have been proposed by different groups [1,2]. A hypothetical scheme of B-cell differentiation (Figure 1) is used in our institute. The range of reactivity of each antibody is based on antigen expression on fresh cells taken from patients with B-cell tumors and B-cell lines [3–7].

With the exception of Burkitt lymphoma, attempts to culture lymphomas have been mostly unsuccessful. The most common problem is the overgrowth of Epstein-Barr virus (EBV) positive lymphoblastoid cell lines from B-

J.R.W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol. III, 61–79.

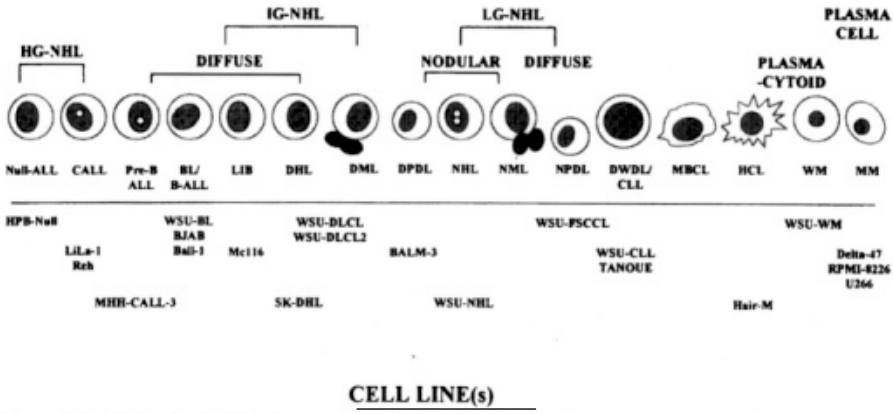

Figure 1. A hypothetical scheme of B-cell differentiation. Stages are arranged on top, from the most immature (stem cell) on the far left to the most mature (plasma cells) on the far right. ALL – Acute lymphoblastic leukemia; CALL – Common ALL; BL – Burkitt's lymphoma; LIB – Large cell immunoblastic lymphoma; DHL – Diffuse histiocytic lymphoma; DML – Diffuse mixed lymphoma; DPDL – Diffuse poorly differentiated lymphocytic lymphoma; NHL – Nodular histiocytic lymphoma; NML – Nodular mixed lymphoma; NPDL – Nodular poorly differentiated lymphocytic lymphoma; DWDL/CLL – Diffuse well differentiated lymphocytic lymphoma/Chronic lymphocytic leukemia; MBCL – Monocytoid B cell lymphoma; HCL – hairy cell leukemia; WM – Waldenstrom's macroglobulinemia; MM – Multiple myeloma; HG-WHL – High grade NHL; IG-NHL – Intermediate grade NHL; LG-NHL – Low grade NHL.

lymphocyte precursors contaminating the original cultured tumor cells [8,9]. Many B-cell lines reported in the literature have been established through EBV infection [10]. Although such B-cell lines may represent the original malignant phenotype, it remains unclear whether the incorporation of the EBV in the genome altered the genetic and biological characteristics. B-cell lines which are EBV-positive show features typical of lymphoblastoid cell lines [10–13]. Such concerns make EBV-transformed cell lines unsuitable for preclinical investigation [14].

Since 1986, we have established more than ten cell lines from B-cell tumors. All of these cell lines were established without the aid of exogenous mitogens, growth factors or viral transformation and all are EBV-negative. The success rate of establishing a B-cell line is approximately 10% [15]. There are no clear predictive factors for successful establishment of such a cell line. However, tumor cells derived from serous effusions appear to have a better chance of continuous growth in vitro. In our experience, 60% of the B-cell lines were established from either a pleural effusion or ascites fluid.

When a fresh specimen is received, mononuclear cells are isolated by Lymphoprep (Nycomed Pharma AS, Oslo, Norway) density centrifugation (density of 1.077 g/ml and osmolality of 280 mOsm). Cells are washed twice

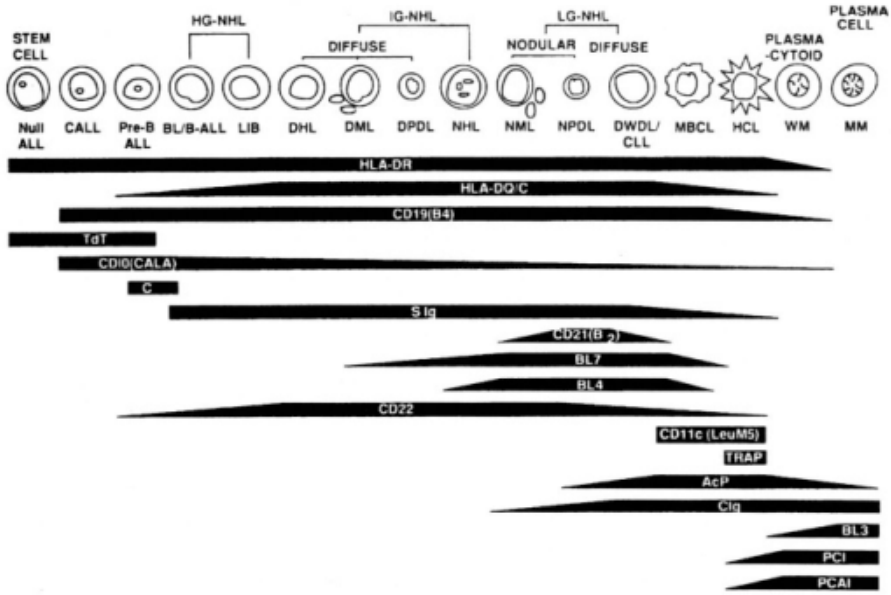

Figure 2. Representative cell line(s) for B-cell tumors (bottom). Stages of B-cell tumors (on top) are the same as in Figure 1.

with either phosphate buffered saline (PBS) or Hanks balanced salt solution (HBSS) and then plated at densities ranging from half a million to ten million cells per ml in RPMI-1640 medium supplemented with 5–30% fetal bovine serum (FBS) for each cell density. Cell density and FBS concentration play a major role in the establishment of a successful B-cell line.

Figure 2 shows examples of various types of B-cell tumors together with the associated stages of the B-cell differentiation pathway. We have also listed other representative line(s) for the corresponding tumor type. The scheme presented in Figure 2 is hypothetical. The nodular (follicular) B-lymphomas are believed to be more mature than the diffuse lymphomas. However, the difference between the small cell and large cell lymphomas (or the poorly differentiated lymphocytic and histiocytic, according to the Rappaport classification) may be related to transformation rather than differentiation [16].

2. EARLY STAGES OF B-CELLS

[Examples: Early pre-B-ALL (HPB-Null); cALL (Lila-1, MHH-CALL, REH); pre-B-ALL(Ball, Km-3, Laz 221); B-ALL (MN-60)].

The classification of B-lineage ALL can be assessed by determining the immunophenotype of cells using monoclonal antibodies to leukocyte anti-

Table 1. Immunologic classification of acute lymphoblastic leukemia

Subtype	Profile of antigen expression
Early pre-B	CD19+, CD22+, CD79a+, CD10+/−, CD7−, CD3−, cIgμ−, sIgκ−, sIgλ−
Pre-B	CD19+, CD22+, CD79a+, CD10+/−, CD7−, CD3−, cIgμ+, sIgμ−, sIκ−, sIgλ−
Transitional pre-B	CD19+, CD22+, CD79a+, CD10+/−, CD7−, CD3−, cIgμ+, sIgμ+, sIgκ−, sIgλ−
B-cell	CD19+, CD22+, CD79a+, CD10+/−, CD7−, CD3−, cIgμ+, sIgμ+, sIgκ+ or sIgλ+

cIg – cytoplasmic immunoglobulin; sIg – surface immunoglobulin; μ (mu) – heavy-chain protein; κ (kappa) – light-chain protein; λ (lambda) – light-chain protein.

gens known to be present during the stages of early pre-B, pre-B, transitional pre-B and B-cell lineages (Table 1).

CD7 and CD3 antibodies are used to rule out the possibility of T-ALL. In cellular differentiation along the B-lymphocyte pathway, the initial commitment of the putative pluripotent stem cell to the B-cell lineage may normally occur early in development in fetal liver [17]. Cells present in the mouse fetal liver at 12 days of gestation possess cytoplasmic immunoglobulin M (cIgM) but lack detectable surface immunoglobulins (sIg) [18].

Additional evidence indicates that these cells, which have been called pre-B cells, are direct precursors of sIg-positive cells [19]. This pattern of B-lymphocyte differentiation from pre-B cells (cyIg+, sIg–) to B cells (sIg+) is now known to occur in man [20]. The synthesis of μ-chains precedes the appearance of immunoglobulin light chains in these cells [20].

The phosphoprotein B1 is a human lymphocyte cell surface marker detected by the CD20 cluster of monoclonal antibodies [22]. During ontogeny, this molecule is first expressed by a subset of pre-B cells. Approximately 50% of pre-B-cell acute lymphoblastic leukemias (ALL) express the CD20 molecule [23]. This indicates that CD20 appears late in pre-B-cell development [22] and persists on mature B cells throughout the resting and activated stages of B-cell development. The CD20 antigen is not expressed on either normal or neoplastic plasma cells [24].

The molecular basis of ALL is likely to be more complex than previously thought. Genetic aberrations resulting in increased cellular proliferation, diminished cell differentiation, and abrogation of the normal process of programmed cell death (apoptosis) may all play a role in the development of the leukemic phenotype. Chromosome translocations have been well studied in ALL and have provided a major contribution towards understanding the biology of this malignancy. Germline mutation in known tumor suppressor

Table 2. Recurring chromosomal translocations in B-ALL

Translocation	Gene	Function	Frequency
t(9;22)(q34;q11)	BCR	unknown	adults: 20%
	ABL	tyrosine kinase	children: 4%
t(1;19)(q23:p13)	E2A	bHLH transcription factor	adults: 2%
	PBX1	hemeotic	children: 5%
t(11;v)(q23;v)	MLL	trithorax-like	adults: 3%
		variable	75% in infants
t(12;21)(p13;q22)	TEL	Ets-like transcription factor	adults: 3%
	AML1	runt-like transcription factor	children: 25%
t(17;19)(q22;p13)	E2A	bHLH transcription factor	<1%
	HLF	bZip transcription factor	
t(5;14)(q31;q32)	IL-3	cytokine	<1%
	IgH	Ig enhancer	
t(8;14)(q24;q32)	MYC	bHLH transcription factor	2–5%
	IgH	Ig enhancer	
t(8;22)(q24;q11)	MYC	bHLH transcription factor	<1%

genes such as RB and p53 are rare in ALL. Gene overexpression through chromosomal translocation is very common in ALL, likely due to the highly recombinogenic immunoglobulin. The best example of this is the *BCR*/ABL chimeric protein which results from the fusion of the *BCR* gene on chromosome 22 to the ABL tyrosine kinase on chromosome 9 as a consequence of the Philadelphia chromosome (t(9;22)). Other chromosomal translocations in ALL are listed below in Table 4 [25–42].

Clinically, ALL is a disease of childhood. Patients usually present with symptoms and signs of bone marrow failure. Common manifestations include fever and infection secondary to neutropenia, bleeding secondary to thrombocytopenia and weakness, tachycardia, dyspnea on exertion due to anemia. The disease has a tendency to involve the central nervous system (unlike acute myelogenous leukemia). Based on light microscopic features of lymphoblasts, ALL is classified into three categories according to the French-American- British (FAB) classification, L1, L2 and L3. ALL-LI is the most common type accounting for 60–70% of all cases and has the best prognosis. L1 blasts are small in size with indistinct nucleoli. L2 is the next most common subtype consisting of a mixture of small (like L1) and large blasts. L3 consist of large blasts with distinct nucleoli and deep blue cytoplasm that has characteristic vacuoles. ALL-L3 cells are identical to Burkitt's lymphoma cells; it is the least common subtype accounting for 1% of all ALL cases. It

Table 3. EBV-negative human B cell lines: clinical characterization

Cell line[a]	Donor age[b]/ sex	Diagnosis[c]	Specimen site	Year est.	Culture medium[d]	Source
Null acute lymphoblastic leukemia (null-ALL)						
HPB-Null	47 yr, M	ALL	ND	1981	90% RPMI 1640 with 10% FBS	43
SUP-B15	10 yr, M	acute precursor ALL	bone marrow	1988	80% McCoy's 5A + 20% FBS	44
Common acute lymphoblastic leukemia (cALL)						
LiLa-1	ND	cALL	peripheral blood	1992	90% RPMI 1640 with 10% FBS	22
LK63	ND	cALL	peripheral blood	1992	90% RPMI 1640 with 10% FBS	22
MHH-CALL-2	15 yr, Caucasian	cALL	peripheral blood	1995	80% RPMI 1640 with 20% FBS	45
697	12 yr, M	cALL		1979	90% RPMI 1640 with 10% FBS	46
MHH-CALL-4	10 yr, Caucasian	cALL	peripheral blood	1993	80% RPMI 1640 with 20% FBS	45
REH	15 yr, F	cALL	peripheral blood	1977	90% RPMI 1640 with 10% FBS	47
Pre-B-acute lymphoblastic leukemia (pre-B-ALL)						
MHH-CALL-3	11 yr, F	pre-ALL	bone marrow	1993	80% RPMI 1640 with 20% FBS	45

Continued on next page

Table 3. (continued)

Cell line[a]	Donor age[b]/ sex	Diagnosis[c]	Specimen site	Year est.	Culture medium[d]	Source
B-acute lymphoblastic leukemia (B-ALL)						
380	15 yr, M	ALL (FAB L3)	peripheral blood	1983	RPMI 1640 with 10-20% FBS	48,49
Ball1	75 yr, M	ALL	peripheral blood	1977	90% RPMI 1640 with 10% FBS	50
KM-3	12 yr, M	ALL	peripheral blood	1977	90% RPMI 1640 with 10% FBS	51
Laz 221	24 yr, F	ALL	peripheral blood	1978	80% RPMI 1640 with 20% FBS	52
NALL-1	14 yr, M	ALL	peripheral blood	1977	80% RPMI 1640 with 15% FBS	50
NALM-6	24 yr, M	ALL	peripheral blood	1979	90% RPMI 1640 with 10% FBS	53
NALM-6-B	24 yr, M	ALL	peripheral blood	1979	90% RPMI 1640 with 10% FBS	53
NALM-6-MI	24 yr, M	ALL	peripheral blood	1979	90% RPMI 1640 with 10% FBS	53
NALM-12	ND	ALL	peripheral blood	1979	90% RPMI 1640 with 10% FBS	5,54
NALM-16	12 yr, F	ALL	peripheral blood	1976	90% RPMI 1640 with 10% FBS	55
NALM-26	24 yr, M	ALL	peripheral blood	1994	90% RPMI 1640 with 10% FBS	56
RPMI-8382	16 yr, F	ALL	peripheral blood	1972	90% RPMI 1640 with 10% FBS	57,58
RPMI-8392	16 yr, F	ALL	peripheral blood	1972	90% RPMI 1640 with 10% FBS	57,58
RPMI-8422	16 yr, F	ALL	peripheral blood	1972	90% RPMI 1640 with 10% FBS	57,58
RPMI-8432	16 yr, F	ALL	peripheral blood	1972	90% RPMI 1640 with 10% FBS	57,58
RPMI-8442	16 yr, F	ALL	peripheral blood	1972	90% RPMI 1640 with 10% FBS	57,58
RT	16 yr, F	ALL	bone marrow	1989	80% RPMI 1640 with 20% FBS	59

Continued on next page

Table 3. (continued)

Cell line[a]	Donor age[b]/ sex	Diagnosis[c]	Specimen site	Year est.	Culture medium[d]	Source
B-non-Hodgkin's lymphoma (B-NHL) refer to the next chapter (non-Hodgkin's B-lymphoma)						
Hairy Cell Leukemia (HCL)						
Hair-M	86 yr,M	HCL	peripheral blood	1983	RPMI 1640 with 10% FBS	56
HCL-Z1	ND	HCL	spleen	1985	RPMI 1640 with 10% FBS	11
Waldenstrom macroglobulinemia (WM)						
WSU-WM	51 yr, M	WM	pleural fluid	1993	RPMI 1640 with 20% FBS	6,14,15,60
Others						
MN-60	20 yr, M	ALL-Burkitt's type	peripheral blood	1982	F-10 medium with 20% FCS	61
NC-37	ND	lymphoblasts	peripheral blood	1973	McCoy's medium with 20% FCS	62
RS4;11	32 yr, F	lymphoid	bone marrow	1985	α-modified MEM with 10% FBS	63
SMS-SB	16 yr, F, black	lymphoblasts	peripheral blood	1977	RPMI 1640 with 15% FBS	64
TOM-1	54 yr, F	leukemia	bone marrow	1987	RPMI 1640 with 20% FBS	65
CJ	54 yr, F	nodular small cleaved lymphoma	lymph node	1989	RPMI 1640 with 20% FBS	59

[a] Cell lines names are given as listed in the original literature.
[b] Age of donor at time of cell line establishment.
[c] Diagnosis is indicated as given in the original reference.
[d] Cell line might also grow in other culture medium.
BM – bone marrow, PB – peripheral blood, ND – not determined.

is associated with mature B-cell phenotype (sIgM+), t(8;14) and has the least favorable prognosis.

In general, ALL in adults has less favorable prognosis than children for reasons that remain unknown. Although earlier studies have indicated a worse outcome in T-cell ALL, others have shown equal results using intensive chemotherapy protocols. Chemotherapy is the mainstay of treatment in ALL with three distinct phases including induction, consolidation and maintenance. The clinical and immunophenotypic characterization of the ALL cell lines, listed in Tables 3-5, represent very well the early stage of B-cells. However, the genetic characterization for these ALL cell lines (table 4) is complicated and therefore it is difficult to reach a reasonable conclusion regarding each stage (early pre-B, pre-B, transitional pre-B and B-cell lineages, Table 5).

3. INTERMEDIATE STAGES OF B-CELLS

Certain surface antigens are expressed in intermediate stages of the B-cell differentiation pathway. These are CD21 (B2) [66], BL4 [2] and BL7 [6]. Such markers are very useful in determining the position of the cells along the B-cell differentiation pathway. The CD11c and CD22 are two markers that were first reported in 1985 [67]. While CD11c is expressed on monocytes and CD22 on B-lymphocytes, the co-expression of the two antibodies was initially thought to be specific for hairy cell leukemia (HCL). Since then, however, a new subset of non-Hodgkin's lymphoma (NHL), the monocytoid B-cell lymphoma (MBCL), has been described that also co-expresses CD11c and CD22 [68,69]. Both HCL and MBCL also express acid phosphatase (AcP). However, such expression can be inhibited by tartrate (tartrate sensitive) in MBCL but not in HCL [70]. The most common forms of NHL are follicular small cleaved cell lymphoma (about 40% of cases) and follicular mixed small cleaved and large cell lymphomas (about 20–40% of cases) [71]. These follicular lymphomas express the B-cell antigens CD19, CD20 and CD22 and are CD5-negative. The expression of antigens varies among other forms of NHL. More than 80% of splenic lymphoma with villous lymphoma (SLVL) cases are CD24+ and FMC7+ and express membrane CD22 [72]. Mantle cell NHL is almost always CD5+ and CD43+ [73] and demonstrates overexpression of cyclin Dl (unlike other B-cell NHLs except for some cases of SLVL [74]).

Table 4. EBV-negative human B cell lines: genetic characterization

Cell line	Primary reference	Doubling time	EBV status	Karyotype
Null acute lymphoblastic leukemia (null-ALL)				
HPB-Null	*Cancer* 47: 1812, 1981	24–30 hrs	Negative	ND
SUP-B15	*Cancer Res* 48: 2876, 1988	60 hrs	Negative	human pseudodiploid karyotype; 46<2n>XY, der(1)t(1;1)(p11;q31), der(4)t(1;4)
Common acute lymphoblastic leukemia (cALL)				
LiLa-1	*Leukemia Res* 16: 655, 1992	ND	Negative	46XX, –9- t(1;19)(q23;p13), +der(9)t(1;9)(q11;p11)
LK63	*Leukemia Res* 16: 655 1992	13 hrs	Negative	45, X, –X (or Y), +8, –9, –17, t(1;19)(q23;p13), +der(9)t(9;17)(p11;p11)
MHH-CALL-2	*Br J Haematol* 89: 771, 1995	100 hrs	Negative	human hyperdiploid karyotype with 13% polyploidy; 52(50–52)<2n>X;+21
697	*Blood* 60: 1305, 1982	30–40 hrs	Negative	human near diploid karyotype; 46(45–48)<2n>XY, t(1;19)(q23;p13)
MHH-CALL-4	*Br J Haematol* 89: 771, 1995	80–100 hrs	Negative	human hypodiploid karyotype with 4% polyploidy; 45(42–46)<2n>X
REH	*Nature* 267: 841, 1977	70 hrs	Negative	human pseudodiploid karyotype; 46(44–47)<2n>X, –X, +16, del(3), t(4;12;21;16)

Continued on next page

Table 4. (continued)

Cell line	Primary reference	Doubling time	EBV status	Karyotype
Pre-B-acute lymphoblastic leukemia (pre-B-ALL)				
MHH-CALL-3	*Br J Haematol* 89: 771, 1995	50 hrs	Negative	human pseudodiploid karyotype; 46(45–46)<2n>XX, del(6)(q15), der(19)t(1;19)
B-acute lymphoblastic leukemia (B-ALL)				
380	*PNAS* 81: 7166, 1984	24–30 hrs	Negative	t(8;14;18)(q24;q32;q21), +der(14) t(8;14;18)(q24;q32;q21); corres. karyotype
Ball1	*J Natl Cancer Inst* 59: 93, 1977	24–28 hrs	Negative	
KM-3	*Int J Cancer* 19(5): 521, 1977	100 hrs	Negative	
Laz 221	*Cancer Res* 38(5): 1362, 1978	80 hrs	Negative	45, XX, –9, –12, +(9q12q)
NALL-1	*Cancer* 40(5): 2131, 1977	72–88 hrs	Negative	chromosome # of 43 with 50 metaphases, neither X or Y chromosome
NALM-6	*Int J Cancer* 23: 174, 1979	36 hrs	Negative	human near-diploid karyotype; 46(43–47)<2n>XY, t(5;12)(q33.2;p13)
NALM-6-B	*Int J Cancer* 23: 174, 1979	36 hrs	Negative	pseudodiploid karyotype; 46 XY, t(5q–/12p+);mar Y
NALM-6-MI	*Int J Cancer* 23: 174, 1979	36 hrs	Negative	pseudodiploid karyotype; 46 XY, del(5q);mar Y
NALM-12	*J Exp Med* 158(5): 1757, 1983	30–36 hrs	Negative	
NALM-16	*Immunology* 35(2): 333, 1978	30–36 hrs	Negative	
NALM-26	*Hum Cell* 7: 221, 1994	ND	Negative	
RPMI-8382	*J Natl Cancer Inst* 53: 655, 1974	ND	Negative	
RPMI-8392	*J Natl Cancer Inst* 53: 655, 1975	ND	Negative	
RPMI-8422	*J Natl Cancer Inst* 53: 655, 1977	ND	Negative	
RPMI-8432	*J Natl Cancer Inst* 53: 655, 1978	ND	Negative	
RPMI-8442	*J Natl Cancer Inst* 53: 655, 1979	ND	Negative	
RT	*Blood* 75: 1311;1990	ND	Negative	52, XY, +7, +12, +13, t(1;17)(q22;p12), t(2;12)(q37;q13), +del(3)(p22>

Continued on next page

Table 4. (continued)

Cell line	Primary reference	Doubling time	EBV status	Karyotype
B-non-Hodgkin's lymphoma (B-NHL) refer to the next chapter (non-Hodgkin's B-lymphoma)				
Hairy cell leukemia (HCL)				
Hair-M	*Nippon Ketsueki Gakkai Zasshi* 46: 1222, 1983 (in Japanese)	ND	Negative	46XY, –11, –14, +2M
HCL-Z1	*Exp Cell Biol* 53: 61, 1985	ND	Negative	ND
Waldenstrom macroglobulinemia (WM)				
WSU-WM	*Blood* 81: 3034, 1993	16 hr	Negative	46–54, XY, t(8;14)(q24;q32), t(12;17)(q24;q21), 2P–, extra copies of 1, 3, 4, 7
Others				
MN-60	*Leukemia Res* 6: 685, 1982	25 hrs	Negative	t(8q–;14q+)(1q+, 6q–)
NC-37	*Lancet* i, 93, 1973	ND	Negative	
RS4;11	*Blood* 65: 21, 1985	60–70 hrs	Negative	46XX, i(7q), t(4;11)(q21;q23)
SMS-SB	*J Immunology* 126: 596, 1981	48 hrs	Negative	47XX with 1 or 2 submetacentric marker chromosomes. Ph1(–)
TOM-1	*Blood* 69: 990, 1987	50–60 hrs	Negative	human hyperdiploid karyotype with 7% polyploidy;47(47–48)<2n>X;
CJ	*Blood* 75: 1311, 1990	ND	Negative	51, XX, +X, –4, +12, +del(2)(p21), +del(2)(q32), +del(3)(q21), +del(3)(q21)

Table 5. EBV-negative human B cell lines: immunophenotypic characterization

Cell	Immunophenotype	Availability
Null acute lymphoblastic leukemia (null-ALL)		
HPB-Null	CD3−, CD10+, CD19+, HLA-DR+	
SUP-B15	CD3−, CD10+, CD13+, CD19+, CD20−, CD34+, CD37+, HLA-DR+, sm/cylambda−, smkappa−, cykappa+	
Common acute lymphoblastic leukemia (cALL)		
LiLa-1	CD10+, CD19+, CD20−, CD21−, CD11b−, CD54−, HLA-DR+, HLA-DQ−, smIgM−	
LK63	CD10+, CD19+, CD20−, CD21−, CD11b−, CD54−, HLA-DR−, HLA-DQ−, smIgM+	
MHH-CALL-2	CD3−, CD10+, CD13+, CD19+, CD20+, CD37−, HLA−DR+, sm/cyIgG+, sm/cylambda−	DSMZ
697	CD3−, CD10+, CD13−, CD19+, CD20+, CD37+, HLA-DR+, sm/cyIgM+, sm/cylambda−	
MHH-CALL-4	CD3−, CD10+, CD13+, CD19+, CD20+, CD37+, HLA-DR+, sm/cyIgG−, sm/cylambda−	DSMZ
REH	CD3−, CD10+, CD13−, CD19+, CD20+, CD37−, CD71+, CD138+, HLA-DR+, sm/cyIgG−, sm/cyIgM−, sm/cylambda−, sm/cykappa−	ATTC, DSMZ
Pre-B-acute lymphoblastic leukemia (pre-B-ALL)		
MHH-CALL-3	CD3−, CD10+, CD13+, CD19+, CD37−, HLA-DR+, sm/cyIgG−, sm/cyIgM−	DSMZ

Continued on next page

Table 5. (continued)

Cell	Immunophenotype	Availability
B-acute lymphoblastic leukemia (B-ALL)		
380	CD3−, CD10+, CD13−, CD19+, CD37+, HLA-DR+, sm/cyIgM−, sm/cyIgG−	ATTC, DSMZ
Ball1	CD10+, CD19−, CD20+, CD21−, CD11b−, CD54+	
KM-3	ND	
Laz 221	smIg−	
NALL-1	ND	
NALM-6	CD3−, CD10+, CD13−, CD19+, CD34−, CD37−, CD138+, HLA-DR+, sm/cyIgG−, smIgM−, cyIgM+, sm/cylambda−, sm/cykappa−	DSMZ
NALM-6-B	CD3−, CD10+, CD13−, CD19+, CD34−, CD37−, CD138+, HLA-DR+, sm/cyIgG−, sm/cyIgM+, sm/cylambda−, sm/cykappa−	
NALM-6-MI	CD3−, CD10+, CD13−, CD19+, CD34−, CD37−, CD138+, HLA-DR+, sm/cyIgG−, smIgM−, cyIgM+, sm/cylambda−, sm/cykappa−	
NALM-12	CD3−, CD10+, CD13−, CD19+, CD34−, CD37−, CD138+, HLA-DR+	
NALM-16	CD3−, CD10+, CD13−, CD19+, CD34−, CD37−, CD138+, HLA-DR+	
NALM-26	T cell associated CD5 and Myeloid cell associated CD13 antigens, IL-7 receptor + (CDw127)	
RPMI-8382	CD3−, CD10+, CD13−, CD19+, CD34−, HLA-DR+	
RPMI-8392	CD3−, CD10+, CD13−, CD19+, CD34−, HLA-DR+	
RPMI-8422	CD3−, CD10+, CD13−, CD19+, CD34−, HLA-DR+	
RPMI-8432	CD3−, CD10+, CD13−, CD19+, CD34−, CD37−, CD138+, HLA-DR+	
RPMI-8442	CD3−, CD10+, CD13−, CD19+, CD34−, HLA-DR+	
RT	CD19+, CD20+, CD10+, cIg+, CD2−, CD3−, smIg−	

Continued on next page

Table 5. (continued)

Cell	Immunophenotype	Availability
B-non-Hodgkin's lymphoma (B-NHL) refer to the next chapter (non-Hodgkin's B-lymphoma)		
Hairy cell leukemia (HCL)		
Hair-M	cy and smgIG-kappa, E−, IgGFcR−, IgMFcR−, C3R−, OKT-9+, OKT10+, OKT1+, MCS-1+, TdT−, CD10−, B1+, B7+, CD3−	
Waldenstrom macroglobulinemia (WM)		
WSU-WM	CD3−, CD19+, CD20+, CD22+, HLA-DR+, CD10+, smIgM+, smIg-lamda+	Author, DSMZ
Others		
MN-60	CD10+, HLA-DR+, HLA-A-C+, sm/cyIgG−, smIgM+, cyIgM−, smlambda+, cylambda−, sm/cykappa−	
NC-37	ND	
RS4;11	CD4−, CD8−, CD10−, CD19+, CD20−, CD71+, HLA-DR+, sm/cyIgG−	
SMS-SB	CD10−, HLA-DR+, sm/cylambda−, sm/cykappa−, smIgM−, cyIgM+, sm/cyIgG−	
TOM-1	CD3−, CD10+, CD13+, CD19+, CD20+, CD34+, CD37−, HLA-DR+, sm/cylambda−, sm/cykappa−	
CJ	CD19+, CD20+, CD10+, sIg+, HLA-DR+, CD2−, CD3−, Tac(IL-2R)−	

(+), strong, definite protein expression (more than 50% cells are positive); (+/−), moderately (20−50% cells are positive); (−/+), weak expression (less than 20% cells are positive; (−), no protein expression.
ATTC – American Tissue Type Collection; DSMZ – Deutsche Sammlung Mikroorganismen und Zellkuturen GmbH.

4. MATURE B-CELLS

[Examples: Hair-M for Hairy cell leukemia; WSU-WM for Waldenstrom's Macroglobulinemia].

The conventional marker of the most mature B-cells, the plasma cells, is the intense cyIg expression and secretion. Surface antigens that are associated or restricted to the Ig-secreting stage are BL3, PC1, and PCA1 [1,75,76].

4.1. Hairy Cell Leukemia (HCL)

[Example, HCL-Z1 cell line].

HCL (Leukemic reticuloendotheliosis) is an indolent lymphoid malignancy first described by Bouroncle in 1958. Over the last two decades, HCL cell lines have increased dramatically the understanding of the biology of this disease. They have also led to the discovery of a new retrovirus and the isolation and cloning of granulocyte-macrophage colony-stimulating factor [77,78]. HCL is a rare disorder, it develops more often in men than in women, and its incidence peaks during the fifth decade of life. The neoplastic HCL cells are derived from B-cells [1,76]. Typical HCL display several surface antigens, including immunoglobulins of both light- and heavy-chain types, characteristic of B-cells [79]. The most specific marker is the expression of B-ly7 with CD19, which can be used to differentiate HCL from CLL. HCL also expresses CD11c with CD19 and, usually, CD25 with CD19. CD5+ cells are highly atypical in HCL [80]. Some cases of HCL show overexpression of cyclin Dl [74]. While the hair-like surface projections place them closer to the monocytic lineage [81], these unusual surface features of hairy cells are not seen in normal or neoplastic B-lymphocytes. Several cell lines from HCL patients had been established; however, all of them are EBV-positive, and therefore show features typical of lymphoblastoid cell lines. In vivo, hairy cells are usually EBV-negative. The cardinal features of HCL are splenomegaly which is usually associated with pancytopenia, and occasional characteristic lymphocytes bearing hair-like cytoplasmic extensions. Unfortunately, the clinical, genetic and immunophenotypic characterization of the two cell lines, Hairy-M and HCL-1, listed under hairy cell leukemia, do not provide enough information to consider them as a perfect model representing hairy cell leukemia.

4.2. Waldenstrom's Macroglobulinemia (WM)

[Example, WSU-WM cell line].

The presence of CD20 and CD22 antigens distinguishes WM from multiple myeloma (MM). As in CLL, CD 19 and CD21 are expressed [58,70].

The differential diagnostic criteria are the overproduction of monoclonal IgM protein and diffuse infiltration of bone marrow and occasionally of spleen, liver or lymph nodes [82]. The histology is that of malignant B-cells with the appearance of plasmacytoid lymphocytes; however, many patients have only well-differentiated lymphocytes, as in CLL [83,84]. In 1944, Jan Waldenstrom described two patients with epistaxis, anemia, retinal hemorrhage, hyperglobulinemia, and infiltration of the bone marrow by lymphocytes [85]. The disease that he described and now bears his name results from malignant proliferation of plasmacytoid lymphocytes (or lymphocytoid plasma cells) producing a monoclonal IgM protein, now known as the macroglobulin. WM is closely related to other mature lymphocytic or plasmacytic neoplasms such as CLL, low-grade NHL, HCL, and MM [86]. Clinically, although CLL, NHL, HCL and MM have different pathologic manifestations (contributing to their different nomenclature), they are almost always of B-cell origin, affect elderly people, and have similar natural history characterized by chronic course. The median survival of patients with WM is reported to be 5 years [87]; it is almost never seen in people younger than 40 [88]. The WSU-WM cell line is EBV-negative and carries the 8; 14 chromosomal translocation with a unique breakpoint of chromosome 8 downstream of exon 3 of the c-MYC proto-oncogene, which is rearranged. The cells grow in liquid culture, soft agar, athymic nude mice, and severe combined immune deficient (SCID) mice [14]. The WSU-WM cell line is the only cell line in the literature that clinically and immunophenotypically partly represents Waldenstrom's macroglobulinemia.

ACKNOWLEDGEMENTS

This work was supported by Grant CA79837 from the US National Cancer Institute and from the Leukemia Society of America grant No. 6323-99. The authors would like to thank Dr. Ishtiaq Ahmad for his technical help.

References

1. Anderson KC, Bates MP, Slaughenhoupt B, et al. *J Immunol* 132: 3172, 1984.
2. Hashimi L, Wang CY, Al-Katib A, et al. *Cancer Res* 46: 5431, 1986.
3. Hokland P, Ritz J, Schlossman SF, et al. *J Immunol* 135: 1746, 1985.
4. Loken M, Shah VO, Dattilio KL, et al. *Blood* 70: 1316, 1987.
5. Wang CY, Azzo W, Al-Katib A, et al. *J Immunol* 133: 684, 1984.
6. Al-Katib A, Wang CY, Bardales R, et al. *Hematol Oncol* 3: 271, 1985.
7. Small TN, Keever CA, Weiner-Fedus, et al. *Blood* 76: 1647, 1990.
8. Nilsson K, Ponten JJ. *Cancer* 15: 321, 1975.

9. Nilsson K, Klareskog L, Ralph P, et al. *Hematol Oncol* 1: 277, 1983.
10. Findley HW Jr, Cooper MD, Kim TH, et al. *Blood* 60: 1305, 1982.
11. Lang AB, Odermatt BF, Gut DR, et al. *Exp Cell Biol* 53: 61, 1985.
12. Miyoshi I, et al. *Cancer* 47: 60, 1981,
13. Saxon A et al. *J Immunol* 120: 777, 1978.
14. Al-Katib A, Mohammad R, Hamdan M, et al. *Blood* 81: 3034; 1993.
15. Al-Katib A, Mohammad RM. *Basic and Clinical Applications of Flow Cytometry*, Valeriote FA, Nakeff A, Valdivieso M (eds.), Kluwer Academic Publishers, Boston, USA, 1992.
16. Rosenberg SA, Bernard CW, Brown BW Jr, et al. *Cancer* 49: 2112, 1982.
17. Hozier J, Lindquist L, Hurwitz R, et al. *Int J Cancer* 26: 281, 1980.
18. Raff MC, Megson M, Owen JJT, Cooper MD. *Nature* (Lond.) 259: 224, 1976.
19. Owen JJ, Wright DE, Habu S, Raff MC, Cooper MD. Studies on the generation of B lymphocytes in fetal liver and bone marrow. *J Immunol* 118(6): 2067–73, 1977.
20. Perl ER, Volger LB, Okos AJ, Crist WM, Lawton AR 3rd, Cooper MD. B lymphocyte precursors in human bone marrow: An analysis of normal individuals and patients with antibody-deficiency states. *J Immunol* 120(4): 1169–75, 1978.
21. Levitt D, Cooper MD. *Cell* 19:617,1980.
22. Salvaris E, Novotny JR, Welch K, et al. *Leukemia Res* 16: 655, 1992.
23. Nadler LM, Korsmeyer SJ, Anderson KC, et al. *J Clin Invest* 74: 332, 1984.
24. Boyd AW. *Pathology* 19: 329, 1987.
25. Quesnel B, Preudhomme C, Philippe et al. *Blood* 85: 657, 1985.
26. Stranks G, Height SE, Mitchell P, et al. *Blood* 85: 893, 1995.
27. Hebert J, Cayuela J, Berkely J, et al. *Blood* 84: 4038, 1994.
28. Okuda T, Shurtleff SA, Valentine MB, et al. *Blood* 85: 2321, 1995.
29. Croce C, Nowell P. *Blood* 65: 1, 1985.
30. Apian P, Lombardi D, Ginsberg A, et al. *Science* 250: 1426, 1990.
31. Brown L, Cheng J-T, Siciliano M, et al. *EMBOJ* 9: 3343, 1990.
32. Nourse J, Mellentin J, Galili N, et al. *Cell* 60: 535, 1990.
33. Raimondi SC. *Blood 81:* 2237, 1993.
34. Dedera D, Waller E, LeBrun D, et al. *Cell* 74: 833, 1993.
35. Inaba T, Inukai T, Yoshihara T, et al. *Nature* 382: 541, 1996.
36. Thirman MJ, Gill HJ, Burnet RC, et al. *N Engl J Med* 329: 909, 1993.
37. Golub T, Goga A, Barker G, et al. *Mol Cell Biol* 16: 4107, 1996.
38. Suto Y, Sato Y, Smith SD, et al. *Cancer* 18: 254, 1997.
39. Papadopoulos P, Ridge SA, Boucher CA, et al. *Cancer Res* 55: 34, 1995.
40. Buijs A, Sherr S, van Baal S, et al. *Oncogene* 10: 1511, 1995.
41. Romana SP, Poirel H, Leconiat M, et al. *Blood* 86: 4263, 1995.
42. Rubnitz JE, Downing JR, Pui CH, et al. *J Clin Oncol* 15: 1150, 1997.
43. Nakao Y, Tsuboi S, FujitaT, et al. *Cancer* 47(7): 1812, 1981.
44. Naumovski L, Morgan R, Hecht F, et al. *Cancer Res* 48: 2876, 1988.
45. Tomeczkowski J, Yakisan E, Wieland B, et al. *Br J Haematol* 89: 771, 1995.
46. Findley HW Jr, Cooper MD, Kim TH, et al. *Blood* 60: 1305, 1982.
47. Minowada J. Markers of human leukemia-lymphoma cell lines. Reflect haematopoietic cell differentiation. In: Serrou B and Rosenfeld C (eds.). *Human Lymphocyte Differentiation.* Elsevier/North-Holland Biomedical Press, 1978.
48. Pegoraro L, Palumbo A, Erikson J, et al. *PNAS* 81: 7166, 1984.
49. Pegoraro L, Malavasi F, Bellone G, et al. *Blood* 73: 1020; 1989.
50. Hiraki S, Miyoshi I, Masuji H, et al. *J Natl Cancer Inst* 59: 93, 1977.

51. Schneider U, Schwenk HU, Bornkamm G. Int *J Cancer* 19(5): 521, 1977.
52. Lazarus H, Barell EF, Krishan A, et al. *Cancer Res* 38(5): 1362, 1978.
53. Hurwitz R, Hozier J, LeBien T, et al. *Int J Cancer* 23: 174, 1979.
54. Wang CY, Al-Katib A, Lane CL, et al. *J Exp Med* 158(5): 1757, 1983.
55. Han T, Minowada J. *Immunology* 35(2): 333, 1978.
56. Matsuo Y, Adachi T, Tsubota T. *Hum Cell* 7: 221, 1994.
57. Huang CC, Hou Y, Woods LK, et al. *J Natl Cancer Inst* 53: 655, 1974.
58. Huang N, Kawano MM, Mahmoud MS, et al. *Blood* 85: 3704, 1995.
59. Ford RJ, Goodacre A, Ramirez I, et al. *Blood* 75: 1311; 1990.
60. Al-Katib A, Wang CY, Koziner B. *Cancer Res* 45: 3058, 1985.
61. Roos G, Adams A, Giovanella B, et al. *Leukemia Res* 6: 685, 1982.
62. Pattengale PK, Smith RW, Gerber P. *Lancet* 2: 93, 1973.
63. Stong RC, Korsmeyer SJ, Parkin JL, et al. *Blood* 65: 21, 1985.
64. Smith RG, Dev VG, Shannon WA Jr. *J Immunology* 126: 596, 1981.
65. Okabe M, Matsushima S, Morioka M et al. *Blood* 69: 990, 1987.
66. Nadler LM, Stashenko P, Hardy R, et al. *J Immunol* 126: 1941, 1981.
67. Schwarting R, Stein H, Wang CY. *Blood* 65: 974, 1985.
68. Sheibani K, Burke JS, Swartz WG, et al. *Cancer* 62: 1531, 1988.
69. Traweek ST, Sheibani K, Winberg CD, et al. *Blood* 73: 573, 1989.
70. Burke JS, Sheibani K. *Leukemia* 1: 298, 1987.
71. Eyre HJ, Farver ML. Holleb AI, Fink DJ, Murphy GP (eds.) *American Cancer Society Textbook of Clinical Oncology.* Atlanta, Ga: ACS Co., p377, 1991.
72. Matutes E, Morilla R, Owusu-Ankomah K, et al. *Blood* 83: 1558, 1994.
73. Weisenburger DD, Armitage JO, *Blood* 87: 4483, 1996.
74. De Boer CJ, Schuuring E, Dreef E, et al. *Blood* 86: 2715, 1995.
75. Anderson KC, Park EK, Bates MP, et al. *J Immunol* 130: 1132, 1983.
76. Anderson KC, Bates MP, Slaughehoupt BL, et al. *Blood* 63: 1424, 1984.
77. Glaspy JA, Golde DW. *Oncology* 4: 25, 1990.
78. Antman KS et al.N Engl *J Med* 319:593, 1988.
79. Golomb HM, Braylan R, Polliak A. et al. *Br J Haematol* 29: 455, 1975.
80. Palackdharry CS. Skeel RT & Lachant NA (eds.) *Handbook of Cancer Chemotherapy,* 4th edn. Boston, Mass: Little, Brown & Co., p425, 1995.
81. Golomb HM, Braylan R, Polliak A, et al. *Br J Haematol* 29: 455, 1975.
82. *Dorlands Illustrated Medical Dictionary,* 27th edn. Philadelphia, Pa: WB Saunders Co., p965, 1988.
83. Dimopoulos MA, Alexanian R. *Blood* 83: 1452, 1994.
84. Oken MM. Skeel RT, Lachant NA (eds.) *Handbook of Cancer Chemotherapy,* 4th edn. Boston, Mass: Little, Brown & Co., 1995.
85. Waldenstrom J. *Acta Med Scand* 127: 216, 1944.
86. Bergsagel DE: Macroglobulineamia, in Williams WJ, Beutler E, Erslev AJ, Lichtman (eds.), *Hematology,* 3rd edn. New York, NY, McGraw-Hill, p1 104, 1983.
87. Pappenberger R, Sommerfeld W, Hoffmann-Fezer G. *Scand J Haematol* 31: 359, 1983.
88. Kyle RA, Garton JP. *Mayo Clin Proc* 62: 719, 1987.

Chapter 4

Multiple Myeloma Cell Lines

Helena Jernberg-Wiklund and Kenneth Nilsson
Section of Pathology, Rudbeck Laboratory, Uppsala University, 751 85 Uppsala, Sweden. Tel: +46-18-611-0252; Fax: +46-18-558-931; E-mail: Helena.Jernberg_Wiklund@genpat.uu.se

1. INTRODUCTION

Multiple myeloma (MM) is an incurable B cell malignancy characterized by the clonal expansion of malignant plasmablasts/plasma cells in the bone marrow. Among B cell tumors, MM exhibits some unique characteristics. The immunoglobulin (Ig) isotype of MM cells is generally IgG or IgA, suggesting that MM is derived from a post- switched B cell. This and the fact that the Ig of MM cells is somatically hypermutated suggests that the critical transformation steps occur in the germinal centre of the lymph node, perhaps driven by antigenic stimulation.

MM is essentially localized to the bone marrow and only rarely, and after progression, will MM disseminate, systemically in the form of a PCL but also to pleural or peritoneal cavities, forming effusions at these sites. MM cells interact via cell-cell contacts or soluble factors with normal cells in the bone marrow environment, including stromal cells, osteoblasts, osteoclasts and cells of normal hematopoiesis. Adhesion molecules expressed on MM cells are involved in these interactions and in the MM cell-extracellular matrix (ECM) interactions. The area of cell-cell interaction via adhesion molecules has recently attracted attention and a number of reports have demonstrated the importance of cell-cell contact for the production of cytokines mediating growth and survival in the bone marrow [1]. Since a number of MM cell lines have been established which are highly dependent on growth factors for their maintenance in vitro, paracrine growth/survival factor influence is a likely mechanism crucial for the establishment of MM cell lines.

Until 1980, only two MM cell lines had been established, RPMI 8226 [2] and U-266 [3]. To date at least 81 established true MM cell lines from 73 patients have been reported (see Table 2). The vast majority of these cell lines have been established in non-stirred suspension cultures from patients with advanced, often terminal, disease by explanting MM

J.R.W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol.III, 81–155.

cells from bone marrow, pleural effusion, peripheral blood or ascites. From BM biopsies obtained from non-progressive MM there is usually a requirement for either feeder cell layers of autologous BM, adherent monolayers of murine plasmacytoma cell lines [4–6], conditioned medium from PHA-stimulated leukocytes (PHA-LCM) or rIL-6 [7] alone or in combination with GM-CSF [8,9].

MM bone marrow stromal cells, ECM proteins, cytokines and other soluble factors provide the necessary anchorage and cell-cell contacts for MM establishment as continuous cell lines. The major explanations for the difficulty in establishing MM cell lines are the slow proliferative activity of MM cells and the requirement for anchorage dependence on feeder layer stromal cells. Additionally, there is at least a 30% risk of the outgrowth of Epstein Barr positive lymphoblastoid cell lines (LCLs) with less stringent growth requirements, which will overgrow the MM cells [10,11]. The understanding of the nature of feeder cells and fibroblast conditioned medium, and the discovery of IL-6 as a major growth factor of MM cells in vitro, have greatly improved the previously low success rate (2–5%) in the attempts to establish MM cell lines [12–14].

In discriminating true MM cell lines from EBV positive LCLs derived from the patient's non-neoplastic B cells, several markers are available (Table 1). It is essential to examine a putative MM cell line within 3 months of primary culture, as several of the hallmarks of lymphoblastoid cell lines, including polyclonality, diploidy, and lack of tumorigenicity can alter during prolonged culture [15]. It is not sufficient that data on new cell lines are obtained only 1–2 months after setting up the culture. The cells must be checked at least at 6 and 12 months. Hallmarks for authentic MM cell lines are EBV negativity, the presence of chromosomal abnormalities identical to those of the primary tumor cells, Ig rearrangements identical to the MM clone in vivo, Ig production identical to the primary tumor, and expression of characteristic surface markers (CD19, CD20, CD138, CD11a, CD49e). A complete analysis can distinguish the authentic MM cell lines from EBV positive LCLs and define useful models for studies of the biology of MM in vitro.

Authentic MM cell lines and highly purified primary MM cells have allowed the investigation of the biology of MM. However, in several studies two types of established cell lines, authentic MM lines and EBV+ non-malignant lymphoblastoid cell lines, have been used, making several of the conclusions in these studies irrelevant to the biology of MM [10,11,16–18].

How can we then discriminate the authentic MM cells from the EBV+ lymphoblastoid cell lines using immunophenotypical markers? Eight discriminant surface markers were selected by the European Myeloma Research Network to discriminate between MM and LCL; CD 19, CD20, sIg, CD11a,

Table 1. Comparison of the characteristics of newly established LCLs and MM cell lines and normal plasma cells

Markers	EBV+ LCLs	MM	Normal plasma cells
EBV status	+	–	–
Aneuploidy	–	+	–
Polyclonality	+	–	+
Tumorigenicity in nude mice	–	+/–[b]	–
Colony formation in agar	–	+/–[b]	–
sIg[a]	+	–	–
Immunophenotype: Surface markers			
CD19[a]	+	–	+
CD20[a]	+	–	
CD11a[a]	+	–	+
CD49e[a]	+	–	–
CD38[a]	–/+	+	+
CD28[a]	–	+[b]	–
CD138[a]	–	+	+
CD40		+[b]	+
CD56[a]	–	+[c]	–

[a] Selected discriminating immunophenotypical markers of EBV+ LCLs.
[b] Expression/feature related to advanced disease.
[c] Expression lost with aggressive disseminating disease.

CD49e, CD38, CD28 and CD138 [18] (see Table 1). Strong expression of CD 19 and CD20 was found on the EBV+ cell lines HS- Sultan and ARH-77 and other MM-derived cell lines with a lymphoblastoid phenotype [17,19]. The LCLs, however, failed to express CD138 (syndecan-1/B-B4). This was confirmed by other studies demonstrating weak or absent staining of CD 138 in LCLs (ARH-77, HS- Sultan, MC/CAR, and IM9). The LCLs also failed to express CD28, but were positive for sIg and the adhesion markers CD11a and CD49e. In addition, CD38 expression was observed in HS-Sultan and MC/CAR, while ARH-77 stained positive for PCA-1 (Tables 1 and 2b).

In contrast, the MM cell lines RPMI 8226, JJN-3, U-266, NCI-H929 as well as XG-1, XG-2, XG-6, LP-1, and OPM-2 express CD28 and CD 138 but lack the expression of CD 19, CD20, sIg and the adhesion molecules CD11a and CD49e. In RPMI 8226, weak expression of CD49e was detected. With the exception of U-266 and XG-6, CD38 was expressed in all the MM cell lines mentioned above (Table 3).

Table 2a. Multiple myeloma cell lines: clinical characterization

Cell line[a]	Patient age/sex	Diagnosis[b]	Treatment status[c]	Specimen site[d]	Authentication[e]	Year est.	Culture medium[f]	Ig production of cell line	Other requirements for establishment[m]	Primary ref./ availability[n]
ACB-885	39 M	IgG/κ MM	T	PE	yes		DMEM+5%FCS		20% hu Pl, 10% PHA-LCM later 10% huPl, 7 5% PHA-LCM	20 (probably unavailable)
ACB-1085	39 M	IgG/κ MM, sister cell line/ same origin as of ACB-885	T	PE	yes					20 (probably unavailable)
AD3	69 M	IgA/κ plasmacytoma/ hyperamylasemia/subclone of FR4	D	ascites	yes	1986	RPMI1640+10%FCS	IgA/κ 0.87 μg/72h	no additional feeder cells	21
AMO1	64 F	IgA/κ plasmacytoma		ascites	yes	1985	RPMI1640+7%FCS	IgA/κ	Macrophage feeder layers later no feeder layers	22
ANBL-6	58 F	MM/PCL BJP	R	PB	yes*	1992	RPMI1640+10%FCS		1 ng/ml IL6	23
ANBM-6	58 F	MM/PCL BJP same origin as ANBL-6/phenotypically indistinguishable from ANBL-6/ sister cell line	R	BM	yes	1992	RPMI1640+10%FCS		1 ng/ml IL6	23
delta-47	72 M	IgD/λ extraosseus MM	T	ascites	yes	1986	RPMI1640+20%FCS	IgD/λ 2 μg/72 h/ milj cells	no feeder/growth factors	24
DOBIL-6	65 F	nonsecr. MM/hypercalcaemia	R	BM	yes*	1995	RPMI1640+10%FBS	nonsecretory		25
DP-6	78 F	IgA/λ MM	T	PB	yes	1994	RPMI1640+10%FCS	IgA/λ	1–5 ng/ml IL-6	26
EJM	58 F	IgG/λ extramedullary MM/ PCL	T	PE	yes	1988	IMDM/Ham+20%FCS	IgG/λ 5–10 μg/24 h/ milj cells	initially adherent cells or 1–2% EwSarGF (IL-1+IL-6)	27
FLAM-76	77 M	nonsecr. κ type MM/PCL no BJP	T	PB	yes	1990	RPMI1640+10%FCS	nonsecretory	10% human cord serum, later 4 ng/ml rIL-6	28
FR4	69 M	IgA/κ plasmacytoma/ hyperamylasemia	D	ascites	yes	1986	RPMI1640+10%FCS	IgA/κ 0.555 μg/72 h		21
GM2132 (=RPMI 8226)		IgG/λ MM		PB	yes		RPMI1640+10%FCS			29
HL407[g]		IgA MM	D	BM	yes	1987	RPMI1640+10%FBS	IgA/λ	BM stromal feeder layer	6
ILKM2		IgG/κ MM		BM	yes	1987	RPMI1640+8%FCS	IgG/κ	BM macrophage/fibroblasts 3 mo later allog. MQ/MDF/rIL-6 (2 ng/ml)	5

Continued on next page

Table 2a. (continued)

Cell line[a]	Patient age/sex	Diagnosis[b]	Treatment status[c]	Specimen site[d]	Authentication[e]	Year est.	Culture medium[f]	Ig production of cell line	Other requirements for establishment[m]	Primary ref./ availability[n]
ILKM3		IgG/κ MM		BM	yes	1987	RPMI1640+8%FCS	κ	BM macroph/fibrobl, 3 mo later allog. MQ/MDF/rIL-6 (2 ng/ml)	5
JIM-1[h]	60 M	IgA/λ MM		PE	yes		Dexter medium		autol. PE fluid, autol stromal feeder layer, later IMDM (Ham's F12+20%FCS)	30
JIM-2		(subclone of JIM-1)			yes					30
JIM-3		(subclone of JIM-1)			yes					30
JIM-4		(subclone of JIM-1)			yes					30
JJN-1[i]	57 F	IgA/κ PCL	D	BM	yes	1987	Isc/Ham+20%FCS RPMI1640+15%FCS	IgA/κ	coculture with XG-Ag8.653 plasmacytoma	4
JJN-2	57 F	IgA/κ PCL (subclone of JJN)	D	BM	yes	1987	Isc/Ham+20%FCS RPMI1640+15%FCS	κ	derived from JJN-1+ ESG (IL-6), 0.5% ESG (IL-6)	4
JJN-3		IgA/κ PCL (subclone of JJN)		BM	yes					27
Karpas 620	77 F	IgG/κ PCL	D	PB	yes	1987	RPMI1640+20%FCS	κ	no feeder cells	31
Karpas 707	53 M	IgG/λ MM	R	BM/PB	yes	1981	RPMI1640+10%FBS	λ ~10–50 μg/ml		32
KAS-6/1	54(56) F	IgG/κ MM	T	PB	yes	1994	RPMI1640+10%FCS	IgG/κ	1–5 ng/ml IL-6	26
KHM-1A	53 M	IgA/λ MM/hyperamylasemia	T	PE	yes	1986	RPMI1640+10%FCS	IgA/λ		33
KHM-1B	53 M	IgA/λ MM/hyperamylasemia sister cell line/same origin as KHM-1A	T	PE	yes	1986	RPMI1640+10%FCS	IgA/λ		33
KHM-11	52 M	IgA/κ BJP MM (AMHL)	T	PE	yes*	1992	RPMI1640+10%FCS			34
KMM-1	62 M	BJP/λ plasmacytoma	T	plasma-cytoma	yes	1980	RPMI1640+20%FCS RPMI1640+10%FCS	λ		35,36,RCB
KMS-5	80 M	IgD/λ MM	ND	PE	yes	1982	RPMI1640+10%FCS	nonsecretory		36
KMS-11	67 F	IgG/κ MM	ND	PE	yes	1987	RPMI1640+10%FCS	κ		36
KMS-12-PE (same patient as KMS-12-BM	64 F	nonsecr. MM	ND	PE	yes	1987	RPMI1640+10%FCS	nonsecretory		36,JCRB

Continued on next page

Table 2a. (continued)

Cell line[a]	Patient age/sex	Diagnosis[b]	Treatment status[c]	Specimen site[d]	Authentication[e]	Year est.	Culture medium[f]	Ig production of cell line	Other requirements for establishment[m]	Primary ref./ availability[n]
KMS-12-BM (same patient as KMS-12-PE	64 F	nonsecr. MM	ND	BM	yes	1988	RPMI1640+10%FCS	nonsecretory		36 JCRB
KMS-18	58 M	IgA/λ BJP MM–BJP/λ PCL	R	PB	yes	1995	RPMI1640+15%FCS	λ	later RPMI1640 +10%FCS	37
KP-6	53(54) M	IgG/κ PCL	T	PE	yes	1994	RPMI1640+10%FCS	IgG/κ	1–5 ng/ml IL-6	26
KPMM2	76 F	IgG/λ MM plasmacytoma	R	ascites	yes	1991	RPMI1640+20%FCS	IgG/λ 10 μg/72h/ milj cells	4 ng/ml rIL6	38
L363	36 F	IgG PCL	D	PB	yes	1977	RPMI1640+20%FCS	nonsecretory		39,DSMZ
LB-831	36 F	IgG/κ MM stageIIIA	T	PE	yes	1983	M3	IgG/κ 69 μg/72 h/ milj cells	L15, insulin, transferrin, 5%FCS, autologous adherent feeder layer	40
LB-832	36 F	IgG/κ MM stageIIIA sister cell line/same origin as LB-831	T	PE	yes	1983	M3	IgG/κ 69 μg/72 h/ milj cells	L15, insulin, transferrin, 5%FCS, autologous, adherent feeder layer	40
LB 84-1	45 M	IgA/κ MM BJP/κ	R	BM	yes*	1983	RPMI1640+15%FCS M3	IgA/λ (+), κ−, nonsecr.	L15, insulin, transferrin, 5%FCS	79
LOPRA-1[j]	62 F	IgA/κ MM Stage IIA extramedullary plasmacytoma	T	ascites	yes	1982	Iscove's+20%FCS	IgA/κ	autologous ascites fluid or human AB serum or CM from mouse peritoneal cells/cloned by limiting dilution	41
LP-1	56 F	IgG/λ MM–PCL	R	PB	yes	1986	IMDM+20%FCS	IgG/κ 50 μg/24 h/ milj cells	5 μg/ml transferrin+ 5 μg/ml porcine insulin	43,DSMZ
MEF-1	67 F	IgG/κ BJP MM	D	BM	yes*		IMDM+20%FCS	κ		44
MM.1	42 F	IgA/λ MM	T	PB	yes	1986	RPMI1640+20%FCS	λ-light chain 4–9 μg/24 h/ milj cells	no feeder or growth factors	45
MM5.1[k]	55 M	IgA/κ MM stage IIIA	R	BM	yes	1993	RPMI1640	κ	12.5% horse serum, 12.5%FCS normal irr. BM/stromal cell feeder layer	46
MM5.2	55 M	MM (subclone of MM5.1)	R	BM	yes		RPMI1640+10%FCS	κ	1 year after the initiation of MM5.1	46
MM-A1	39 M	IgG/λ stage IIIA MM	D	PE	yes	1988	RPMI1640+20% human plasma, 5%PHA-LCM or 10 μg/ml rIL-6	IgG/λ		7
MM-C1	50 M	IgG/λ stage IIIA MM	D	BM	yes	1988	RPMI1640	IgG/λ	20% huPl, 5% PHA-LCM or 10 μg/ml rIL-6	7

Continued on next page

Table 2a. (continued)

Cell line[a]	Patient age/sex	Diagnosis[b]	Treatment status[c]	Specimen site[d]	Authenti-cation[e]	Year est.	Culture medium[f]	Ig production of cell line	Other requirements for establishment[m]	Primary ref./ availability[n]
MM-M1		IgG/κ MM			yes			nonsecretory		47
MM-S1	56 F	BJP/λ stage IIIA MM	T	PE	yes	1983	RPMI1640	λ	20% huPl, 5% PHA-LCM or 10 μg/ml rIL-6 later 10% FBS+ rIL-6 (10 U/ml)	7,48
MM-Y1	61 F	IgG/κ stageIIIA MM	T	BM	yes	1988	RPMI1640	IgG/λ	20% huPl, 5% PHA-LCM or 10 μg/ml rIL-6	7
MT3	51 F	IgA/κ PCL	D	PB	yes	1985	RPMI1640+20%FCS	IgA/κ 40–50 μg/ 24 h/milj cells		49
NCI-H929	62 F	IgA/κ MM	R	PE	yes	1984/85	RPMI1640+10%FCS	IgA/κ >80μg/24 h/ milj cells	or 10–20% pleural fluid or ACL-3 (RPMI1640 suppl with insulin, transferrin, EGF, BSA)	50, DSMZ
NOP-2	47 M	BJP/λ MM extramedullary plasmacytoma	T	plasma-cytoma	yes	1986	RPMI1640+20%FCS	λ	later 10%FCS	51
OCI-My1[l]		advanced MM			yes		IDMEM		20% hu Plasma, PHA-LCM	52
OCI-My2		advanced MM			yes		IDMEM		20% hu Plasma, PHA-LCM	52
OCI-My3		advanced MM			yes		IDMEM		20% hu Plasma, PHA-LCM	52
OCI-My4		advanced MM			yes		IDMEM		20% hu Plasma, PHA-LCM	52
OCI-My5		IgA/λ advanced MM	T	PB	yes		IDMEM	λ	20% hu Plasma, PHA-LCM primary cells cloned in 0.9% methylcellulose	52–54
OCI-My6		advanced MM			yes		IDMEM		20% hu Plasma, PHA-LCM	52
OCI-My7		advanced MM			yes		IDMEM		20% hu Plasma, PHA-LCM	52
OH-2	52 F	IgG/MM terminal stage	R	PE	yes	1984	RPMI1640+10%FCS	IgG/κ 60 μg/72h/0.5 milj cells	TNF+IL-6 (5 ng/ml), later 10% huA-serum+ IL6 (2 ng/ml)	55
OPM-1	56 F	IgG/λ MM – terminal PCL G/λ–λ	R	PB	yes	1982	RPMI1640+10%FCS	λ		56
OPM-2	56 F	IgG/λ MM – terminal PCL	R	PB	yes	1982	RPMI1640+10%FCS	λ		56, DSMZ
PCM6	60 F	IgG/λ BJP MM	R	PB	yes	1992	IMDM+20%FCS	IgG/λ/ BJP	30U/ml rIL-6	57
RPMI 8226	61 M	IgG/λ MM BJP		PB	yes	1966	RPMI1640+10%FCS	λ 15 μg/24 h/ milj cells		2,58, DSMZ, ATCC, JCRB

Continued on next page

Table 2a. (continued)

Cell line[a]	Patient age/sex	Diagnosis[b]	Treatment status[c]	Specimen site[d]	Authentication[e]	Year est.	Culture medium[f]	Ig production of cell line	Other requirements for establishment[m]	Primary ref./ availability[n]
SK-MM-1	55 M	PCL	T	BM	yes		RPMI1640+10%FCS	κ 0.9 μg/48 h/ 0.3 milj cells	no feeder	59
SK-MM-2	54 M	PCL	T	PB	yes		RPMI1640+10%FCS	κ 1.1 μg/48 h/ milj cells	no feeder	59, DSMZ
TH	32 M	κ chain MM (plasmoblastic)	D	BM	yes		IMDM+10%FCS	IgG/κ		60
U-1957	60 M	IgG/κ PCL	T	PE	yes*	1983	RPMI1640+20%FCS	IgG/κ	feeder cells	61
U-1958	60 M	IgG/κ PCL sister cell line/same origin as U-1957	T	PE	yes	1983	RPMI1640+20%FCS	IgG/κ 1.5 μg/24 h/ milj cells	feeder cells	61
U-1996	70 F	κ extramedullary MM with leukemic course (PCL)	D	ascites	yes	1983	RPMI1640+20%FCS	κ	feeder cells	61
U-2030		IgA/κ MM		PE	yes*	1983	RPMI1640+10%FCS	nonsecretory	(1) 30%CM of hematopoietic cell lines, fibroblasts, (2) grid organ, (3) feeder cells	62
U266BI (U-266)[p]	53 M	IgE/λ MM/PCL	T	PB	yes	1968	F10+10%NCS	IgE/λ 4–9 μg/24 h/ milj cells	feeder cells	3, ATCC, DSMZ
UCD-HL46]		IgG/κ Stage IIIB MM		BM	yes	1988	RPMI1640+10%FBS	IgG/κ 15 ng/24 h/ milj cells	stromal cell adh. later independent of adh. and dependent of CM	63
UMJF-2[o]	74 M	IgG/λ BJP MM/diffuse plasmacytosis		BM	yes	1988	IMDM+20%FCS	IgG/λ	RPMI1640+15%FBS	64
UTMC-2	72 F	IgA/κ MM	T	PE	yes	1993	RPMI1640+10%FBS	IgA/κ 4 μg/24 h/milj		65
XG-1	M	IgA/κ PCL (2ndary)		PB	yes*		RPMI1640+10%FCS		rIL-6(1ng/ml)+GM-CSF(10ng/ml)	9
XG-2	F	IgG/λ PCL (stageIII)		PE	yes*		RPMI1640+10%FCS		rIL-6+GM-CSF	9
XG-3P	F	λ PCL (primary)	D	PB	yes*		RPMI1640+10%FCS		rIL-6+GM-CSF	9
XG-3E	F	λ PCL stage III	R	PE	yes*		RPMI1640+10%FCS		rIL-6+GM-CSF	9
XG-4	M	IgG/κ PCL (2ndary)		PB	yes*		RPMI1640+10%FCS		rIL-6+GM-CSF	9
XG-5	F	λ PCL (2ndary)		PB	yes*		RPMI1640+10%FCS		rIL- 6+GM-CSF	9
XG-6	F	IgG/λ PCL (2ndary)		PB	yes*		RPMI1640+10%FCS		rIL-6+GM-CSF	9
XG-7	F	IgA/κ PCL (2ndary)		PB	yes*		RPMI1640+10%FCS		rIL-6+GM-CSF	9

Continued on next page

Table 2a. (continued)

Cell line[a]	Patient age/sex	Diagnosis[b]	Treatment status[c]	Specimen site[d]	Authenti-cation[e]	Year est.	Culture medium[f]	Ig production of cell line	Other requirements for establishment[m]	Primary ref./ availability[n]
XG-8	F	IgA/λ PCL (2ndary)		PB	yes*		RPMI1640+10%FCS		rIL-6+GM-CSF	9
XG-9	M	IgA/κ PCL (2ndary)		BM	yes*		RPMI1640+10%FCS		rIL-6+GM-CSF	9

[a] Cell lines are named as indicated in original literature (later given names in parenthesis; sister cell lines derived independently from the same patient of origin, and subclones originating from the in vitro growth of the parental cell line are indiated. [b] Diagnosis as indicated in the original literature. MM – multiple myeloma, PCL – plasma cell leukemia. [c] Treatment status as indicated in original literature. D – at diagnosis, T – during therapy, R – at relapse, ND – not determined. [d] Specimen site; BM – bone marrow, PB – peripheral blood, PE – pleural effusion. [e] Based on the criteria mentioned in Table 1, the authentication was determined by EBV negativity, and * Ig rearrangements, structural abnormalities or immunophenotype consistent with the fresh tumor cells. [f] Culture medium as indicated in original literature. [g] Subclone of HL407 (HL407E (early) was obtained during in vitro growth and progression from stroma cell dependent, feeder cell CM/IL-6 dependent growth to IL-6 independent autonomous growth in the HL407L (late) cell line. [h] Several subclones of JIM-1 were established (JIM-2, JIM-3, JIM-4 only distinguishable by the expression of bcl-2). [i] Subclones were obtained in the JJN cell line by in vitro cultivation in Ewing Sarcoma Growth Factor (EwSarGF) (i.e. IL-1+ IL-6). The JJN-3 line has retained IL-6 sensitivity. [j] LOPRA-1 was cloned by limiting dilution with the use of mouse peritoneal cells as feeder cells and several myeloma clones were obtained, two of which were further propagated in vitro in serum-free medium HB104TM (LOPRA-1/4 and LOPRA-1/5) differing only in the expression of cIg. LOPRA1/4 stained negative for cIg and subsequent Ig production. [k] The subclone MM5.2 was obtained at 1 year after initiation of the parental cell line MM5.1. [l] The OCI-My1-5 cell lines were obtained from BM or PB samples from advanced MM. Six of these were obtained by colony formation of fresh tumor cells in methyl cellulose, and one directly initiated in liquid suspension. No detailed data is available concerning the origin or karyotype of each individual cell line [54]. [m] huPl – human plasma; PHA-LCM – conditioned medium from PHA stimulated leukocytes; EwSarGF/ESG – Ewing sarcoma growth factor (IL1 and IL6), MQ/MDF – macrophage/MQ derived factors. [n] DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen. RCB – RIKEN Cell Bank. JCRB – Japanese Cell Resource Bank. ATCC – American Type Culture Collection. [o] EBV-positive (Drexler, personal communication). [p] Unfortunately U-266 has two other names. Both SKO 007 (Brodin et al. *J Immunol Methods* 60: 1, 1983) and AF10 [66] are sublines of and should be regarded as identical to U-266. Also note that GM2132 = RPMI 8226.

Table 2b. EBV+ LCLs phenotypic characteristics*

Cell line	EBV status	Doubling time/Ig production	Cytochemistry	Heterotrans-plantability in nude mice	B-cell/plasma cell associated marker	Activation marker/ other	Adhesion marker	Cytogenetic karyotype	Ref.
ARH-77	EBNA+	110 h 3.8–4.7 d IgG/λ 12.1 μg/ milj cells	Acid mucopolysaccaride–, lipids–, fats–, esterase–, SBB–, ORO–, NASDCAE–		CD10– CD19+ CD20+ CD21± CD38– PCA-1+ sIg+	HLA-DR+ CD28(+)	CD11a+ CD11b– CD11c–CD18+ CD29+ CD40+ CD49b– CD49d+ CD49e+ CD49c+ CD49f+ CD54+ CD56–	45–46 no marker chr	50,51, 67–69
Fr/FRAVEL	EBNA–, later EBNA+	62 h IgG/κ			CD10+ CD19+ CD20+ CD21+ CD23– CD24– CD37+ PCA-1– sIg– cIg–	HLA-DR+	CD11a+ CD11b+ CD11c– CD18+ CD29+ CD49b– CD49d+ CD49c+ CD49f+ CD54+ CD56–		69–71
GM1312	EBV+				CD20+ CD38–	OKIa+			29
GM1500	EBV+				CD20+ CD38–	OKIa+			29
HS-Sultan	EBV+				CD20+ CD21+ CD19+ CD10+ PCA-1+ CD38+ B-B4+ sIg+	HLA-DR+ CD28– CD40+	CD11a+ CD49e+		17,18, 50,72
IM-9	EBV+				CD19+ CD20+ CD38– B-B4+	CD28– CD40(+)	CD11a(+) CD49e+		17,18,72
MC/CAR	EBV+ IgG/κ nonsecretory	19.4 h	MGP+, SBB-, PAS(+), ANAE+, BGLU+, AcP+, TdT–	in nude mice 2/2, clonable in agarose	CD10– CD19+ CD20+ CD23– CD24– CD38+ B-B4(+) sIg+	OKIa1+ HLA-DR+ CD25– CD28– CD45+ CD30–	CD11a+ CD49e(+) CD56–	46, XY	17,18, 71–73

* Phenotype of some frequently used EBV positive LCLs.

Table 3. Multiple myeloma cell lines: immunophenotypic characterization[a]

Cell line	T-/NK-/myelomonocytic cell marker	B-cell/plasma cell associated marker	Activation marker	E/Fc/complement receptor/other	Adhesion marker	Ref.
ACB-885	CD2– CD3– CD4– CD8a–	CD10– CD20– CD21– CD38+	HLA-DR–			20
AD3		CD10(+) CD19(+) CD20+ CD38+ PCA-1+ sIg– cIg+	HLA-DR+	Fcγ–		21
AMO1	CD2– CD3– CD4+ CD5– CD8– CD13– CD33–	CD10– CD19– CD20– CD21– CD38+ PCA-1+ cIg+		CD34–	CD11b– CD11c–	22
ANBL-6 (ANBM-6)	CD2– CD3– CD5–	CD10+ CD19– CD20– CD38+ cIg+ CD21– sIg– CD73– CD23–	CD40+ CD25+	CD45–	CD11a– CD11b– CD18– CD29+ CD44+ CD49a+ CD49e– CD54+ CD56–	23 76
delta-47	CD4(+) CD5– CD8– CD13–	CD10– CD19– CD38+ PCA-1– sIg– cIg+	HLA-DR– CD30+	E– EA– EAC–		24
DOBIL-6	CD2– CD3– CD4– CD5– CD7– CD8– CD13– CD33–	CD10– CD19+ CD20+ CD21– CD38+ PCA-1+ sIg– cIg+	HLA-DR+ CD40+	CD45RA– CD45RO+	CD11a/18– CD29+ CD44+ CD49d+ CD49e– CD54+ CD56–	25
DP-6	CD2– CD4– CD5– CD14– CD16– CD25– CD33+	CD10– CD19– CD20– CD38+ CD72– CD73+ CD80– CD81– PCA-1+ UV-3+	CD28+ CD40+ CD40L–	CD95+ CD126+ CD130+ CD45+	CD11a+ CD44+ CD54+ CD56+	26

Continued on next page

Table 3. (continued)

Cell line	T-/NK-/myelomonocytic cell marker	B-cell/plasma cell associated marker	Activation marker	E/Fc/complement receptor/other	Adhesion marker	Ref.
EJM		CD9+ CD10– CD19– CD20(+) CD21(+) CD38+ CD75+ CD78+ cIg+	HLA-DR+	CD32(FcRII)+ CD71+	CD11a– CD11b– CD11c– CD29(+) CD44+ CD49b– CD49d+ CD49e– CD49c± CD49f± CD54+ CD56–	27 69 77
FLAM-76	CD2– CD3– CD4– CD5– CD8– CD13– CD15– CD33–	CD10– CD19– CD20– CD38+ PCA-1– sIg– cIg+	Ia– Ki67+ CD30–	CD32–	CD11a– CD18– CD44+ CD54+	28
FR4		CD10+ CD19(+) CD20(+) CD38+ PCA-1+ sIg– cIg+	HLA-DR+			21
GM2132 (=RPMI 8226)		CD20– CD38+ OKIa–				29
HL407	CD2– CD3– CD4– CD5– CD7– CD8– CD13– CD14– CD15– CD33– CD16– Leu7–	CD9– CD10– CD19– CD20– CD21– CD22– CD23– CD24– PCA-1+ MM4+ CD38+ cIg+	HLA-DR–	CD25– FcR–	CD56–	6
ILKM2		CD10– CD19– CD20– CD21– OKIa-1– PCA-1+ CD38+ cIg+				5
ILKM3		CD10– CD19– CD20– CD21– OKIa-1– PCA-1+ CD38+ cIg+				5

Continued on next page

Table 3. (continued)

Cell line	T-/NK-/myelomonocytic cell marker	B-cell/plasma cell associated marker	Activation marker	E/Fc/complement receptor/other	Adhesion marker	Ref.
JIM-1(-2-3)		CD38+ PCA-1+			CD29+ CD44+ CD49b– CD49d+ CD49e– CD49c± CD49f– CD54+ CD56+ CD58+	30
JJN-1		CD9– CD10– CD19– CD20– CD21– CD22– CD23– CD24+ CD37– CD38+ cIg/sIg+	MHCclassII+ CD25–	CD39++ CDw40– CD45R–		4
JJN-2		CD9– CD10– CD19– CD20– CD21– CD22– CD23– CD24+ CD25– CD37– CD38+ cIg/sIg+	MHCclassII–	CD39++ CDw40– CD45R–		4
JJN-3		CD10+ CD19– CD20(+) CD21– PCA-1– sIg– cIg–	HLA-DR+		CD11a– CD11b– CD11c– CD18–CD29+ CD49b– CD49d+ CD49e– CD49c± CD49f± CD54+ CD56–	69 77
Karpas 620	CD1– CD3– CD4– CD5– CD7– CD8– CD14– CD15– CD33–	CD10– CD20– CD37+ CD38–	HLA-DR+	CD45–		31
Karpas 707		CD10– CD19– CD20– CD21– PCA-1+	HLA-DR(+)		CD11a+ CD11b– CD11c– CD18+ CD29+ CD49b– CD49d+ CD49c+ CD49f+ CD54+ CD56+	69

Continued on next page

Table 3. (continued)

Cell line	T-/NK-/myelomonocytic cell marker	B-cell/plasma cell associated marker	Activation marker	E/Fc/complement receptor/other	Adhesion marker	Ref.
KAS-6/1	CD2– CD4– CD5– CD14– CD16– CD33–	CD10– CD19– CD20– CD38+ CD72– CD73– CD80– CD81– PCA-1+ UV-3+	CD25– CD28+ CD40+ CD40L–	CD95+ CD126+ CD130+ CD45–	CD11a– CD44+ CD54+ CD56–	26
KHM-1A	CD2–	CD10– CD20– PCA-1+	HLA-DR+			33
KHM-1B	CD2–	CD10– CD20– PCA-1+	HLA-DR+			33
KHM-11	CD3– CD4– CD5– CD7+ CD8– CD13–	CD10– CD19– CD20-CD25– CD38+ PCA-1+ cIg+	HLA-DR–	CD45RA+ CD45RO+	CD49d+ CD49e–	34
KMM-1	CD4– CD5– CD8a–	CD20+ CD21+ PCA-1+ CD38+ sIg– cIg+	HLA-DR(+)	CD71+ E– EA-EAC–		35 36
KMS-5	ND	CD20(+) CD21(+) PCA-1(+) CD38(+) sIg-cIg+ CD10+	HLA-DR(+)	CD71+	CD11a+ CD54+	78 36
KMS-11	ND	CD20(+) CD21 (+) PCA-1+ CD38(+) sIg+cIg+	HLA-DR(+)	CD71+		36
KMS-12-PE	ND	CD20(+) CD21(+) PCA-1+ CD38+	HLA-DR(+)	CD71+		36
KMS-12-BM	ND	CD20+ CD21+ PCA-1+ CD38+	HLA-DR(+)	CD71+		36
KMS-18	CD1(+) CD2(+) CD3(+) CD4(+) CD5(+) CD7(+) CD8(+) CD13(+) CD14(+) CD33(+) CD36(+)	CD10(+) CD19(+) CD20(+) CD23+ CD38(+)	HLA-DR+ CD30(+)	CD34(+)		37

Continued on next page

Table 3. (continued)

Cell line	T-/NK-/myelomonocytic cell marker	B-cell/plasma cell associated marker	Activation marker	E/Fc/complement receptor/other	Adhesion marker	Ref.
KP-6	CD2– CD4– CD5– CD14– CD16– CD25– CD33–	CD10– CD19– CD20– CD38+ CD72+ CD73+ CD80+ CD81– PCA-1+ UV-3+	CD40+ CD40L– CD28+	CD95+ CD126+ CD130+ CD45+	CD11a– CD44+ CD54+ CD56+	26
KPMM2	CD3– CD4– CD5– CD7– CD8– CD13– CD14– CD33–	CD10– CD19– CD20– CD22– CD38+ PCA-1+ BL3+ sIg+	HLA-DR(+) CD25–	CD34– CD42b– CD45+ CD45RA– CD45RO– CD62– CD63+ CD69– CD71+ glycophorinA– CD61– CD62L–	CD11a– CD11b– CD11c– CD18– CD29+ CD44+ CD54+ CD56+ CD58+ CD49d+ CD49e–	38
L363	CD2– CD7– CD13– CD14– CD15– CD16– CD17+ CD37+	CD9– CD10– CD19– CD20± CD21– CD23– CD24– CD38+ B-B4+ PCA-1± sIg–	HLA-DR+ CD28+ CD40– CD25– CD30–	E– EA– EAC– EBNA–, EA–, VCA– CD45+ CD30–	CD11a– CD11b– CD11c– CD18– CD29+ CD49b– CD49d+ CD49e– CD49c+ CD49f+ CD54+ CD56+	69 17,18, 72 71
LB-831	T cell antigens–	CD10+ (later CD10–) CD20+ CD21– cIg+	HLA-DR+			40
LB-832	T cell antigens–	CD10+ CD20+ CD21– cIg+	HLA-DR+			40
LB 84-1	CD1– CD2– CD3– CD4+ CD5– CD7– CD8– CD13±	CD9– CD10– CD19+ CD20+ CD21– CD22– CD23– CD24–	HLA-DR+ Ki-67+ CD25–	CD45+	CD11a– CD11b– CD11c± CD18– CD29+ CD49b– CD49d+	79 71

Continued on next page

Table 3. (continued)

Cell line	T-/NK-/myelomonocytic cell marker	B-cell/plasma cell associated marker	Activation marker	E/Fc/complement receptor/other	Adhesion marker	Ref.
	CD14– CD15± CD16– CD17+ Leu M3+ Leu7– Leu8– Leu9–	CD38+ CD37+ PCA-1+ PC-1+			CD49c+ CD49f+ CD54+ CD56±	69
LOPRA-1	CD1– CD2– CD3– CD4– CD5– CD6– CD7– CD8– CD13–	PCA-1+ CD24(+) cIg+ CD10– CD19– CD20– CD21– CD22– CD23– CD37– CD38+ CD39– sIg–	HLA-ABC+ CD30– HLA-DR– CD25– CD28+	CD45– CD71+		41
LP-1	CD2– CD3– CD16–	CD10– CD19– CD20– CD21– CD38+ PCA-1– PC1– sIg+ B-B4+	HLA-DR+ CD25– CD28+ CD40+ CD40L–	CD35(+)	CD11a– CD11b– CD11c– CD18– CD29+ CD49b– CD49d+ CD49e– CD49c+ CD49f+ CD54+ CD56+	43 69 17,18, 72
MEF-1	CD2– CD5– CD7– CD13– CD14–	CD19– CD20– CD21– CD22(+) CD23– CD24 (+) CD38– PCA-1– sIg–	HLA-DR– HLA-ABC+ CD28+	CD34– CD41a– CD45(+)	CD44+ CD56– CD71+	44
MM.1		CD10– CD20– CD19– PCA-1+ CD38+ sIg– T11– LN-1+	HLA-DR+	CD71+		45
MM5.1	CD3– CD4– CD8– CD14– CD16–	CD10– CD19– CD20– CD21– CD38+ B-B4+ cIg+	HLA-DR– B7–	CD40– CD45–	CD11a– CD11b– CD11c– CD18– CD29+ CD49a– CD49b– CD49c– CD49d+ CD49e– CD49f– CD44+ CD54+ CD56– CD58–	46

Continued on next page

Table 3. (continued)

Cell line	T-/NK-/myelomonocytic cell marker	B-cell/plasma cell associated marker	Activation marker	E/Fc/complement receptor/other	Adhesion marker	Ref.
MM-A1	CD3– CD13–	CD10– CD19– CD20– CD38+ PCA-1+ cIg+	HLA-DR–			7
MM-M1	CD3– CD13–	CD10– CD19– CD20– CD38– PCA-1 (+) cIg–	HLA-DR–			47
MM-C1	CD3– CD13–	CD10– CD19– CD20– CD38+ PCA-1+ cIg+	HLA-DR–			7
MM-S1	CD3– CD13–	CD10– CD19– CD20– CD38– PCA-1+ cIg+	HLA-DR–	CD41a– glycophorinA–		48 7
MM-Y1	CD3– CD13–	CD10– CD19– CD20– CD38+ PCA-1+ cIg+	HLA-DR–			7
MT3	CD1a– CD3– CD7– CD13– CD14– CD33–	CD10(+) CD19– CD20– CD21– CD24– PCA-1+ CD38+ cIg/sIg+	HLA-DR(+)	CD71+		49
NCI-H929	CD5– L1– L2– L3– L4– L7– L8– L11– L12– LeuM1– LeuM3–	CD10– CD19– CD20– CD21– CD38+ B-B4+ PCA-1+ cIg+ sIg+	HLA-DR– I2– TdT– CD25– CD28+	CD71+	CD11a– CD49e–	50 17,18,72
NOP-2	CD2– CD3– CD7– CDw14– CDw15– CD24–	CD10– CD19– CD20– CD21– CD38+ PCA-1+ sIg– cIg+	HLA-B(classI)+ HLA-DR– CD25–		CD11–	51

Continued on next page

Table 3. (continued)

Cell line	T-/NK-/myelomonocytic cell marker	B-cell/plasma cell associated marker	Activation marker	E/Fc/complement receptor/other	Adhesion marker	Ref.
OH-2	CD2– CD4– CD5– CD14–	CD10– CD19– CD20– CD38+ PCA-1+ B-B4+			CD56+	55
OPM-1	CD1a– CD3– CD4– CD8–	CD9– CD10– CD19– CD20– CD21–	HLA-DR–	E– EA– EAC–	CD11a– CD11b– CD11c– CD18–	51 69
		CD24– CD38+ PCA-1– sIg–			CD29+ CD49b– CD49d+ CD49c+ CD49f+ CD54– CD56+	
OPM-2	CD1a– CD3– CD4– CD8–	CD9– CD10– CD19– CD20– CD24–	CD28+	E– EA– EAC–	CD11a– CD49e–	51
		CD38+ B-B4+ sIg–				17,18,72
PCM6	CD13–	CD19– CD10– PCA-1– CD38+	Ia–			57
RPMI8226	CD1a– CD2– CD3– CD4– CD7– CD8–	CD9+ CD10– CD19– CD20– CD21–	HLA-DR– CD40+	CD45–	CD11a(+) CD18± CD29+ CD49b–	51
		CD22– CD24– CD38+ OKIa-1(+) PCA-1+ B-B4+ sIg–	CD28+		CD49d+ CD49e+ CD54+ CD56±	71 17,18, 69,72
SK-MM-1	CD2– CD25– CD33– M195–	CD10– CD19– CD20+CD21– BL3+ CD38– PCA-1– sIg–	HLA-DR–	EAC–		59
SK-MM-2	CD2– CD13– CD33–	CD10– CD19– CD20+ CD21– BL3+ CD38+ PCA-1+ sIg–	HLA-DR–	EAC–		59
TH	CD5–	CD9+ CD10(+) CD19– CD20(+)	HLA-DR+ CD40–	CD34–		60
		CD22– CD23– CD24– CD38+ PCA-1+ cIg+	CD25(+) CD28+	CD45– CD6(+)		

Continued on next page

Table 3. (continued)

Cell line	T-/NK-/myelomonocytic cell marker	B-cell/plasma cell associated marker	Activation marker	E/Fc/complement receptor/other	Adhesion marker	Ref.
U-1957		CD19– CD20– CD21– CD38+ PCA-1+ PC-1– cIg+	CD25–	E– Fcγ+ C2–		61
U-1958		CD19– CD20– CD21– CD38+ PCA-1+ PC-1– cIg+	CD25–	E– C2–		61
U-1996		CD19– CD20– CD21– CD38+ PCA-1+ PC-1– cIg+	CD25–	E– Fcγ– C2–		61
U-2030		CD10– CD19– CD20– CD21– PCA-1+	CD25+			62
		PC-1– CD38+ cIg– sIg–	HLA-DR–			
U-266	CD2– CD7-CD13– CD14– CD15– CD16– CD17–	CD10– CD19– CD20– CD21– CD23– CD24– CD37– CD38–	HLA-DR+ CD40– CD28+	CD71+ CD45–	CD11a– CD11b– CD11c– CD18–	51
		B-B4+ PCA-1(+) sIg–	CD25–		CD29± CD49b– CD49d+ CD49e–	50
					CD49c± CD49f± CD54+ CD56–	17,18,69 71,72,77
UCD-HL461	CD1– CD2– CD3– CD4– CD5+ CD7– CD8– CD14– CD13– CD16– CD33– Leu7–	CD9(+) CD10+ CD19+ CD20+ CD21(+) CD22+ CD24+ cIg– sIg+ CD38+ PCA-1+ MM4+	HLA-DR+ I2– CD25+	CD35(+)	CD11– CD56–	63
UMJF-2[b]	CD2– CD3– CD4+ CD5– CD7– CD8– CD13– CD16(+) CD33(+)	CD10– CD19+ CD20+ CD38+ PCA-1+		CD57(+) CD34–	CD11b– CD11c–	64

Continued on next page

Table 3. (continued)

Cell line	T-/NK-/myelomonocytic cell marker	B-cell/plasma cell associated marker	Activation marker	E/Fc/complement receptor/other	Adhesion marker	Ref.
UTMC-2	CD3– CD4– CD5– CD8– CD13–	CD10– CD19– CD20– CD38+ sIg– cIg+	HLA-DR–	CD45RA–	CD29– CD29+ CD54+ CD56– CD11a/CD18–	65
XG-1	CD2– CD3– CD5– CD13– CD14– CD15– CD16– CD33–	CD10+ CD19– CD20– CD21+ CD23+ CD24– CD37+ CD72– B-B4+ CD38+ cIg+ sIg–	CD25– CD28+ B7+ CTLA-4– CD30+ CD40– CD71+ HLA-I+ HLA-II–	CD32– CD57– CD65– CD77+	CD11a– CD11b– CD11c– CD18– CD44+ CD49b– CD49d+ CD49e– CD49f+ CD54+ CD56+ CD58+ PNA+	9,17 18,72
XG-2	CD2– CD3– CD5– CD13– CD14– CD15– CD16– CD33+	CD10– CD19– CD20– CD21+ CD23+ CD24– CD37– CD72– B-B4+ CD38+ cIg+ sIg–	CD25– CD28+ B7– CTLA-4– CD30– CD40+ CD71+ HLA-I+ HLA-II+	CD57– CD65– CD77+	CD11a+ CD11b– CD11c– CD18+ CD44+ CD49b– CD49d+ CD49e± CD49f+ CD54+ CD56– CD58+ PNA+	9,17 18,72
XG-3P	CD2– CD3– CD5– CD13– CD14– CD15– CD16– CD33+	CD10+ CD19– CD20– CD21+ CD23– CD24– CD37+ CD72– B-B4+ CD38+ cIg+ sIg–	CD25– CD28+ B7– CTLA-4– CD30– CD40+ CD71+ HLA-I+ HLA-II–	CD57+ CD65– CD77+	CD11a– CD11b– CD11c– CD44+ CD49b– CD49d+ CD49e+ CD49f+ CD54+ CD56+ CD58+ PNA+	9,17 18,72
XG-3E	CD2– CD3– CD5– CD13– CD14– CD15– CD16– CD33+	CD10– CD19– CD20– CD21– CD23– CD24– CD37– CD72– B-B4+ CD38+ cIg+ sIg–	CD25– CD28+ B7+ CTLA-4– CD30– CD40+ CD71+ HLA-I+ HLA-II–	CD57+ CD65– CD77+	CD11a– CD11b– CD11c– CD44+ CD49b– CD49d+ CD49e+ CD49f+ CD54+ CD56+ CD58+ PNA+	9,17 18,72

Continued on next page

Table 3. (continued)

Cell line	T-/NK-/myelomonocytic cell marker	B-cell/plasma cell associated marker	Activation marker	E/Fc/complement receptor/other	Adhesion marker	Ref.
XG-4	CD2– CD3– CD5– CD13– CD14– CD15– CD16– CD33–	CD10– CD19– CD20– CD21– CD23– CD24– CD37+ CD72– B-B4+ CD38+ cIg+ sIg–	CD25– CD28+ B7– CTLA-4– CD30– CD40+ CD71+ HLA-I+ HLA-II–	CD32– CD57+ CD65– CD77+	CD11a– CD11b– CD18+ CD44+ CD49b– CD49d+ CD49e– CD49f– CD54+ CD56– CD58+ PNA+	9,17 18,72
XG-5	CD2– CD3– CD5– CD13– CD14– CD15– CD16– CD33+	CD10+ CD19– CD20– CD21+ CD23– CD24– CD37+ CD72– B-B4+ CD38+ cIg+ sIg–	CD25– CD28+ B7+ CTLA-4– CD30+ CD40– CD71+ HLA-I+ HLA-II–	CD32– CD57– CD65– CD77+	CD11a– CD11b– CD11c– CD18– CD44+ CD49b– CD49d+ CD49e+ CD49f– CD54+ CD56– CD58+ PNA+	9,17 18,72
XG-6	CD2– CD3– CD5– CD13– CD14– CD15– CD16– CD33–	CD10– CD19– CD20– CD21+ CD23– CD24– CD37+ CD72– B-B4+ CD38± cIg+ sIg–	CD25– CD28+ B7– CTLA-4– CD40+ CD71+ HLA-I+ HLA-II–	CD32– CD57– CD65– CD77+	CD11a– CD11b– CD11c– CD18– CD44+ CD49b– CD49d+ CD49e± CD49f– CD54+ CD56– CD58+ PNA+	9,17 18,72
XG-7	CD2– CD3– CD5– CD13– CD14– CD15– CD16–	CD10– CD19– CD20– CD23– CD24– CD37– CD72– B-B4+ CD38+ cIg+ sIg–	CD25– CD28+ B7– CTLA-4– CD40– CD71+ HLA-I+ HLA-II–	CD32– CD57– CD65– CD77+	CD11a– CD11b– CD11c– CD18– CD44+ CD49b– CD49d+ CD49e+ CD49f+ CD54+ CD56+ CD58+ PNA+	9,17 18,72

Continued on next page

Table 3. (continued)

Cell line	T-/NK-/myelomonocytic cell marker	B-cell/plasma cell associated marker	Activation marker	E/Fc/complement receptor/other	Adhesion marker	Ref.
XG-8	CD2– CD3– CD5– CD13– CD14– CD15–	CD10– CD19– CD20– CD21– CD23+ CD24– CD37– CD72– B-B4+ CD38+ cIg– sIg–	CD25– CD28+ B7+ CTLA-4– CD30+ CD40+ CD71+ HLA-I+ HLA-II–	CD32+ CD57– CD65– CD77+	CD11a– CD11b– CD11c– CD18+ CD44+ CD49b– CD49d+ CD49e+ CD49f+ CD54+ CD56– CD58+ PNA+	9,17 18,72
XG-9	CD2– CD3– CD5– CD13– CD14– CD15– CD33–	CD10– CD19– CD20– CD23+ CD24– CD37– CD72– B-B4+ CD38+ cIg– sIg–	CD25– CD28+ B7– CTLA-4– CD30+ CD40– CD71+ HLA-I+ HLA-II–	CD32– CD57– CD65– CD77+	CD11a+ CD11b– CD11c– CD18+ CD44+ CD49b– CD49d+ CD49e– CD49f– CD54+ CD56– CD58+ PNA+	9,17 18,72

[a] + ; strong, definite protein expression, mostly >10–20% cells positive; (+) = weak protein expression, qualitatively and quantitatively (commonly <10% cells positive); – = no protein expression; ± = contradictory results.

[b] EBV positive (Drexler, personal communication).

In summary, the EBV+ LCL type of non-neoplastic B cell line is generally recognizable by the surface marker profile CD 19+, CD20+, CD138–, CD28–, CD11a+, CD49e+ and CD56–, while the phenotype of MM is characterized by the expression of CD19–, CD20–, CD138+, CD28+, CD11a–, CD49e– and CD56+/– (Table 1).

2. CLINICAL CHARACTERIZATION

Cell lines have been established from bone marrow, peripheral blood, plasmacytoma, pleural effusions and ascites of patients with advanced disease at a terminal stage (Table 2). During the course of the disease from the chronic to the acute phase, the proliferative compartment progressively increases [74]. In the late phases, MM cells frequently invade extramedullary sites, from which the majority of the cell lines are derived (62 characterized cell lines derived from 54 patients).

The data listed in Table 2 include a few cell lines derived from bone marrow of MM in the absence of extramedullary spread (DOBIL-6, HL407, ILKM2, ILKM3, LB 84-1, MEF-1, MM5.1, MM-C1, MM-Y1, TH, UCD-HL461, UMJF-2.). Interestingly, stage III disease was found in four of these patients. In the case of LB 84-1, MEF-1 and UMJF-2, Bence-Jones protein (BJP) was present in the MM patients and diffuse plasmacytosis was described in UMJF-2. The DOBIL-6 is derived from a nonsecretory MM. Three additional cell lines were established from nonsecretory MM, but these were from disseminated disease (FLAM-76 and KMS-12-PE and KMS-12-BM, the two latter lines being derived from different sites in the same patient). A few cell lines were derived from plasmacytomas (AMO1, delta-47, FR4 and its subline AD3, KMM-1, KPMM2, LOPRA-1, NOP-2).

3. IMMUNOPHENOTYPICAL CHARACTERIZATION

The established MM cell lines share common phenotypic characteristics with freshly isolated MM cells, including Ig gene rearrangements, a unique myeloma protein and the requirement for stromal cell-derived growth factors, including IL-6 and IGF-I. Also several surface markers are expressed on continuous MM cell lines and fresh MM cells, although some can differ in expression.

The CD38 marker of normal plasma cells is also expressed on MM cells. However, CD38 is not restricted to the plasma cell compartment, nor is it B-cell lineage specific. PCA-1 is expressed on malignant plasma cells of MM patients, with some specificity for disseminating tumor cells escaping the

bone marrow compartment. CD28 is expressed on fresh MM cells and on many MM cell lines [17,72,75] (Table 3). The CD28 molecule expressed on MM cells was recently proved to be functional due to its binding to its receptor B7, inducing activation of PI-3 kinase in MM cells [75], CD19 is expressed on normal plasma cells, while MM cells are usually negative.

The combined expression of phenotypic markers can be useful for the authentication of MM cell lines. Normal plasma cells are CD28– , but CD40+. Immunophenotypic studies have also demonstrated the expression of CD40 on primary cells of MM and on established cell lines [72]. The expression of CD40 is related to progression and was seen in 7/13 cell lines examined (XG-2, XG-3, XG-4, XG-6, XG-8, RPMI 8226, LP-1), although with the exception of XG-2 expression was weak, and negative in XG-1, XG-5, XG-7, U-266 and L363 (Table 3). All MM cell lines examined express CD28, a feature of advanced disease which is not expressed during the chronic phase of MM [72]. It is interesting that in the ANBL-6 cell line, the expression of CD40 and signaling via CD40 may induce autocrine IL-6 production and IL-6 autocrine growth stimulation [80]. Other reports also show enhanced secretion of IL-6 as a result of CD40L stimulation in MM cell lines [68].

CD138 (syndecan-1/B-B4) is a transmembrane heparan sulfate proteoglycan that binds ECM components and growth factors, such as fibroblast growth factor (FGF). It mainly mediates adhesion to collagen type I of ECM [81,82]. Similar to CD38, the expression of CD138 is a feature both of normal and malignant plasma cells derived from patients with MM. It has been suggested that the immature phenotype expressing CD45+, CD 19– does not express CD138, while the more mature phenotype CD45–, CD19– does express CD138. Syndecan-1 may participate in the homing to or maintenance of tumor cells in the marrow, as well as in the cytokine-mediated growth regulation of MM cells. The fact that CD-138 is not presented on other hematopoietic, or lymphoid cells in patients with MM, while present on MM cells, emphasizes the usefulness of this marker in the purification and selection of primary cells for studies of the biology of MM [81].

The integrins are important cell adhesion molecules in MM. The very late antigens (VLA) are adhesion molecules belonging to the integrin β family with a possible role in maintaining the tumor cells within the bone marrow [77]. Ligands of the ECM consist of collagen and laminin (VLA-2 (CD49b)), and fibronectin (VLA-4 (CD49d) and VLA-5 (CD49e)). Binding of MM cells to bone marrow stromal cells and osteoblasts through VLA-4 (CD49d)/VCAM-1 may induce IL-6 production in the stromal cells [83]. Immature subpopulations of MM are negative for VLA5 (CD49e), while mature cells are positive [84]. Normal and malignant plasma cells express VLA-4, but not other VLAs, including VLA-5 (CD49e) [77]. MM cell lines JIM-1, JIM-3, JJN-3, EJM, U-266 are negative for CD49e, and only RPMI 8226 was

positive [77]. The cell lines were positive for VLA-4 (CD49d), while other VLAs (CD49a, CD49b) were generally not expressed. The results of Drew et al. [77] were confirmed by Kim et al. [85] comparing one authentic MM (U-266) with an EBV+ LCL (IM-9), and by Ohmori et al. [25] in the phenotypic characterization of the DOBIL-6 cell line. The expression of VLA-6 (CD49f) is heterogeneous in MM cells, while VLA3 (CD49c) does not appear to be expressed. However, the expression of CD49c and CD49f in MM cell lines seems to be highly variable [69,77].

The $\beta 2$ family of integrins includes the lymphocyte function associated antigen (LFA-1) (CD18/CD11a), Macl (CD18/CD11b) and p150.95 (CD18/CD11c). The pattern of $\beta 2$-integrin expression is heterogenous in normal and malignant plasma cells from MM patients. Authentic MM cell lines (L363, JJN-3, U-266, LP-1, Karpas 707, OPM-1, EJM, LB 84-1) are negative or weak for $\beta 2$ integrins, while LCLs (ARH-77 and Fr/Fravel) are positive for CD18, CD11a, CD11b and CD11c [69] (Tables 2 and 3). The expression of the $\beta 2$ integrins CD18/CD11a was determined in malignant plasma cells from MM patients [86]. In normal plasma cells and in MM cells from patients with inactive disease CD11a expression was absent, indicating that LFA expression correlated with proliferation [78,86]. However, these data were not confirmed by others [1, 17, 18], showing a decreased expression of CD11a in MM cells as compared to normal plasma cells. These data suggest the lack of expression of LFA-1 (CD11a) is associated with highly proliferative and highly malignant MM cells. The other $\beta 2$ integrins, including CD11b and CD11c, are not expressed on fresh MM cells and cell lines derived from MM [69,77,85].

CD56 is an isoform of the neural adhesion molecule N-CAM. It belongs to a group of adhesion molecules (the Ig-superfamily) which also includes the intercellular adhesion molecule ICAM-1 (CD54). CD54 is one of the ligands of LFA-1 [69]. CD54 is invariably expressed by normal plasma cells, freshly isolated MM cells and established MM cell lines (L363, JJN-3, U-266, LP-1, Karpas 707, OPM-1, EJM, LB 84- 1) [69]. The expression of CD54 was also demonstrated in KMS-5 [78], DOBIL-6 [25], DP-6, KAS-6/1, KP-6, FLAM-6, KPMM2, UTMC-2 and the XG1- 9 cell lines (Table 3). CD54 was also detected in the LCLs ARH77 and Fr/Fravel (Table 2).

In line with the use of the combined expression pattern of CD28 and CD40, the expression of CD 19 and CD56 can be used to determine the authenticity of MM lines. Normal plasma cells are frequently CD 19+ and CD56–, while MM cells are almost invariably positive for CD56. The CD 19+ CD56– phenotype was never detected in MM cells. CD56 (NKH- 1/Leu19) binds heparan sulfate of the ECM, which may be present in the BM environment, and this would be consistent with the finding that loss of CD56 may be associated with a more aggressive disseminating tumor [17,87,88]. The

CD56 was expressed in XG-1 and XG-7 and absent or weakly expressed in the XG-3, RPMI 8226, L363 and LP-1 MM cell lines [72] (Table 3). These data were confirmed by van Riet et al. [69], who showed the absence of CD56 expression in L363, JJN-3, U-266 and EJM cell lines. However, the LP-1, Karpas 707, OPM-1 and LB 84-1, KPMM2, KP-6, KAS-6/1, DP-6, JIM and DOBIL-6 lines stained positively for CD56. Interestingly, the CD56 expression has been suggested to be absent in cell lines derived from MM patients with plasma cell leukemia (PCL) [69,88,89]. CD56 was also absent in the LCLs Fr/Fravel and ARH-77, which express CD19 (Table 2).

4. CYTOKINE-RELATED CHARACTERIZATION

The proliferation and survival of MM cells in vivo is dependent on cytokines and a fine-tuned network of cell-cell and cell- extracellular matrix interactions [87,90,91]. In recent years IL-6 has been identified as an important factor regulating growth and survival of MM cells in vitro and in vivo. The study by Kawano et al., suggesting that IL-6 was an autocrine growth factor in MM, stimulated extensive studies of the role of this cytokine. IL-6 can induce growth of primary cells derived from MM patients in about 50% of cases [12,13]. The importance of IL-6 in the pathogenesis of MM was further emphasized by the anti-tumor effects of monoclonal IL-6 antibodies used in the treatment of MM patients [92]. The frequency of establishment of MM cell lines has considerably increased with the use of IL-6-producing feeder cells or recombinant growth factors (IL- 6, GM-CSF, and TNFα). Yet there is still no tissue culture technique that allows consistent success in the establishment of MM cell lines from the bone marrow of early stage MM patients [5,6,40,61,63].

It is difficult to provide a coherent picture of the capacity of MM cell lines to produce and secrete cytokines, and to express cytokine receptors, since there are few reports on the characterization of cytokine production and the response to cytokines in authentic MM cell lines. Also, the sensitivity of methods used varies (RT-PCR, northern blots, ELISA and bioassays). In addition, few studies take account of the changes in expression of cytokine and corresponding receptors that occur during progression in culture [6,93–95], These difficulties are exemplified by the IL-6 dependent cell lines established with feeder cells (U-266, HL407) [6,95]. In these cell lines a weak expression of IL-6 mRNA, but no detectable IL-6 protein was found. During in vitro progression, the expression of IL-6mRNA increased and IL-6 secretion was induced in U-266 (–1984) and HL407 (late), in some cases coinciding with autonomous growth and survival in the absence of exogenous IL-6. Also the

IL-6R mRNA and the number of IL-6 receptors increased during a decade of continuous in vitro growth [94,95].

The relevance of an autocrine loop in vivo and in vitro has also been disputed [26]. The proposal for an autocrine loop in MM cells [12] was challenged by Portier et al. [96], who did not detect IL-6 mRNA in highly purified MM cells. However, recent studies using RT-PCR and in situ analysis have demonstrated the expression of IL-6mRNA in bone marrow plasma cells of MM patients [97,98].

In MM cell lines, IL-6mRNA was detectable in U-266, and antisense strategies and antibodies against IL-6R inhibited cell proliferation, indicating an autocrine growth stimulation in this cell line [19,94,95,99,100]. In addition to U-266 and HL407, further cell lines have been demonstrated to express IL-6mRNA and secrete IL-6 protein (OCI-My2, OCI-My3, DOBIL-6, DP-6, KP-6, KPMM2, MEF-1), while others express IL-6mRNA but no detectable protein (OCI-My1, OCI-My6, OCI- My7, ANBM-6, KAS-6/1, UTMC-2) (Table 4). In a few of these cell lines, autocrine IL-6 stimulation has been suggested [26,38,52,65]. This notion has also been supported in some cases by antisense strategies [129]. Most cell lines are negative for IL-6 mRNA, but do express IL-6R mRNA. The presence of functional receptors has in some cases been confirmed by binding assays and/or by flow cytometry analysis [9,26,38,65,95,109].

Numerous reports have established IL-6 as the major growth factor for primary cells and established cell lines (reviewed by Hawley and Berger [130]). Most MM cell lines can be stimulated by IL-6 (Table 4). Several IL-6 dependent cell lines have been described (ANBL-6, DP-6, FLAM-76, HL407 (early), ILKM2, ILKM3, JJN-1, KAS-6/1, KP-6, MM5.1, MM-A1, MM-C1, MM-Y1, MM-S1, OH-2, PCM6, U-1958, U-266-1970, XG-1, XG-2, XG-3, XG-4, XG-5, XG-6) (Table 4). As described above for HL407 and U-266, in DP-6 and KAS-6/1 the dependence on IL-6 decreased during long term in vitro culture.

Other members of the IL-6 type cytokine family, IL-11, leukemia inhibitory factor (LIF), oncostatin M (OM) and ciliary neurotrophic factor (CNTF) can induce proliferation in MM cells [122, 131], The IL-11, LIF, OM and CNTF share the same transducer chain (gp130) and induce similar biological effects as IL-6 (reviewed by Klein [92]). In two IL-6 dependent cell lines (XG-4, XG-6) proliferation was induced, while two other cell lines (XG-1 and XG-2) were unresponsive. This correlated with the expression of IL-11R and LIFRβ in the responsive cell lines [122]. In particular LIF-, Oncostatin M-, IL-11-, or CNTF- dependent MM cell lines have been established [92,122,125]. However, in a previous study, the absence of IL-11R, IL-11 and response to IL-11 was reported in two cell lines autonomous for growth (U-266, RPMI 8226) [118]. Established MM cell lines have been suggested

Table 4. Multiple myeloma cell lines: cytokine-related characterization

Cell line	Cytokine receptor expression[a]	Cytokine production[d]	Proliferation response to cytokines[b]	Growth arrest/apoptosis response to cytokines[c,d]	Dependency on cytokines	Ref.
ANBM-6/ ANBL-6	Flow cytometry: IGF-IR+	RT-PCR: IL-6 mRNA+ ELISA: IL-6–	3H-TdR: IGF-I+, IL-6+	IFNα+ (inh. of IL-6 ind. growth)	IL-6	101,102
DOBIL-6	RT-PCR: IL-6R/gp80 mRNA+ IL-6R mRNA+	RT-PCR: IL-6 mRNA+ IL-6+ (5440 pg/100 000 cells/ml/96 h), IL-1α–, TNFα– RT-PCR: PTHrP mRNA+	IL-6+	neutr. anti-IL-6 ab+ (growth inh.) serum depletion + (apoptosis)	25	
DP-6	Flow cytometry: IL-6R/gp80 (CD126)+ gp130 (CD130)+ IGF-IR+	RT-PCR: IL-6 mRNA+ ELISA/bioassay: IL-6+	3H-TdR: IL6+, IL-1α+, IL-1β+, IL-3+, IGF-I+. IL-10+, TNFα+, IL-2(+), IL-11(+), IL-12–, IL-13(+) GM-CSF(+), LIF–, OSM–, IL-4(+), CNTF–, IFNγ(+)	TGFβ+ (growth inh.) IFNα + (growth inh.) anti-IL-6R ab+ (growth inh.), (<1 yr after initiation of cell line) anti-IL-6R ab(+) (growth inh.), (>1 yr after initiation of cell line) anti-IGF-IR + (growth inh.)	IL-6, later partly dependent	26,103 101,102
EJM	RT-PCR/binding assay: IGF-IR+	RT-PCR/RIA: IGF-I+ Northern: IL-1β mRNA– Immunoassay: GM-CSF–	3H-TdR: IL6+, IL-1(+) IL-3–, IL-7–, TNFα– IGF-I– IGF-I truncated analogue+ (truncated IGF-BP site)	IFNα+ (growth inh.) anti-IGF-IR ab+ (growth inh.)		27 8 104 105,106

Continued on next page

Table 4. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[d]	Proliferation response to cytokines[b]	Growth arrest/apoptosis response to cytokines[c,d]	Dependency on cytokines	Ref.
FLAM-76		bioassay: IL-6–	3H-TdR: IL-6(+)	IFNα+ (growth inh.)	IL-6	28
HL407	RT-PCR: IL-6R mRNA+	RT-PCR: IL-6 mRNA+ bioassay: IL-6– In HL407L, late subclone of IL-6 independent cell line: bioassay: IL-6+ > 2 μg/ml IL-6	IL-6+, IL-1–, IL-2–, IL-3–, IL-4–, IL-5–, IL-7–, G-CSF–, GM-CSF–, IGF-I+ In HL407L, late IL-6 independent subclone: IL-6–, IGF-I+	anti-IGF-IR ab+ (growth inh.)	IL-6, later independent of stromal cells/ independent of IL-6 (HL407L)	6 105, 106
ILKM2		IL-6– (in supernatant)	3H-TdR: IL-1α+, IL-1β+ IL-2–, IL-3–. IL-4–, IL-6+ IFNα+, IFNβ–, IFNγ–, EGF–, TNFα(+), G-CSF–, GM-CSF–, MDF (Macrophage CM)+	anti-IL-6 ab+ (inh. MDF induced growth)	IL-6	5
ILKM3		IL-6– (in supernatant)	3H-TdR: IL-1α–, IL-1β– IL-2–, IL-3–, IL-4–, IL-6+ IFNα–, IFNβ–, IFNγ–, TNFα–, G-CSF–, GM-CSF–, EGF–, MDF (Macrophage CM)+	anti-IL6 ab+ (partly inh. MDF induced growth)	IL-6	5

Continued on next page

Table 4. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[d]	Proliferation response to cytokines[b]	Growth arrest/apoptosis response to cytokines[c,d]	Dependency on cytokines	Ref.
JJN-1	IL-2R–		3H-TdR: IL-2–, IL-6+		IL-6	4
JJN-2	IL-2R–		3H-TdR: IL-2–, IL-6+		IL-6	4
JJN-3		Northern: IL-1β mRNA–	IL-6+			27
		Immunoassay: GM-CSF–				8,104
Karpas 707	Northern: IL-6 mRNA+ RT-PCR: IL-6R mRNA+ RT-PCR/binding assay: IGF-IR+	Northern: IL-6mRNA– RT-PCR/ RIA: IGF-1+ RT-PCR/RIA: IL-6–	IL-6– IGF-1+	anti-IGFIR ab+ (growth inh.)		14,107; 105–106, Georgii-Hemming per. comm.
KAS-6/1	Flow cytometry:	RT-PCR: IL-6 mRNA+	3H-TdR: IL-6+, IL-1α(+),	TGFβ(+) (growth inh.)	IL-6, later less	
	IL-6R/gp80(CD126) +	ELISA/bioassay: IL-6–	IL-1β(+), IL-3+, IGF- I+,		dependent on	26,103
	gp130 (CD130)+, IGF-IR+	RT-PCR: CNTF mRNA+,	IL-10+, TNFα+, IL-2(+),		exogenous IL-6	101,102
	RT-PCR: LIFRβ mRNA+	OSM mRNA+, IL-11	IL-11+, IL-12–, IL-13(+)			
		mRNA–, LIF mRNA–	GM-CSF(+), LIF+, OSM+, IL-4(+),			
		ELISA: OSM+	CNTF–, IFNγ+, IFNα+			
KHM-1A		Northern: IL-6 mRNA+ TNFα mRNA+	TNFα+, IL-6+	anti-TNF-α ab– (growth inh.)		108
KHM-11		bioassay: IL-6+ (200 pg/ml)	IL-6+, IL-11–, GM-CSF–			34

Continued on next page

Table 4. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[d]	Proliferation response to cytokines[b]	Growth arrest/apoptosis response to cytokines[c,d]	Dependency on cytokines	Ref.
KP-6	Flow cytometry: IL-6R/ gp80(CD126)+, gp130(CD130)+, IGF-IR+	RT-PCR: IL-6mRNA+ ELISA/bioassay: IL-6+ ELISA: OSM–	3H-TdR: IL6+, IL-1α (+), IL-1β(+), IL-3+, IGF- I+, IL-10+, TNFα+, IL-2+, IL-11+, IL-12–, IL-13(+) GM-CSF(+), LIF–, OSM+, IL-4(+), CNTF–	IFNγ+ (growth inh.) IFNα+ (growth inh.) TGFβ+ (growth inh.) anti-IL-6R ab+ (growth inh. <1 yr after initiation of cell line) anti-IL-6R ab– (growth inh. >1 yr after initiation of cell line)	IL-6, later more dependent on exogenous IL-6	26,103 101,102
KPMM2	Flow cytometry: IL-6R+ RT-PCR: IL-6R mRNA+	ELISA: IL-6+ (79.7 pg/ml) PCR: IL-6 mRNA+	3H-TdR: IL-6+, IL-11–, OSM–, IL-3–, SCF–, GM-CSF–, IL-1α–, IL-2–, IL-4–, IL-5–, IL-7–, IL-8–, IL-9–, IL-10–, EPO–, TNFα–, TGFβ–	IFNα+ (growth inh.) IFNγ+ (growth inh.) anti-IL-6R ab (PM1)+ (growth inh.) anti-IL-6 ab (SK2)+ (growth inh.)		38
L363	Northern: IL-6R mRNA– Northern: IL-6R/gp130 mRNA+ RT-PCR: IL-6R mRNA+ IL-6R+ (Flow cytometry/bind. assay)	Northern: IL-6 mRNA–	IL-6– 3H-TdR: IL-6/sIL-6R+			14,107 109
LB-831	EGFR–	Immunoassay: GM-CSF–				40,8

Continued on next page

Table 4. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[d]	Proliferation response to cytokines[b]	Growth arrest/apoptosis response to cytokines[c,d]	Dependency on cytokines	Ref.
LB-832	EGFR–					40
LP-1	RT-PCR/bind. assay: IGF-IR+ RT-PCR: IL-6R mRNA+	RT-PCR/RIA: IGF-I+ RT-PCR/ELISA: IL-6–	IL-6+ (tyr phosph. of Shc) IGF-I+	anti-IGF-IR ab+ (growth inh.)		110; 105,106, Georgii-Hemming per. commun.
MEF-1	RT-PCR: IL-6R mRNA–	RT-PCR: IL-6 mRNA+ bioassay: IL-6, 18 pg/ml/48h				44
MM5.1	RT-PCR: LIFRβ mRNA+ In subclone MM5.2: LIFRβ mRNA+		3H-TdR: OSM+, LIF–, SCF–, IL-6–, IL-10–, IL-11–, GM-CSF–, G-CSF–, IL-6/sIL-6R+		CM of BM stromal cells, later independent (subclone MM5.2)	46
MM-A1	Flow cytometry: IL-6R+	Northern: IL-6 mRNA– ELISA: IL-6–	3H-TdR: IL-6+, IL-1α–, IL-2–, IL-3–, IL-4–, IL-5–, G-CSF–, GM-CSF–		IL-6	7
MM-C1	Flow cytometry: IL-6R+	ELISA: IL-6–	3H-TdR: IL-6+, IL-1α–, IL-2–, IL-3–, IL-4–, IL-5–, G-CSF–, GM-CSF–		IL-6	7

Continued on next page

Table 4. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[d]	Proliferation response to cytokines[b]	Growth arrest/apoptosis response to cytokines[c,d]	Dependency on cytokines	Ref.
MM-M1		Northern: IL-6 mRNA– RT-PCR: IL-6 mRNA– bioassay: IL-6–	3H-TdR: IL-6+			47
MM-S1	EPO rec.+ Flow cytometry: IL-6R+	Northern: IL-6 mRNA– RT-PCR: IL-6 mRNA– ELISA: IL-6–	EPO+, 3H-TdR: IL-6+, IL-1α–, IL- 2–, IL-3–, IL-4–, G-CSF–, GM-CSF–, IL-5+	anti-IL-6 ab– (growth inh.)	IL-6 (partly dependent)	7,111 47
MM-Y1	Flow cytometry: IL-6R+	Northern: IL-6 mRNA– ELISA: IL-6–	3H-TdR: IL- 6+, IL-1α–, IL-2–, IL-3–, IL-4–, IL-5–, G-CSF–, GM-CSF–		IL-6	7
OPM-1		Northern: TGFβ1 mRNA+		Dexamethasone–, TGFβ1–, TGFβ2+ (growth inh.)		56 112
OPM-2		Northern: TGFβ1 mRNA–		Dexamethasone+ (apoptosis) TGFβ1+, TGFβ2+ (growth inh.)		56 112
OCI-My1	Northern: IL-6R mRNA+	Bioassay/ELISA: IL-6– RT-PCR: IL-6 mRNA+ ELISA: IL-1β– Northern: IL-1β–, GM-CSF–	IL-6– (colony formation)	anti-IL-6 ab– (inh. of prol.)		52

Continued on next page

Table 4. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[d]	Proliferation response to cytokines[b]	Growth arrest/apoptosis response to cytokines[c,d]	Dependency on cytokines	Ref.
OCI-My2	Northern: IL-6R mRNA+	Bioassay/ELISA: IL-6+ RT-PCR: IL-6 mRNA+ ELISA: IL-1β– Northern: IL-1β–, GM-CSF–	IL-6– (colony formation) 3H-TdR: IL-6+			52
OCI-My3	Northern: IL-6R mRNA+	Bioassay/ELISA: IL-6+ RT-PCR: IL-6 mRNA+ ELISA: IL-1β– Northern: IL-1β–, GM-CSF–	IL-6– (colony formation)	anti-IL-6 ab– (inh. of prol.)		52
OCI-My4	Northern: IL-6R mRNA+	Bioassay/ELISA: IL-6– RT-PCR: IL-6 mRNA– ELISA: IL-1β– Northern: IL-1β–, GM-CSF–	IL-6+ (colony formation) 3H-TdR: IL-6+			52
OCI-My5	Northern: IL-6R mRNA+	Bioassay/ELISA: IL-6– RT-PCR: IL-6 mRNA– ELISA: IL-1β– Northern: IL-1β–, GM-CSF–	IL-6– (colony formation)	anti-IL-6 ab– (inh. of prol.)		52
OCI-My6	Northern: IL-6R mRNA+	Bioassay/ELISA: IL-6– RT-PCR: IL-6 mRNA+ ELISA: IL-1β– Northern: IL-1β–, GM-CSF–	IL-6+ (colony formation) 3H-TdR: IL-6+			52

Continued on next page

Table 4. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[d]	Proliferation response to cytokines[b]	Growth arrest/apoptosis response to cytokines[c,d]	Dependency on cytokines	Ref.
OCI-My7	Northern: IL-6R mRNA+	Bioassay/ELISA: IL-6– RT-PCR: IL-6 mRNA+ ELISA: IL-1β– Northern: IL-1β–, GM-CSF–	IL-6+ (colony formation) 3H-TdR: IL-6+			52
OH-2	Flow cytometry: p55/p75 TNFR +		3H-TdR: TNFα+, LTα(+)	TGFβ+ (growth inh.)	IL-6 and/or TNFα	55
			IL-12–, M-CSF–, GM-CSF–, IL-1β–, IL-3–, IL-7–, IL-6+ IL-6/TNFα+	neutr. anti-TNF ab– (IL-6 ind. prolif.) neutr. anti-IL-6 ab– (TNF ind. prolif.)		
		113				
PCM6		RIA: PDGF–, EGF–, IL-1β–			IL-6	57
RPMI8226	RT-PCR: IL-6R/gp80 mRNA–	RT-PCR: IL-6 mRNA–	3H-TdR: IL-6–	TNF+		114
	IL-6R mRNA+	RT-PCR/Northern: IL-6 mRNA–	3H-TdR: GM-CSF+			66
	RT-PCR/bind. assay: IL-6R+	Northern: M-CSF mRNA+ (3.5 kb)	3H- TdR: IL-11–			25
	bind. assay: TNFR+	Northern: IL-1β mRNA–				47
	IGF-IR+	Immunoassay: GM-CSF–				115,116
	bind. assay: IL-11R–	Northern/bioassay: IL-11–				117
	RT-PCR: GM-CSFRα mRNA+,					104
	GM-CSFRβ mRNA+					8,19,118

Continued on next page

Table 4. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[d]	Proliferation response to cytokines[b]	Growth arrest/apoptosis response to cytokines[c,d]	Dependency on cytokines	Ref.
TH		IL-1–, IL-6–, TNFα–	3H-TdR: IL-1–, IL-2–, IL-4–, CD28–, IL-6+ IL-6+ (stim. Ig production) IL-6+ (increased expression of CD28 and CD38)			60
U-1958	Northern:IL-6R mRNA+, IFNγR+	Northern:IL-6 mRNA–	IL-6+, IL-1β–, IL-2–, TNFα–, GM-CSF–, IGF-I±	IFNα+ (growth inh.) IFNγ+ (growth inh.)	IL-6	14,61, 107
			IL-3–, insulin–	anti-IGF-IR ab+ (growth inh.)		105,106
U-1996	Northern: IL-6R mRNA+, IFNγR+	Northern: IL-6 mRNA–	IL-6+	IFNα– (growth inh.) IFNγ– (growth inh)		14,61, 107
U-266	Northern: IL-6R mRNA+ IFNγR+ RT-PCR:IL-6R/gp80 mRNA– IL-6R+ (binding assay)	In IL-6 indep. U-266-1984: Northern: IL-6 mRNA+ RT-PCR: IL-6 mRNA+ prot/bioassay: IL-6+	In IL-6 indep. U-266-1984: IL-6–, IL-1β–, TNFα–, IL-2– 3H-TdR:IL-6/sIL-6R– IGF-I–	In IL-6 indep. U-266-1984: anti-IL-6R ab(PM1)+, IFNα+ IFNγ– (growth inh) anti-IGF-IR ab+ (growth inh)	IL-6 (U-266-1970) later indep. of IL-6 (U-266-1984)	8,19,25, 47,61, 66,95, 104–107, 109,115, 116, 118–121

Continued on next page

Table 4. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[d]	Proliferation response to cytokines[b]	Growth arrest/apoptosis response to cytokines[c,d]	Dependency on cytokines	Ref.
	Northern: IL-6R/gp130 mRNA+	Northern: M-CSF mRNA+ (3.5 kb)	3H- TdR: IL-11–			
	IGF-IR+	/biol.assay M-CSF+	IL-1–, IL-2–, IL-3–,			
	bind assay: IL-11R–	RT-PCR: hrIL-17 mRNA+	IL-4–, IL-5–, IL-7–,			
	Northern: IL-11R–	Northern: IL-1β mRNA–	GM-CSF–			
		Northern/bioassay: IL-11–				
		In IL-6 dep. U-266-1970:	In IL-6 dep. U-266-1970:	In IL-6 dep. U-266- 1970:		
		Northern: IL-6 mRNA+	IL-6+	anti-IL-6R ab(PM1)+, IFNα+,		
		prot/bioassay: IL-6–	3H-TdR: IL-1β–, TNFα–	IFNγ+ (growth inh)		
			IGF-I–	anti-IGF-IR ab+ (growth inh)		
UTMC-2	Flow cytometry: IL-6R+	ELISA: IL-6–	IL-6+, IL-1β–, IL-4–,	IL-6 antisense+ (growth inh)		65
		RT-PCR: IL-6 mRNA+,	TNFα–, TNFβ–			
		TNFα mRNA–, IL-1β mRNA–				
XG-1	Flow cytometry:	Northern/bioassay: IL-6–	IL-6/GM-CSF+,	IL-6 antagonist+ (growth inh.,	IL-6	9,122
	IL-6R(gp80)+ (gp130)+	RT-PCR/ELISA: OM +,	3H-TdR: CNTF–, LIF–,	apoptosis)		123
	RT-PCR/Flow cytom.:	IL-6+, LIF–,	IL-11–,			
	LIFRβ–,	IL-11–, IL-10–	IL-6/IL-3+, OM–			124,125
	IL-10R+, IL-11R–,	Northern: IL-1β–	IFNα+			126
	CNTFRα–	Immunoassay: GM-CSF–				104
						8,127
						128

Continued on next page

Table 4. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[d]	Proliferation response to cytokines[b]	Growth arrest/apoptosis response to cytokines[c,d]	Dependency on cytokines	Ref.
XG-2	Flow cytom: IL-6R(gp80)+ (gp130)+ RT-PCR/Flow cytom.: LIFRβ–, IL-10R+, IL-11R–, CNTFRα–	Northern/bioassay: IL-6– RT-PCR/ELISA: OM+, IL-6–, LIF–, IL-11–, IL-10– Northern: IL-1β–	IL-6/GM- CSF+ 3H-TdR: CNTF–, LIF–, IL-11–, IL-6/IL-3+, OM– IFNα+		IL-6	9,122 8 126 104 127,128 124,125
XG-3P	Flow cytom: IL-6R(gp80)+ (gp130)+	Northern/bioassay: IL-6– Northern: IL-1β mRNA–	IL-6/GM-CSF+ IL-6/IL-3+		IL-6	9,122 104
XG-3E	Flow cytom: IL-6R(gp80)+ (gp130)+	Northern/bioassay: IL-6– Northern: IL-1β mRNA–	IL-6/GM-CSF+ IL-6/IL-3+			9,122 104
XG-4[e]	Flow cytom: IL-6R(gp80)+ (gp130)+ RT-PCR/Flow cytom: LIFRβ+, IL-10R+, IL-11R+, CNTFRα–	Northern/bioassay: IL-6– RT-PCR/ELISA: OM –, IL-6±, LIF–, IL-11–, IL-10+ Northern: IL-1β–	IL-6/GM-CSF+ 3H-TdR: CNTF+, LIF+, IL-11+, OM+ IFNα+		IL-6	9,122 124,125 126 104 127,128
XG-5	Flow cytom: IL-6R(gp80)+ (gp130)+	Northern/bioassay: IL-6–	IL-6/GM-CSF+		IL-6	9,122

Continued on next page

Table 4. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[d]	Proliferation response to cytokines[b]	Growth arrest/apoptosis response to cytokines[c,d]	Dependency on cytokines	Ref.
	RT-PCR: CNTFRα mRNA–	Northern: IL-1β–	IFNα+			126
			IL-6/IL-3+			104,125
XG-6	Flow cytom: IL-6R(gp80)+ (gp130)+ RT-PCR/Flow cytom: LIFRβ+, IL-10R+, IL-11R+, CNTFRα–	Northern/bioassay: IL-6– RT-PCR/ELISA: OM –, IL-6–, LIF–, IL-11–, IL-10+	IL-6/GM-CSF+ IL-6/IL-3+ 3H-TdR: CNTF–, LIF+, IL-11+, OM+		IL-6	9,122 127,128 124,125
XG-7	Flow cytom: IL-6R(gp80)+ (gp130)+ RT-PCR: CNTFRα mRNA+	Northern/bioassay: IL-6– RT-PCR/ELISA: OM+, IL-6±, LIF–, IL-11–, IL-10+	IL-6/GM- CSF+ IL-6/IL-3+		IL-6 independent	9,122 127,128 124,125
XG-8	Flow cytom: IL-6R(gp80)+ (gp130)+ RT-PCR: CNTFRα mRNA+	Northern/bioassay: IL-6–	IL-6/GM-CSF+ IL-6/IL-3+			9,122 125
XG-9	Flow cytom:IL-6R(gp80)+ (gp130)+ RT-PCR: CNTFRα mRNA+	Northern/bioassay: IL-6–	IL-6/GM-CSF+ IL-6/IL-3+			9,122 125

[a] Receptor expression at mRNA (RT-PCR , Northern) or at protein level; binding assay, or flow cytometry analysis. + = expression, – = no expression.

[b] Effects of cytokines on proliferation or growth as measured by thymidine incorporation (3H-TdR) or cell counting. + = induction of proliferation/growth, – = no effect.

[c] Effects of cytokines on growth inhibition or apoptosis. + = growth inhibitory or apoptosis inducing effect, (–); no effect.

[d] PTHrP; parathyroid hormone-related protein, MDF; Macrophage derived conditioned media (CM), OSM/OM; oncostatin M.

[e] Cell line responded by 3H-TdR incorporation to IL-6, LIF, OM and was by RT-PCR suggested to express CNTFRα [125,128].

to be classifiable into three different categories: (1) those dependent on IL-6 only, (2) those responding to some other member of the family of IL-6 type cytokines (OM, LIF), and (3) those independent of an IL-6 type cytokine [14,26,103,122,132].

The IL-6 unrelated growth factor IL-10 can induce proliferation in MM cells [127]. IL-10 has been suggested to induce proliferation via the up-regulation of IL-11R expression and the IL-11 signal transduction pathway [124,127,128]. Also, the expression of LIFRβ has been suggested to be induced by IL-10. Thus, relating back to the classification of MM cell lines, exogenous IL-10 will induce proliferation in MM cell lines dependent on IL-6 only by inducing functional LIFRβ expression. Furthermore, an autocrine IL-10 loop may be part of the growth regulation of MM cell lines stimulated by the IL-6 type family of cytokines [124]. IL-10 was produced by some MM cell lines (XG-4, XG-6, XG-7) but not in others (XG-1, XG-2). This finding was supported by the presence of both autocrine IL-6 and an autocrine OM loop in one cell line (XG-7) growing autonomously without the addition of exogenous IL-6. As IL-10 and/or OM is frequently produced in freshly isolated MM cells, these cell lines seem to be representative of MM cells in vivo. The primary cells were therefore suggested to be responsive to OM by upregulating LIFR expression in response to IL-10. However, the relevance of these findings to growth in vivo is not known [92,103,124].

Insulin-like growth factors (IGFs) produced by bone marrow stromal cells also influence growth and survival in MM. IGF-I has an anti-apoptotic effect on MM cells, via PI3-K activation of PKB/Akt and subsequent phosphorylation of the *Bcl-2* related Bad protein. Bad is thereby sequestered and cannot initiate apoptosis [133,134]. IGF-I may improve the survival of CD138 (syndecan-l/B-B4) positive MM cells in vitro, and a recent report suggests that IGF-I is an important chemoattractant in MM [105,135]. IGF-I was reported to be an important growth and/or survival factor in MM cell lines, possibly acting in some cell lines by an autocrine mechanism [105]. All the cell lines examined express functional IGF-IR (LP1, Karpas 707, EJM, HL407 (early), HL407 (late), U266-1970 and U-266-1984) and IGF-ImRNA and protein (LP-1, Karpas 707, EJM) [105,106] (Table 4). IGF-IR was also found to be expressed on U-266 and RPMI 8226 [115,116]. Using IL-6 dependent MM cell lines (DP-6, ANBL-6, KAS-6/1 and KP-6), IGF-I was also demonstrated to potentiate the IL-6 stimulated proliferative response, perhaps via autocrine IGF-I production [102].

Several other cytokines stimulating the growth of MM cells have been identified. In contrast, no consistent pattern of MM cell responsiveness to cytokines has been demonstrated. Together, such cytokines (G-CSF, GM-CSF, IL-5, TNFα TGFβ) may act as indirect MM cell growth factors either by inducing IL-6 production or IL-6R expression (IL-3) [92,130,136]. In line with

the need for feeder cells for growth and survival, cytokine production by MM of IL-1α/IL-1β, TNFα/β and TGFβ has also been suggested to stimulate paracrine IL-6 production by bone marrow stromal cells [8,137,138].

GM-CSF was added to IL-6 to establish the XG (1-9) cell lines [8,9]. Increased DNA synthesis was reported in RPMI 8226 in response to GM-CSF [117]. However, using immunoassays, GM-CSF was not expressed in the MM cell lines (RPMI 8226, U-266, JJN-3, EJM, LB-831, XG-1) [8] (Table 4). A truncated form of monocyte-macrophage (M)-CSF that is functionally active has been identified in malignant plasma cells and in cell lines (RPMI 8226 and U-266) [66]. A synergistic growth promoting effect of IL-3 with IL-6 was demonstrated in several cell lines [9,139,140]. In some IL-6 dependent cell lines TNFα induces proliferation (DP-6, KAS- 6/1, KP-6) and in one case a TNFα dependent MM cell line has been described (OH-2) [55].

IL-1 is considered important in MM as it plays a role in osteoblast activation (OAF). More importantly, IL-1β may control IL-6 production and the expression of adhesion molecules of stromal cells implicated in homing [104]. Some controversy exists concerning the synthesis of IL-1 by MM. IL-1 production was not detected in CD138 (syndecan-1) positive cells, while the expression of IL-1 and IL-6 was found in the CD 138 negative population of non-myeloma cells [141]. However, using in situ analysis, weak expression was observed of IL-1β and of both IL-1α and IL-1β in 10/31 and 22/31 samples of freshly isolated MM cells [104]. In cell lines, IL-1β transcript was not found in nine MM cell lines (including XG-1, XG-2, XG-3, XG-4, XG-5, EJM, JJN-3, RPMI 8226, U-266) (Table 4).

The use of IFNα in patients is controversial [130,142]. The heterogenous response of MM cells to IFNα in vitro may reflect the variable response in vivo. Some in vitro studies have shown a growth stimulatory effect of IFNα on primary MM cells and MM cell lines [126,143], but growth inhibition and cytotoxicity has also been described [144,145]. This effect seems to be dose dependent. Low concentrations of IFNα are stimulatory and high concentrations are inhibitory [144]. In the MM cell lines U-1958 and U-266-1970, a growth inhibitory effect of IFNα was seen at both low and high concentrations, while U-1996 was refractory to IFNα [107]. In U-266, the growth inhibition by IFNα was suggested to be due to the loss of surface IL-6R and a downregulation of gp130 disrupting the autocrine loop [121]. The IL-6 dependent cell lines KP-6 and ANBL-6 are also growth-inhibited by IFNα [101]. IFNα stimulated proliferation of 4/5 established cell lines (XG-1, XG-2, XG-4, XG-5) initially dependent on IL-6 for growth [126]. In these cell lines IFNα stimulation was suggested to lead to autonomous growth by autocrine IL-6 production [126]. The stimulatory effect of IFNα has been confirmed in another cell line, KP-6, that is growth stimulated by IFNα [26,101]. However, the mechanism of growth stimulation has been

challenged, as upregulation of IL-6 or IL-6 R was not considered to be a pathway for IFNα stimulated growth [101].

IFNγ generally inhibits growth in primary cells derived from MM patients and in IL-6 dependent MM cell lines [107, 146, 147].

5. GENETIC CHARACTERIZATION

Cytogenetic studies show aneuploidy in MM. Extensive structural and numerical chromosomal alterations have been observed, including loss of chromosomes and chromosome fragments in 30–50% of the cases and the presence of marker chromosomes [148–150]. Fluorescence in situ hybridization has identified cytogenetic abnormalities in 80–90% of bone marrow cells from patients with MM [151,152].

The hallmark genetic lesion in many B cell tumors is a translocation, often involving a proto-oncogene, to the Ig heavy chain (IgH) locus (14q32), or less frequently involving one of the IgL loci [91]. A 14q32 translocation and/or illegitimate switch recombination fragments (ISRF) were seen in 19/21 cell lines analyzed, indicating that the translocation to the IgH locus is an almost universal event in MM cell lines [153].

Compared to other B cell tumors, MM is genetically very heterogenous. This heterogeneity is obvious both between and within MM clones and is reflected by the diversity of chromosomal partners to the IgH locus, including 1p13, 6p25, 8q24, 12q24, 16q23, 21q22, 11q23, 1q21, 3p11, 6p21, 7q11 and 18q21, as deduced from karyotypes of tumors and cell lines [91,153]. The translocation breakpoints cloned from eight MM lines were demonstrated to involve six different loci; 4p16 (JIM-3, KMS-11), 6 (SK-MM-1), 8q24 (KMM-1), 11q13 (SK-MM-2, KMS-12, U- 266), 16q23 (JJN-3), and 21q22 (KMM-1) (Table 5) [153,154].

The IgH translocation most frequently involves 11q13 (*cyclin D1*), 4p16 (*FGFR3*) and 16q23 (*c-maf*). In the established MM cell lines these three loci are involved in about 25% of the lines and are associated with ectopic expression of cyclin D1, fibroblast growth factor receptor 3 (FGFR3) gene and the *c-maf* oncogene, respectively. In 2/3 MM cell lines overexpressing cyclin D1, there is a translocation t(11;14)(ql3;q32). The expression of cyclin D1 mRNA was found in MM-M1, KMS-12 and SK-MM-2. However, in the MM-M1 cell line the candidate translocation breakpoint 11ql3 into the switch region of IgH loci was not identified [156]. FLAM-76 and U-266 overexpress cyclin D1 mRNA, and in U-266 the translocation involves the switch region of IgH loci [153]. The overexpression of cyclin D1/PRAD1 gene and an amplification of the cyclin Dl gene in the absence of t(l 1;14) was demonstrated in the KHM-11 cell line, while it was absent in cells of

Table 5. Multiple myeloma cell lines: genetic characterization

Cell line	Cytogenetic karyotype	Unique translocations, translocations involving 14q32 and/or ISRF[a]	Unique gene alterations abberant gene expression[b]	Ref.
ACB-885	46XY, –1, –2, –6, –7, –8, –10, –12, –13, +21, –22, +8mar t(1;10)(1qter-1q22::10p11.2-10qter), t(2;10)(2qter-–2p11.2::10p11.2-10pter), t(1;9)(1qter-1q11::9p13-9p22), t(2;8)(2pter-2p11.2::8q24-8pter), t(1;9)(1pter-1p32::9p21-9q22), t(3;12)(p?;q24)		elevated c-*myc* mRNA (no RFLP)	20 155
ACB-1085	45XY, –1, –2, –6, –7, –8, –10, –12, –13, +21, – 22, +8mar, no discernable difference from ACB-885		elevated c-*myc* mRNA (no RFLP)	20 155
AD3	109, XXY, –Y, –1, –1, –4, +7, –8, –8, –8, –9, –9, –9, –10, –11, +12 –12, –13, –13, –14, –14, –14, –15, –16, +19, +20, +20, +22, +22, del(1)(q21), del(1)(q21), +der(1)t(1;7)(q11;p11.2), +der(1)t(1;7)(q11;p11.2), +der(1)t(?;1)(1;?) (?p32q22;?), +der(1)t(?;1)(1;?)(?;p32q22;?), der(2) t(2;?)(q23;?), del(5)(q11.2q35), der(5)t(5;?)(q11.2;?), +der(5)t(5;?)(q11.2;?), i(6q), der(6)t(6;?)(q21;?), der(8) dic(1;8)(8pter-8q24.1::1p13-1q42:), +der(8)dic(1;8) (8pter-8q24.1::1p13-1q42:), +der(8)t(1;8)(q25;q24), +der(8)t(8;?)(q24;?), +der(9)t(9;?)(p22;?), der(11) t(11;?)(q21;?), +der(13)t(1;13;?)(13qter-13p11::?:: 1p11-1q42:), +der(13)t(13;?)(p11;?), +der(14)t(8;14) q24.1;q32), +der(14)t(8;14)(q24.1;q32), +der(14)t(8;14) (q24.1;q32), +der(15)t(9;15)(q13;p11), +der(15)(t(9;15) (q13;p11), i(21q), +7mar	14q+, 8q+	germline c-*myc*	21

Continued on next page

Table 5. (continued)

Cell line	Cytogenetic karyotype	Unique translocations, translocations involving 14q32 and/or ISRF[a]	Unique gene alterations abberant gene expression[b]	Ref.
AMO1	pseudodiploid 2p+, 8q+, 10q+, 12q+, 12p–, 14q+, 15p+			22
ANBL-6	near diploid population 44, X-X, –1, t(3;9)(p21;q12), der(6) t(1;6)(q11;q13), der(?8)t(8;?)(?p11.1;?), –9, der(10) t(9;10)(q13;q26), –13, –14x2, der(15)t(15;?)(q1?5;?), –17, –22.+7mar[cp3], near tetraploid population 87-88, XX, –Xx2, –1x2, –5, der(6) t(1;6)(q11;q13)x2, der(?8)t(8;?)(?p11.1;?)x2, –9x2, der(10)t(9;10)(q13;q26)x2, –13x2, –14x3, –15, der(15) t(15;?)(q1?5;?), –17, x2, –22x2, +15-17mar[cp4]	ISRF/translocation into IgH switch region involving 16q23	c-*maf* mRNA overexpression	23 153 170
delta-47	46, X, –Y, –1, +der(1)t(1;?)(p34;?), –2, +der(2)t(2;?)(p23;?), –8, –10, –11, –11, –12, –14, +der(14)+t(14;?)(q34;?), +6mar	14q+, no translocation into IgH switch region		24 153
DOBIL-6	47, X, –X, der(1)t(1;3)(p36.1;p25)t(1;22)(q21;q13.1), der(2)t(2;10)(p11.2;p11.2)del(2)(q32.2q35), inv(3)(q25 q29), add(3)(p25), add(6)(p21.3), +9, der(10)t(2;10)(p11.2; p11.2), der(11)t(6;11)(p21.3;q13.1), t(12;16)(p11.2; q12)der(14)t(11;14)(q13;q32.1), der(17)t(14;17)(q24; q21), i(17)(q10), +18, add(19)(p13.3), der(19)t(17;19)(q21; q13.3), del(20)(q11.2q13.1), der(22)t(1;22)(q21;q13.1)	t(11;14)(q13;q32)	PRAD1-IgH (cyclin D1) mRNA not overexpressed	25
EJM	48X, +1, del(3q), –4, +5, del(6)(q21), +9p, +9p, +11p, +11p, +12q, –13, –13, –14, iso(14q), –15, –15, –16, –16, –18, +18p, +20, –21, –22, +7mar		*p53*mut (ex5) LOH expression of *bcl*-2+	27

Continued on next page

Table 5. (continued)

Cell line	Cytogenetic karyotype	Unique translocations, translocations involving 14q32 and/or ISRF[a]	Unique gene alterations abberant gene expression[b]	Ref.
FLAM-76	43, X, Y, –8, –13, –14, –21, –22, t(11;14)/q13;q32), +2mar	t(11;14)(q13;q32), 14q+, 8q+	cyclin D1mRNA	28
		11q13, no translocation into IgH switch region	overexpression, selective expression of one	153
			c-*myc* allele	157
FR4	67, X, –Y, +3, +7, +9, +11, +11, +12, –13, –13, –14, –15, +16, +17, +18, +19, +20, +22, +22, del(1)(q21), del(1)(q21), +der(1)t(1;7)(q11;p11.2), der(1)t(?;1)(1;?)(?;p32q22;?), del(5)(q22q35), +i(6q), der(8)dic(1;8)(p13;q24.1), +der(8)t(8;?)(q24;?), der(13)t(1;13)(q11;p11), +der(13) t(1;13)(q11;p11), der(14)t(8;14)(q24.1;q32), der(14) t(8;14)(q24.1;q32), der(15)t(9;15)(q13;p11), i(21q), 3mar		germline c-*myc*	21
JIM-1	hypotetraploid karyotype	no detected t(14;18)	expression of *bcl*-2–	30
JIM-2			expression of *bcl*-2(+)	30
JIM-3		14q+, translocation into IgH switch region involving 4p16.3	expression of *bcl*-2+ FGFR3 mRNA–	30 153,156
JIM-4			expression of *bcl*-2–	30
JJN-1	40XX, –7, +der(7)t(7;11)(q32;q13), –9, –10, –11, –12, –13, –14, –16, –20, –20, +mar, del(6)(q25), del(8)(p21), 14q+			4
JJN-2	75XXX, del(Xq), –1, 1q+, Iq–+, –2, –3, del(3)(q23), 5p+, 5p+ del(6)(q15), del(6)(q15), –7, –7, +der(7)t(7;11)(q32;q12), del(8)(p21), del(8)(p21), –9, –9, –10, –10, –11, –11, –12, –12, –13, –13, –14, 14, +14q+, +14q+, –15, –16, –16, –17, –20, del(20)(p11), +4mar			4

Continued on next page

Table 5. (continued)

Cell line	Cytogenetic karyotype	Unique translocations, translocations involving 14q32 and/or ISRF[a]	Unique gene alterations abberant gene expression[b]	Ref.
JJN-3		14q+, translocation into IgH switch region involving	*p53*wt	4
		16q23.1	selective expression of one c-*myc* allele	157 153
			c-*maf* mRNA overexpression	170
			expression of *bcl*-2+	158
Karpas 620	76X, +7 mar der from chr 1, 8, 11, 13, 14, 17 t(1;14)(q11;q32.3), t(1;17)(q11;p13.1), t(1;11) (q32.1;q13.3), t(8;11)(q24.22;q13.3), t(8;14) (q24.1;q32.3), t(11;13)(q13.3;q14.3)+ numerical alter. incl. chr 2, 3, 4, 5, 6, 9, 12, 22 and 7, 15, 16, 18, 19, 20, 21			31
Karpas 707	hypodiploid 45 Ph+, monosomy 6, 12 and 16, 4p+, +6p, monosomy 5, 13, 17 and trisomy 9, +2mar, 45XY, Ph+, 4p+, +6p, –6, –12, –16, t(6q;7q)	Philadelphia chr Ph+ (q1.1)	germline c-*myc*	32
			germline *bcl*-2	120
			expression of *bcl*- 2+	159
KHM-11	81-95 add (X)(q22), add(X)(q12), der(1)t(1;19)(p11;q11) add (1)(q21)x2, der(1)t(1;8)(q10;q10)X2, t(2;5)(q35;q11) x2, dup(3)(q21;p25), i(11)(q10), der(15)t(15;21)(q10, q10) x2, mar1 and mar2	no detectable t(11;14) or t(11;22)	cyclin D1/PRAD amplification and overexpression	160 152
KMM-1	47, X, Y, 1q+, –2, +t(1;2)(cen;cen), +7, 12q+, 14q+, +mar	translocation into IgH switch region involving	c-*myc* mRNA overexpression	35
		21q22.1 and 8q24.13		161 153

Continued on next page

Table 5. (continued)

Cell line	Cytogenetic karyotype	Unique translocations, translocations involving 14q32 and/or ISRF[a]	Unique gene alterations abberant gene expression[b]	Ref.
KMS-5	Triploid, no karyotype available		c-*myc* mRNA overexpression	161
KMS-11	Triploid, no karyotype available	missense mut in codon 373 of FGFR3	c-*maf* overexpression	37
		translocation into IgH switch region involving 4p16.3 and 16q23	selective expression of one c-*myc* allele	161 153,170
			c-*myc* mRNA overexpression	156
			FGFR3 mRNA overexpression	157,168
KMS-12-PE	Hypodiploid 41, no karyotype available	t(11;14)(q13;q32) t(11;9)(q13;q34)	cyclin D1mRNA overexpression	153
		11q13, translocation into IgH switch region involving 11q13 and 14	elevated c-*myc* mRNA	161
KMS-12-BM	Hypodiploid 41, no karyotype available	t(11;14)(q13;q32) t(11;9)(q13;q34)	elevated c-*myc* mRNA	161
KMS-18	42, add(1)(q32), add(10)(q24), add(17)(p11)	t(4;14)(p16.3;q32.3)		37
KPMM2	46, XX, der(1;19)(q10;q10), t(3;14)(q21;q32)-4, t(6;11)(p12;p15), der(10)add(10)(p13)dic(9;10)(q10;q26), +16	t(3;14)(q21;q32)(3q21/CDCL1/BM28)		38
L363	49, X, +8M, –5, –6, +7, –8, –8, 14q+, –22		germline c-*myc*	39
			germline *bcl*-2	120
			expression of *bcl*-2+	159

Continued on next page

Table 5. (continued)

Cell line	Cytogenetic karyotype	Unique translocations, translocations involving 14q32 and/or ISRF[a]	Unique gene alterations abberant gene expression[b]	Ref.
LB-831	t(1;?)(q32;?), t(1;15)(p11;p11), del(3)(p21;p25), t(5;?)(q35;?), del(7)(p15:), t(7;?)(p22;?), ?HSR 7(p22), i(8q), inv(11)(p11;q13), t(13;13)(p12;p12), +3, +4, +4, +5, +6, +8, –9, +10, +11, +14, –16, –17, +18, +19, +20, –22		*p53*mut(ex5)	40 158
LB-832	t(1;?)(q32;?), t(1;15)(p11;p11), del(3)(p21;p25), t(5;?)(q35;?), del(7)(p15:), t(7;?)(p22;?), ?HSR 7(p22), i(8q), inv(11)(p11;q13), t(13;13)(p12;p12), +3, +4, +4, +5, +6, +8, –9, +10, +11, +14, –16, –17, +18, +19, +20, –22			40
LB 84-1	Hyperdiploid -X, +2, –4, –14, +17, +18, +20, +21, +22, del(1)(p36), t(2;?)(37;?), del(3)(q22), dup(3)(q26;q29), dup(3), del(5)(p14), t(5;?)(q35), del(6)(q15), del(6)(q21), del(7)(q31)		germline c-*myc*(ex 1)	79
LOPRA-1	3n+–, 70, XX, –X, –1, –4, –6, –8, –8, –13, –16, +7, +18, +21, +i(1q), +i(1q), +6q–, +3mar			41
LP-1	73, XX, dup(1)(p13;p23), +3, +3, inv4(p12;p16), –4, der5 t(5;?)(q31;?), +5, del(6)(q23), +del(6)(q23), +7, +7, +8, +8, der(9)t(9;12)(q34;q22), +9, +10, +10, +11, der(12)t(12;13)(p13;q11), der(12), –13, –13, +15, +15, +16, +17, +17, +18, +19, +20, +21, +21, +22, +22, +3mar	t(4;14)	altered expr. of c-*myc* /3.2kb c- *myc* transcr. (enz polymorph/RFLP) c-*maf* mRNA expression(+)	42 170
MEF-1	50, X, –X, +der(3)add(3)(p22)add(3)(q13), t(4;6)(q21;p23), +5, add(7)(q36), +8, t(11;14)(q13, q32), +der(14)t(11;14)(q13;q32), der(19)dic(1:19)(p13;p13.3)	t(11;14)	*p53* mut (ex7) cyclin D1 overexpression germline c-*myc*	44

Continued on next page

Table 5. (continued)

Cell line	Cytogenetic karyotype	Unique translocations, translocations involving 14q32 and/or ISRF[a]	Unique gene alterations abberant gene expression[b]	Ref.
MM.1	44, XX, –8, –13, –14, –16, –21, del(1)(p13p22), t(2;?)(q37;?), t(3;?)(p25;?), t(6;?)(q22;?), t(12;14)(q24.3;q32.3), +der(8)t(8;13) (q21;q22), +der(16)t(8;16)(p21.1;q12), +der(21)t(1;21) (q12;p13), t(2;?)(q37;?), del(8) (8pter–8q11;13q31–13qter), clonal additional markers	t(12;14)(q24.3;q32.3)	c-*maf* mRNA overexpression	45
		12q24, translocation into IgH switch region involving		53
		16q23		170
MM-M1	no available karyotype	11q13, no translocation into IgH switch region	cyclin D1 mRNA overexpression	153
			selective expression of one c-*myc* allele	157
MT3	?74, 1q–, 2q+, 3q–, 4q+, 13q–, 14q+, 22q–, mar1, mar2, +DM, –2, –5, –14, –15, +1, +3, +5, +6, +7, +8, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, +21, +22	t(?;22)(?;q1.1-q1.3)	germline c-*myc*	49
NCI-H929	90, XX 8q+ dup(1)(q11-25), t(10q, 12p), del(12p), +1mar	translocation into IgH switch region involving 4p16	germline/rearranged c-*myc*	50
			(no enz polymorph/RFLP)	153
			c-*maf* mRNA expression	168
			FGFR3 mRNA expression	
NOP-2	47(47, X, –Y, *inv dup* del(1)(p13-q21 q21), +6, +7, t(8;22) (q24;q11), t(11;14)(q13;q32), –15, +der(15)t(15;1) (p11;p22)	t(11;14)(q13;q32)		51

Continued on next page

Table 5. (continued)

Cell line	Cytogenetic karyotype	Unique translocations, translocations involving 14q32 and/or ISRF[a]	Unique gene alterations abberant gene expression[b]	Ref.
OCI-My5	no karyotype published	t(14;16), no ISRF/Ig translocation to IgH switch region	*p16* wt (PCR), *p16* protein–	68
			MDM2 protein overexpression	162
			c-*maf* overexpression	153
				170
OPM-1	76, 79 +del(1)(p32), +del(1)(p32), +der(3)t(1;3)(p22;p21), +der(3)t(3;7)(p13;p15), t(4;14)(p16;q13), +del(7)(p15), +der(8)t(1;8)(q12;q22), der(8)t(1;8)(q12;q22), +del(14)(q13), +der(22)t(1;22)(q12;p13)		germline c-*myc*	56
			germline *bcl*-2	120
				159
OPM-2	65~75 +del(1)(p13), +del(1)(p13), +del(1)(q31), +der(1)t(1;2)(p13;p13), +del(5)(q13), +del(10)(q22), +del(10)(q22), +del(10)(q22), t(1;22)(q12;p13), der(22)t(1;22)(q12;p13)	translocation into Ig switch region involving 4p16.3 missense mutations of FGFR3	germline c-*myc*	56
			germline *bcl*-2	120
			selective expression of one c-*myc* allele	157
				168
			FGFR3 mRNA overexpression	159
PCM6	45X, –X, 1p+, 1p–, +1q–, t(2;8)(q23;p23), 3p–, 6q–, 12q–, 14q+, –16, –17, +mar			57
RPMI 8226	58-67, t(1;14)(p13:q32), del(2)(q35), t(3;?)(q29), t(5;6)(p13;p12), del(6)(q15), del(6)(q11), t(9;?)(p24;?), del(11)(q23), t(11;?)(p11;?), del(11)(p11), t(17;?)(q25;?), HSR(21)(q22)	translocation into IgH switch region + (unidentified partner) 1p13, variant translocation t(16;22)	*p53*mut (ex8) LOH	2
			*p16*wt(PCR), *p16* protein–	163
			c-*maf* mRNA overexpression	58
			K-*ras* mutation codon12	162
			MDM2 protein overexpression	153
				170
				158
				164

Continued on next page

Table 5. (continued)

Cell line	Cytogenetic karyotype	Unique translocations, translocations involving 14q32 and/or ISRF[a]	Unique gene alterations abberant gene expression[b]	Ref.
SK-MM-1	33-45X, –X, 1p–, +2, t(4;19), 6p–, t(8q;10q), –9, –10, t(11q; 15q), +11p, t(13p;1q), 14q+, 14p+, –15, –18, –19, –20, +22	14q+, translocation into IgH switch region involving 6 and 6p25	germline c-*myc* IRF4 mRNA overexpression	59 153 165
SK-MM-2	69-84 +13-15mar, +1, +1, 1p–, 1p–, +2, 2q+, 2q+, +3, +5, +6, +6, +6, (+6), 6q–, 6q–, (6q-), +9, +10, +11, +12, 14q+, –14, +15, –17, +19, +20, +21	11q13, translocation into IgH switch region involving 11q13.3	germline c-*myc* cyclin D1 mRNA overexpression	59 153
TH	69-75 XX der(1)(t(1;9)(1qter-1p11::9p21-qter), del(2)(p24) x2, der(5)t(5;18)(p15;q23), inv(8)(p23q13), der(10), t(10;?)(q26;?), der(13)t(13;21)(p11;p11)x2, 14q+, +14q+, 2-4 struct abn chr 18 with breakpoints in 18q21 and 18q23		germline *bcl*-2	60
U-1957	46, del(1)(p22), t(1;16)(q24;q24), t(6;13)(q21;q14), 10p+, del(10)(p12), 14q+, +7			166
U-1958			germline c-*myc* germline *bcl*-2 expression of *bcl*-2+	120 159
U-1996	82, 2xint del(1)(p22-p34), int del(2)(q21-q31), t(3;12), 2x4p+, t(6;?), del(6)(q21), del(8)(p21), del(12)(q24.1), t(13;?), 14q+, 2x14q+, 16q+, 14?, der(16)?		germline c-*myc* germline *bcl*-2 expression of *bcl*-2+	166 120 159
U-2030	aneuploid 60; t(1;12)(q21;p13)x2, t(1;?)(p34;?), del (1)(q11), 1q (cen-ter), int del (3)(q21-q25), t(5;16)(q11;q22), iso (6q), 8p-, t(9;?)(p22;?), 10p+, iso(11q), del(11)(q21), 16q+, 19p+, +12mar			62,166
U266Bl	43-44, 1p+(1qter–p34::?), t(2;?)(2qter–p25::?), 3q+,	translocation into IgH switch region involving 11q13.3	*p53*mut (ex5) LOH	166

Continued on next page

Table 5. (continued)

Cell line	Cytogenetic karyotype	Unique translocations, translocations involving 14q32 and/or ISRF[a]	Unique gene alterations abberant gene expression[b]	Ref.
(U-266)	3q–, 4p–, del(6)q23), 7q+(q36), del(8)(p23), 9q+(9pter– q34 ::?), iso(10q), 11q+(11pter–q22::?), 12p+, del(14)(q24), 16-like, dic		*p16*wt (PCR), *p16* prot– (meth.)	163
			germline c-*myc* (c-*myc* mRNA–)	120
			L-*myc* mRNA	153
			cyclin D1 mRNA overexpression	158
			biallelic loss of Rb1	167
			Rb1 mRNA–	159
			germline *bcl*-2 (1984)	
			expression of *bcl*-2+	
			4-fold ampl *bcl*-2 (1970)	
			overexpression of *bcl*-2+	
UCD-HL461	80-83 X, multiple abnormalities includ. abn 1, der(11), der(13) +1mar			63
UMJF-2[c]	46, XY/47XY, +12/92, XXYY			64
UTMC-2	43 t(1;5)t(2;15) no detailed karyotype	translocation into IgH switch region involving 4p16.3	FGFR3 mRNA expression	65
				153
XG-1	hypodiploid, monosomy 13, t(11;14;?)(q13;q32;?)		*p53* mut (ex5)	9
			N-*ras* mut codon12	164
				158
XG-2	hyperdiploid, t(11;14;?)(q13;q32;?)		*p53* mut(ex5)	9
			K-*ras* mut codon12	164
				158
XG-3P	hyperdiploid der14 t(14;?)(q32;?)		*p53*wt	9
				158

Continued on next page

Table 5. (continued)

Cell line	Cytogenetic karyotype	Unique translocations, translocations involving 14q32 and/or ISRF[a]	Unique gene alterations abberant gene expression[b]	Ref.
XG-3E	hyperdiploid			9
XG-4	hyperdiploid		*p53* mut(ex5)	9
				158
XG-5	hypodiploid, t(11;14)(q13;q32), t(8;14)(q24;q32)		*p53* mut(ex8)LOH	9
				158
XG-6	hypodiploid, 13q–, der14 t(11;14)(q13;q32)			9
XG-7	hypodiploid, monosomy 13	t(6;14)(q32)	IRF4 mRNA overexpression	9
		translocation breakpoint 6p25		165
XG-8	hyperdiploid, 13q–, der14 t(11;14)(q13;q32)			9
XG-9				9

[a] ISRF – illegitimate switch recombination fragment (translocation into IgH switch region involving unknown or identifiable partners). By Southern blot analysis the translocation breakpoints were identified.

[b] RFLP – restriction fragment length polymorphism; mut – mutation; ampl – gene amplification; LOH – loss of the wild type allele.

[c] EBV positive (Drexler, personal communication).

the pleural effusion from which the cell line was established [160]. The t(11;14)(ql3;q32) translocation is associated with aggressive disease and poor prognosis [91].

In four out of 21 MM cell lines the FGFR3 gene is dysregulated by t(4;14)(p16.3;q32.3), and the translocation breakpoint 4pl6 was cloned from these cell lines (KMS-11, JIM-3, NCI-H929, OPM-2) (Table 5). With the exception of JIM-3, the FGFR3 was expressed in KMS-11, NCI-H929, OPM-2 and UTMC2, the latter cell line also harboring the t(4;14). Eight cell lines (RPMI 8226, ark, SK-MM-1, delta-47, H1112, KMM-1, TH and U-266) were negative for the translocation and displayed only weak expression of FGFR3 by RT-PCR (Tables 5 and 7). In ANBL-6, FLAM-76, SK-MM-2, JIM-3, JJN-3, KMS-12, MM-M1, MM.l and OCI-My5, no FGFR3 mRNA was detected [168]. In the KHM-11 cell line, FGFR3 was translocated into the VH domain of the IgH locus and expression of the FGFR3 was confirmed [160,168]. An activating mutation of FGFR3 resulted in progression and ligand independent stimulation seems to occur frequently in MM with t(4;14). In two of the four MM cell lines examined (KMS-11, OPM-2), activation as a result of somatic mutation was found (Table 5). In the MM cell lines LP-1, UTMC-2, NCI-H929, JIM-3 and OPM-2, the IgH translocation simultaneously dysregulates two genes with oncogenic potential; FGFR3 on der(4) and Multiple Myeloma SET domain (MMSET) on der(4) [169].

The t(14;16)(q32.3:q23) was present in 5/21 MM cell lines (KMS-11, MM.l, JJN-3, ANBL-6). These cell lines also overexpress c-*maf*, as do OCI-My5 and RPMI 8226, but no candidate IgH switch translocation breakpoint fragment has been identified. FISH analysis revealed the presence of a t(14;16) in OCI-My5. In RPMI 8226, the c-*maf was* suggested to be involved in variant translocations e.g. t(16;22). Also in the NCI-H929 cell line c-*maf* mRNA was detected. Other cell lines (U-266, ark, SK-MM-1, KMM-1, OPM-2, FLAM-76, delta-47) were negative for expression of c-*maf* [170].

In a few cases the IgH translocation in MM may involve other partners, including 8q24 (c-*myc*) in less than 5% of the cases, 18q21 (*bcl*-2), 11q21-24 (mixed lineage leukemia *MLL*) and 6p21.1 (*IRF4*). The t(6;14)(p25;q32) translocation breakpoint was cloned from the SK-MM-1 cell line and was found to map to 6p25, near IRF4/(multiple myeloma oncogene MUM1)/ICSAT/LSIRF), a member of the interferon regulatory factor (IRF) family of transcription factors. This abnormality was also found in XG-7, but not in other cell lines (RPMI 8226, U-266, EJM, XG-1, XG-2, XG-4, XG-5, XG-6 (Table 5). IRF-4 mRNA was also overexpressed in these cell lines as compared to cell lines not carrying the alterations of the MUM/IRF4 [165]. However, in most of the cases the chromosome partner is not identified (14q+) [154].

This search for genetic alterations involved in the pathogenesis of MM has led to the identification of proto-oncogenes with a possible pathogenetic role. One of the candidates is c-*myc*, deregulated as a consequence of the reciprocal translocation of c-*myc* to one of the Ig loci in another human B cell tumor, Burkitts lymphoma. A similar translocation, resulting in a deregulated c-*myc,* has been implicated in the pathogenesis of rat immunocytoma and murine plasmacytoma [171]. Translocations leading to the juxtaposition of c-*myc* to one of the Ig loci are rare in MM [172-176]. In MM cell lines, only two cases (LP-1 and NCI-H929) of structural c-*myc* rearrangements have been reported [42,50,177]. In the absence of structural alterations, elevated expression of c-*myc* has been reported in freshly isolated MM cells [155,173,178,179]. In the cell lines ACB-885, ACB-985, ACB-1085, RPMI 8226 [155], elevated c-*myc* mRNA in the absence of restriction fragment length polymorphism (RFLP) and/or gene amplifications was reported (Tables 5 and 7). Also, an elevated expression of c-*myc* mRNA and protein was demonstrated in KMM-1, KMS-5, KMS-11, KMS-12PE and KMS-12BM in the absence of detectable translocations or hypomethylation [161]. In one MM cell line (KMM-l), a translocation involving the IgH locus and 8q24 was detected by Southern blotting [154]. In the absence of detectable rearrangements c-*myc* mRNA and protein are highly expressed in MM cell lines (U-1996, Karpas 707, L363 and OPM-1), compared to cell lines of other origin harboring *myc* amplification [120]. In the U-266 cell line and its IL-6 dependent subclone U-266-1970, c-*myc* mRNA was not detected, but L-*myc* mRNA and protein were seen at elevated levels.

Expression from only one c-*myc* allele was seen in 5/20 MM cell lines (JJN-3, KMS-ll, FLAM-76, OPM-2, MM-M1), suggesting a cis-deregulation (possibly due to hypomethylation) [157]. However, these cell lines lack translocation of the c-*myc* gene or changes in the encoded c-*myc* protein. Mechanisms that may deregulate c-*myc* include rearrangements affecting the PVT1 locus or rearrangements of the MLVI4 region located 20kb downstream of c-*myc* [180,181], although this was not confirmed by others [176]. Rearrangements of the MLVI4 region were not identified in RPMI 8226, U-266, JJN-3, SK-MM-1, SK-MM-2, KMM-1, OPM-2, H1112, ark, TH, MM-M1, FLAM-76, KMS-11, MM.1, delta-47, JIM-3, UTMC-2, ANBL-6, OCI-My5 or in the KMS-12 MM cell lines [157]. Altered mRNA turnover resulting in the increased half-life of c-*myc* mRNA as a result of posttranscriptional events was not identified in MM [155]. Moreover, expression of the c-*myc* protein is often elevated independently of c-*myc* mRNA levels, pointing to a deregulated translational control of c-*myc* expression [182].

Rearrangements of *bcl*-2 by translocation t(14;18) and altered expression have been implicated in the pathogenesis of non-Hodgkin's lymphoma [183].

In MM these translocations are rare [176]. *Bcl*-2 is expressed at high but variable levels in MM cell lines and primary MM cells. The JJN-3, EJM, U-266, Karpas 707 and JIM-3 were demonstrated to express elevated levels of *bcl*-2 protein in the absence of a translocation t(14;18) [184]. Levels of *bcl*-2 mRNA and protein are elevated 4-fold in the U-266-1970 subline of U-266, while other lines (U-266, U-1996, U-1958, Karpas 707, L363) express elevated levels of *bcl-2* in the absence of t(14;18) [159]. The high expression of *bcl*-2 by normal plasma cells and in MM cells in the absence of detectable translocations, suggests that this may be a marker of long-lived post-follicular cells rescued from apoptosis during germinal centre selection [159,184]. Overexpression of *bcl*-2 related genes may also result in the rescue of MM cells from apoptosis induced by dexamethasone or IL-6 deprivation and be implicated in the development of chemoresistance [185–187].

Deletion of the retinoblastoma gene Rb1 was found in more than 50% of bone marrow specimens obtained from patients with MM [188]. This is in line with the frequent occurrence of monosomy 13 in MM and deletions involving 13q24. However, nullisomy of chromosome 13 was not found in this study and is only rarely detected in MM [91]. In the U-266 cell line a biallelic loss and absence of Rb protein was reported [167]. As in the case of mutations of *ras* and p53, the Rb alterations seem to be associated with tumor progression rather than constituting an early genetic event [167].

Mutations have been identified in N- and K-*ras* oncogenes and occur in about 30% of newly diagnosed MM patients and in 70% of MM patients at relapse. Mutations in *ras* may be restricted to stage III MM and PCL, suggesting that they may be important during tumor progression [16,164,167,174,175]. A high frequency of activating mutations in codon 61 of the N-*ras* gene in MM was reported [174]. However, others found this mutation occurs in only about 25% of the activating *ras* mutations [164]. Of 10 MM cell lines (RPMI 8226, U-266, LB-831, EJM, JJN-3, XG-1, XG-2, XG-3, XG-4 and XG-5) examined, three (XG-1, XG-2 and RPMI 8226) contained a mutation in codon 12 in K- or N-*ras* [164]. The finding of Portier was confirmed in another study of U-266, EJM and LP-1 cell lines. In none of these cell lines could *ras* mutations be observed [167]. The introduction of a constitutively active N-*ras* into an IL-6 dependent MM cell line ANBL-6 (ANBL-6/Ras) resulted in significant IL-6 independent growth and reduced apoptosis suggesting that activation of *ras* oncogenes may result in growth factor independence and suppression of apoptosis [189].

Mutations of p53 are infrequent in MM (5–10% of patients), and are associated with progressive disease [158,164,190]. In established cell lines, however, the frequency of p53 mutations is 80% [158]. This is in line with previous reports, and suggests that the mutations may arise during tumor progression [191]. Among eight cell lines, five exhibited only the mutant

form, indicative of loss of wild type sequences (U-266, EJM, XG-5, RPMI 8226, LB-831). In three cell lines (XG-1, XG-2 and XG-4), both mutated and wild-type alleles were present. The expression of p53 was found in all cell lines tested and the mutations were not correlated with the expression levels of p53 or with autonomous growth [158]. In the U-266 and EJM cell lines, a p53 mutation involving exon 5 was identified, while the LP1 cell line contained wild-type p53 [167].

Overexpression of the MDM2 gene, binding and inactivating p53, has been reported in MM and in the cell lines RPMI 8226 and U-266 [162].

$p16^{INK4}$ is frequently hypermethylated in MM. This occurs more frequently in advanced disease of MM and in cell lines. Inactivation of $p16^{INK4}$ by hypermethylation may be associated with disseminating disease and development of plasma cell leukemia [192]. In MM, a high expression of $p16^{INK4}$ correlated with the loss of cyclin D1 expression and IL-6 responsiveness and was suggested to be confined to a mature phenotype in MM (CD49e+, MPC-l+) [193].

6. FUNCTIONAL CHARACTERIZATION

The population doubling time (T_{do}) of continuous MM cell lines ranges from 16 h to up to 144 days (Table 6). All MM cell lines determined to be authentic were EBV negative. Some were tested and reported to be free of mycoplasma infection (31/81). The cytochemical staining profile of plasma cell and MM cell lines is characterized by β-glucuronidase (BGLU) and α-naphthyl acetate esterase (ANAE) expression [11]. A typical profile can be described as follows; AcP+, NASDCAE-, ANAE/ANBE(+), BGLU+, while the cell lines tested also generally show weak or negative staining for Px and PAS (Table 6).

The staining of the LCLs qualitatively resembles that of normal B cells with reactivity only with acid phosphatase (AcP), naphthol-AS-D acetate esterase (NASDAE) and BGLU [11] (Table 2).

Most MM cells produce only light chains, either λ or *K* (ILKM3, JJN-2, Karpas 620, Karpas 707, KMM-1, KMS-11, KMS-18, MEF-1, MM.l, MM5.1, MM5.2, MM-S1, NOP-2, OCI-My5, OPM-1, OPM-2, RPMI 8226, SK-MM-1, SK-MM-2, U-1996). A few cell lines produce IgG (n = 17), IgA (n = 13) and in one cell line each, IgD and IgE. The secretion rate of IgG per 10^6 cells/24 h is >80 μg/ml in NCI-H929, 50 μg/ml in LP1, 40–50 μg/ml in MT3, 40 μg/ml in OH-2,23 μg/ml in LB-831 and LB-832, about 20 μg/ml in 15 μg (λ-chains) in RPMI 8226, 5–10 μg/ml in EJM, 4–9 μg/ml(λ-chains) in MM. 1, 4–9μg/ml in U-266,4 μg/ml in UTMC-2, about 3 μg/ml in KPMM2, 1.5 μg/ml in U-1958, about 1 μg/ml in SK-MM-2 cell line and < 1

Table 6. Multiple myeloma cell lines: functional characterization

Cell line	Doubling time T_{do}/generation time T_c	EBV status[a,b]	Cytochemistry	Heterotransplantability into nude mice	Special functional features	Histopathology/cell size/growth pattern in tissue culture	Mycoplasma status	Ref.
ACB-885	30–35 h	EBV–	MGP+, PAS+			ecc. nuclei, basophilic cytopl.		20
ACB-1085	30–35 h	EBV–	MGP+, PAS+			ecc. nuclei, basophilic cytopl.		20
AD3	16 h	EBNA–	AcP+, ANBE+		amylase mRNA+	blastoid nuclei, prominent nucleoli, ER, Golgi zone/ adherent islets, floating clusters		21
AMO1		EBNA–	Px–, ANAE(+), AcP+			plasmablast-plasmacyte		22
ANBL-6		EBNA–				plasmacell morph. ecc. nuclei, basophilic cytopl. bi-, multinucl. cells		23
delta-47	40 h	EBNA–				primitive plasmacytoid morph.		24
DOBIL-6	36 h	EBV–		in nude mice 5/5		basophilic cytopl., ecc. nuclei		25
DP-6		EBNA–						26
EJM	72 h	EBV–	PAS–, SBB–, nonspecific esterase–			plasma cell morph. multinucl. cells./ single cells	neg	27
FLAM-76	N D	EBNA–	AcP+, Px–, SBB– NASDCAE–, PAS–, AlkP–, ANBE (+)			ecc. nuclei, basophilic cytopl. multinucl. (5–10%) cells with eosinophilic cytopl. immature plasma cell morph.		28
FR4	131 h	EBNA–	AcP+, ANBE+		amylase mRNA+	blastoid nuclei, prominent nucleoli, ER, Golgi zone adh. paving stones floating clusters		21
GM2132 (= RPMI 8226)		EBNA–						29
HL407		EBNA–				plasma cell morph., ecc. nuclei prominent Golgi		6
ILKM2	120 h	EBNA–				plasmacytoid features		5
ILKM3	96 h	EBNA–				plasmacytoid features		5
JIM-1		EBNA–				plasmablast morph.	neg	30

Continued on next page

Table 6. (continued)

Cell line	Doubling time T_{do}/generation time T_c	EBV status[a,b]	Cytochemistry	Heterotransplantability into nude mice	Special functional features	Histopathology/cell size/growth pattern in tissue culture	Mycoplasma status	Ref.
JIM-2		EBNA–				plasmablast morph.	neg	30
JIM-3		EBNA–				plasmablast morph.	neg	30
JIM-4		EBNA–				plasmablast morph.	neg	30
JJN-1	10 d	EBNA–				plasma cell/plasmablast morph.		4
JJN-2	3–4 d	EBNA–				mono/bi nucl. cells, plasma cell/plasmablast morph.		4
JJN-3		EBV–						4, 27
Karpas 620		EBNA–	AcP+, dual esterase–, SBB–			large plasmacytoid blast cells		31
Karpas 707	48–72 h	EBNA–	AcP+, NASDCAE+			plasma cell morph. prom. nucleoli and RER, highly dev. Golgi	neg	32
KAS 6/1		EBNA–						26
KHM-1α	5 d	EBNA–				plasmablasts basophilic cytopl.		33
KHM-1B	2 d	EBNA–			amylase activity+	multinucl. plasmablasts basophilic cytopl.		33
KHM-11						plasmablast morph. basophilic cytopl.		34
KMM-1	29 h/36–40 h	EBNA–	Px–, PAS–, MGP+, NASDAE–	nude mice–	0.2% clon. eff (soft agar)	basophilic cytopl. ecc. nuclei immature plasma cell/ single cells	neg	36
KMS-5	24 h	EBNA–	Px–, PAS–, MGP+, NASDAE–	nude mice (s.c.)+	16% clon. eff (soft agar)	basophilic cytopl. ecc. nuclei/ single cells	neg	36
KMS-11	36 h	EBNA–	Px–, PAS–, MGP+, NASDAE–	nude mice–	2.3% clon. eff (soft agar)	basophilic cytopl. ecc. nuclei	neg	36
KMS-12-PE	62 h	EBNA–	Px–, PAS–, MGP+, NASDAE–	nude mice–	<0.1% clon.eff (soft agar)	basophilic cytopl. ecc. nuclei	neg	36
KMS-12-BM	56 h	EBNA–	Px–, PAS–, MGP+, NASDAE–	nude mice–	<0.1% clon.eff (soft agar)	basophilic cytopl. ecc. nuclei	neg	36
KMS-18	72 h	EBV–				plasmacytoid morph. ovoid ecc. nuclei	neg	37
KP-6		EBNA–						26

Continued on next page

Table 6. (continued)

Cell line	Doubling time T_{do}/generation time T_c	EBV status[a,b]	Cytochemistry	Heterotransplantability into nude mice	Special functional features	Histopathology/cell size/growth pattern in tissue culture	Mycoplasma status	Ref.
KPMM2	48 h	EBV–	AcP+, Px–, NASDCAE–, PAS– AlkP–, ANBE–			plasmablast/plasma cell morph.	neg	38
L363	65–75 h	EBNA– EA– VCA–	PAS–, Px–, ANAE– AcP+, BGLU+	in nude mice 0/18		large multinucl. cells/ 9–15 μm/ single cells		39
LB-831	T_c 38.37 h	EBNA(–) (<5%EBNA+) EBV–	AcP+, BGLU+		clonable in plate assay	plasma cell morph. multinucl. cells size heterogeneity	neg	40 91
LB-832	T_c 26.4 h	EBNA(–) (<5%EBNA+) EBV–	AcP+, BGLU(+)		clonable in plate assay	plasma cell morph. bi-, multinucl. cells	neg	40 51
LB 84-1	T_c 34.2 h	EBV–	ANBE+, NASDCAE+			plasma cell morph. multinucl. cells double membr. bound mitochondr.		79
LOPRA-1	30 h	EBNA–	AcP+, BGLU+, PAS(+), unspec esterase(+), Px–, AlkP–		clonable on feeder cells	well- differentiated plasma cells, bi- or mononucl. cells highly developed Golgi	neg	41
LP-1	50 h	EBNA–	AcP+, PAS–		ind. to diff by PWM or TPA	basophilic cytopl. multinucl. cells	neg	43
MEF-1	36 h	EBV–				ecc. nuclei, basophilic cytopl.		44
MM.1	72 h	EBNA–	PAS–, Px–, MGP+, AcP+, ANBE(+), ANAE+, AANAE+		not clonable in soft agar or limiting dilution	ecc. nuclei, bi- or multinucl. cells well developed RER, prom. Golgi, single cell/ small clusters	neg	45
MM.5.1		EBNA–				plasmacytic morph./ small aggregates		46
MM5.2		EBNA–				immature plasmablastic		46
MM-A1	48 h	EBNA–			cloning eff. (methyl cellulose): 0 (–IL6), 1.8% (+IL6)			7

Continued on next page

Table 6. (continued)

Cell line	Doubling time T_{do}/generation time T_c	EBV status[a,b]	Cytochemistry	Heterotransplantability into nude mice	Special functional features	Histopathology/cell size/growth pattern in tissue culture	Mycoplasma status	Ref.
MM-C1	96 h	EBNA–			cloning eff. (methyl cellulose): 0 (–IL6), 0 (+IL6)			7
MM-M1		EBNA–	Px–			plasma cell morph./well dev. RER abundant mitochondria		47
MM-S1	24 h	EBNA–		not transplantable, transplantable subclone S6B45 (+IL6 cDNA)	cloning eff. (methyl cellulose): 0.5% (–IL6), 21.5% (+IL6)	plasmacytoid morph.		111
MM-Y1	49 h	EBNA–			cloning eff. (methyl cellulose): 0 (–IL6), 1.7% (+IL6)			7
MT3	40–50 h	EBNA–	AcP+, BGLU+, PAS–			basophilic cytopl., ecc. polynucli cultured/ 17–40 μm cell size	neg	49 51
NCI-H929	50 h	EBNA–	MGP+, AcP+, ANAE+, BGLU+, PAS–			multinucl. immature plasma cells 20–50 μm cell size single cells/loose clusters	neg	50 51
NOP-2	48–72 h	EBNA–				ecc. nuclei basophilic cytopl.	neg	51
OCI-My1	24 h	EBV–						52
OCI-My2	24 h	EBV–						52
OCI-My3	32 h	EBV–						52
OCI-My4	130 h	EBV–						52
OCI-My5	8 h	EBV–			clonable in methyl cellulose			52 53
OCI-My6	45 h	EBV–						52
OCI-My7	57 h	EBV–						52
OH-2		EBV–				ecc. nuclei, bi- multinucl. cells plasma cell appearance	neg	55

Continued on next page

Table 6. (continued)

Cell line	Doubling time T_{do}/generation time T_c	EBV status[a,b]	Cytochemistry	Heterotransplantability into nude mice	Special functional features	Histopathology/cell size/growth pattern in tissue culture	Mycoplasma status	Ref.
OPM-1	36–42 h	EBNA–	Px–, PAS(+), AcP+			blastoid, convoluted nuclei basophilic cytopl./ clumps		56
OPM-2	30–36 *H*	EBNA–	Px–, PAS(+), AcP+			blastoid nuclei/ single cells		56
PCM6	40–50 h	EBV– (PCR)				periph. nuclei, basophilic cytopl.		57
RPMI8226	27.5 h	EBNA–	ANAE+, BGLU+, AcP(+)		8.08% cloning eff. (soft agar)	immature cellmorph.,		2
						no mature plasma cells,		51
						swollen ER		58
SK-MM-1	32 h	EBNA–	AcP+, MGP+, SBB–			central or ecc. nuclei basophilic. cytopl. Bi-, multi-nucl. cells/28–50 μm cell size	neg	59
SK-MM-2	60 h	EBNA–	AcP+, MGP+, SBB–			basophilic. cytopl. Large ecc. nuclei. Bi- or multinucl. cells/ 22–32 μm cell size	neg	59
TH	24 h	EBNA–				small lymphoid cells/ plasmacytoid cells/blastoid cells, basophilic cytopl.		60
U-1957	48 h	EBNA–	AcP+, NASDCAE—, ANAE+ BGLU+		not clonable in agarose, clonable as single cells on feeder cells	plasmablast/cell morph. multinucl. cells ecc. nuclei/ single cells		61
U-1958	60 h (92–136 h)	EBNA–	AcP+, NASDCAE—, ANAE+ BGLU+		not clonable in agarose, clonable as single cells on feeder cells	plasmablast/cell morph. multinucl. cells ecc. nuclei/ single cells		61
U-1996	48 h	EBNA–	AcP+, NASDCAE—, ANAE+ BGLU+		not clonable in agarose, clonable as single cells on feeder cells	immature plasmablast/cell multinucl. cells ecc. nuclei/ single cells		61
U-2030	36 h	EBNA–	AcP+, ANAE—, BGLU+		clonable in agarose	plasmablasts		62
U266Bl (U-266)	108–144 *h*	EBV– EBNA–	ANAE+, BGLU+, AcP(+)		not clonable in agarose	basophilic cytopl., ecc. nuclei/ 6–16 μm cell size single cells		3,194 51

Continued on next page

Table 6. (continued)

Cell line	Doubling time T_{do}/generation time T_c	EBV status[a,b]	Cytochemistry	Heterotransplantability into nude mice	Special functional features	Histopathology/cell size/growth pattern in tissue culture	Mycoplasma status	Ref.
UCD–HL461	47 h	EBNA–			CM suppress PWM-stim. Ig production of normal PBL	ecc nucl. basoph cytopl./ single cells		63
UMJF–2[c]	67–90 h (24–72 h)	EBV–			UMJF–2 CM suppress PWM-stim. Ig production of normal PBMNC	centr. placed nuclei, basophilic cytopl., plasmablast morph./ 10–12 μm cell size		64
UTMC–2	48 h	EBV–				ecc. nuclei, basophilic cytopl./ single cells		65
XG–1						plasmablastic immature	neg	9 124,125
XG–2						plasmablastic immature	neg	9
XG–3P						plasmacytic differentiated		9
XG–3E						plasmacytic differentiated		9
XG–4						plasmacytic differentiated	neg	9
XG–5						plasmablastic immature	neg	9
XG–6						plasmacytic differentiated	neg	9
XG–7						plasmablastic immature	neg	9
XG–8						plasmacytic differentiated	neg	9
XG–9						plasmablastic immature	neg	9

[a] As for the KHM-11 and XG cell lines (1–9), the EBV status has not been reported. In each of these cell lines the authentication was demonstrated by Ig rearrangements consistent with fresh tumor cells of the patient from which the cell line was derived.

[b] In the OCI-My cell lines 1–7 cell lines, the characterization including EBV-status was made elsewhere [54] with no reference to the individual cell lines and/or designation.

[c] EBV positive (Drexler, personal communication).

Px – Peroxidase; PAS; periodic acid–Schiff; MGP – methyl green protein; ANAE – *alpha*–naphthyl acetate esterase; ANBE – *alpha*-naphtyl butyrate esterase; AcP – acid phosphatase; AlkP – alkaline phosphatase; NASDCAE – naphthol AS-D chloro acetate esterase; NASDAE – naphthol AS-D acetate esterase; SBB – Sudan Black B; ORO – Oil Red O; BGLU – β-glucuronidase.

μg/ml in UCD-HL461. Some MM cell lines are Ig non-producers (n = 9), reflecting the status (nonsecretory) in the MM patient in four cases. In other cases, the Ig production was lost during establishment (Table 2).

Transplantability in nude mice was reported in a few cases (DOBIL-6, KMS-5). Interestingly, heterotransplantability was reported in the subline S6B45 of MM-S1 with ectopic IL-6 cDNA and autonomous growth [48]. A number of the cell lines can be cloned and form colonies in soft agar or methyl cellulose (KMM-1, KMS-5, KMS-12, LB-831, LB-832, MM-S1, RPMI 8226) while MM-A1, MM- Y1, U-1957, U-1958 and U-1996 were only clonable in the presence of IL-6 or feeder cells. Phenotypic alterations (including improved growth rate, development of feeder cell independence, capacity for growth as colonies in soft agar, growth as subcutaneous tumors in nude mice) have also been reported to occur as a consequence of long term cultivation, as exemplified by the U-266(–1984) cell line [93–95].

In one of the cell lines, LP-1, PWM or TPA can induce differentiation. The expression of unique genes (amylase mRNA) associated with the occurrence of hyperamylasemia in the patient, was found in three cell lines (AD3, a subclone of FR4), FR4, and KHM-1B (Table 6).

7. PUTATIVE BUT UNCONFIRMED CELL LINES

As described, MM cell lines are phenotypically very heterogenous. However, they can be distinguished from LCLs (see Table 1). The following criteria should be used to distinguish MM from LCLs: (1) morphology; MM cells have a plasmablast/plasma cell morphology including eccentric nuclei with prominent nucleoli, a prominent RER and Golgi and perinuclear zone, (2) capacity for a high rate of Ig secretion, (3) surface antigen profile, (4) aneuploidy, with numerous structural and numerical aberrations and, most importantly (5) the lack of EBV. In some reports characterization by these criteria is insufficient. Taking these markers into account, this review distinguishes four categories of cell lines; (1) authentic MM cell lines, (2) EBV-positive lymphoblastoid cell lines (LCLs) (ARH-77, Fr/FRAVEL, GM1312, GM1500, HS-Sultan, IM-9 and MC/CAR), and (3) putative, but insufficiently characterized cell lines (ACB-985, ard-1, ark, C23/11, col, Hl12, HGN-5, HSM-2, HSM-2.3, KMM 56, LA 49, mer, Oda, ram). In these cell lines the EBV status and/or the phenotypic characterization or structural abnormalities consistent with the fresh tumor cells were not reported. Without further characterization these are regarded as putative but unconfirmed cell lines (Table 7).

Table 7. Unconfirmed cell lines (not characterized, not verified, EBV status unknown, other)

Cell line*	Patient	Treatment status/ specimen site	Culture medium/ other requirements for establishment	Ig production	Features	Ref.
ACB-985	IgG/κ MM	T/BM			elevated c-*myc* mRNA (no RFLP)	155
ard-1	MM	BM	RPMI1640+10%FCS		B-B4+ CD19– CD45(+), requirement of IL-6 for growth	82
ark	MM	BM	RPMI1640+10%FCS		B-B4+ CD19– CD45–, no requirement for IL-6	82
ARP-1	IgA/κMM	BM	RPMI1640+10%FCS	IgA/κ	CD9+ CD10– CD19– CD38+ CD45+	195
					CD56– cIg+ RT-PCR/Flow cyt: IL6R+	196
					RT-PCR/bioassay IL-6+ (0.4 pg/mL)	
					bcl-2 (low expression) *p53-/-*	
C23/11	MM	PB	RPMI1640+10%FCS no requirement for feeder cells/growth factors	κ/λ	T_{do} 12-15h eccentric nuclei, binucleated grow in clusters	197
col	MM	BM	RPMI1640+10%FCS		B-B4– CD19+ no requirement for IL-6	82
H112	MM				no available karyotype t(11;14), no ISRF/translocation into IgH switch region, cyclin D1 overexpression	153
HGN-5	IgA/κ MM				CD11a+ CD54+, c-*myc* rearrangement	78

Continued on next page

Table 7. (continued)

Cell line*	Patient	Treatment status/ specimen site	Culture medium/ other requirements for establishment	Ig production	Features	Ref.
HSM-2	90 M IgM/κ bi-phenotypic leukemia/ PCL	BM	IMDM+20%FCS adherent monolayers/ 10 U/ml IL-6 cloned by limiting dilution	IgM/κ	Px–, ANBE–, NASDCAE–, PAS+, SBB– Plasmablast/plasma cells/ 15-25 μm cell size Karyotype: 45, 1q+, 3q–, 4q–, 5q–, 7p+, 10p–, 10q+, 19p+, 22p+, –5, –9, –9, –13, –15, –15, –22, –Y MTT assay: IL-6+, IL-1–, IL-2–, IL-3–, IL-4–, IL-5–, GM-CSF– Dependence of cytokine: IL-6+ CD38+ PCA-1+ sIg+ cIg+	139
HSM-2.3	(subclone of HSM-2)		IMDM+5-20%FCS IL-3(100 U/ml)/ IL-6(0.5 U/ml)	IgM/κ	MTT assay: IL-6+, IL-3+ dependence of cytokine: IL-6+, IL-3+ CD10+ CD19+ CD38+ PCA-1+	139
KMM 56	62 M IgD/λ BJP MM	PE	αMEM+20%FCS adherent to fibroblasts/ feeder cells, later feeder independent growth	λ	T_{do}: 36 h Px–, PAS–, E–, EA–, EAC–, sIg– immature plasmacytoid basophilic cytoplasm hyperdiploid	198

Continued on next page

Table 7. (continued)

Cell line*	Patient	Treatment status/ specimen site	Culture medium/ other requirements for establishment	Ig production	Features	Ref.
LA 49	59 F IgD/λ BJP	PE	RPMI 1640+40%FCS fibroblasts overlaid with agar/fibroblast CM	IgD/λ	immature blasts/mature plasmacytes basophilic cytoplasm large ecc. nucleus/15–20 μm cell size colony formation in agar overlay in the presence of fibroblasts polyploid	199
mer	MM	BM	RPMI1640+10%FCS		B-B4– CD19+ no requirement for IL-6	82
Oda	IgD/λ plasmacytoma	localized plasmacytoma			Ig production: IgD/λ	200
ram	MM	BM	RPMI1640+10%FCS		B-B4(+) CD19+ no requirement for IL-6	82
SIK	IgG/κMM	BM	RPMI1640+10%FCS		CD9– CD10– CD19– CD38+ CD45+ CD56– RT-PCR; IL-6R mRNA+, IL-6 mRNA+ Flow cyt: IL-6R–; Bioassay: IL-6+	195
UCLA#1	PCL	PB	RPMI1640+10%FCS		CD38+ IL-6R+, Anti-apoptotic response to IL-6	201

* For all putative but unconfirmed cell lines the insufficient characterization concerns EBV negativity status and in some cell lines also relates to the clinical data, analysis of markers consistent with the fresh tumor cells, immunophenotypes and cytogenetics.

References

1. Helfrich, MH, Livingston, E, Franklin, IM, Soutar, RL. *Blood Rev* 11: 28–38, 1997.
2. Matsuoka, Y, Moore, GE, Yagi, Y, Pressman, D. *Proc Soc Exp Biol Med* 125: 1246–1250, 1967.
3. Nilsson, K, Bennich, H, Johansson, S, Pontén, J. *Clin Exp Immunol* 7: 477–489, 1970.
4. Jackson, N, Lowe, J, Ball, J, Bromidge, E, Ling, NR, Larkins, S, Griffith, MJ, Franklin, IM. *Clin Exp Immunol* 75: 93–99, 1989.
5. Shimizu, S, Yoshioka, R, Hirose, Y, Sugai, S, Tachibana, J, Konda, S. *J Exp Med* 169: 339–344, 1989.
6. Scibienski, R, Paglieroni, T, Caggiano, V, Lemonello, D, Gumerlock, P, Mackenzie, M. *Leukemia* 6: 940–947, 1992.
7. Okuno, Y, Takahashi, T, Suzuki, A, Ichiba, S, Nakamura, K, Fukumoto, M, Okada, T, Okada, H, Imura, H. *Leukemia* 5: 585–591, 1991.
8. Zhang, XG, Bataille, R, Jourdan, M, Saeland, S, Banchereau, J, Mannoni, P, Klein, B. *Blood* 76: 2599–2605, 1990.
9. Zhang, XG, Gaillard, JP, Robillard, N, Lu, ZY, Gu, ZJ, Jourdan, M, Boiron, JM, Bataille, R, Klein, B. *Blood* 83: 3654–3663, 1994a.
10. Nilsson, K, Ponten, J. *Int J Cancer* 15: 321–341, 1975.
11. Nilsson, K. *Hamatol Bluttransfus* 20: 253–264, 1977.
12. Kawano, M, Hirano, T, Matsuda, T, Taga, T, Horii, Y, Iwato, K, Asaoku, H, Tang, B, Tanabe, O, Tanaka, H, et al. *Nature* 332: 83–85, 1988.
13. Klein, B, Zhang, XG, Jourdan, M, Content, J, Houssiau, F, Aarden, L, Piechaczyk, M, Bataille, R. *Blood* 73: 517–526, 1989.
14. Jernberg, H, Pettersson, M, Kishimoto, T, Nilsson, K. *Leukemia* 5: 255–265, 1991a.
15. Nilsson, K, Klein, G. *Adv Cancer Res* 37: 319–380, 1982.
16. Ralph, P. *Cancer* 56: 2544–2545, 1985.
17. Pellat-Deceunynck, C, Barille, S, Puthier, D, Rapp, MJ, Harousseau, JL, Bataille, R, Amiot, M. *Cancer Res* 55: 3647–3653, 1995a.
18. Pellat-Deceunynck, C, Amiot, M, Bataille, R, van Riet, I, van Camp, B, Omede, P, Boccadoro, M. *Blood* 86: 4001–4002, 1995b.
19. Barut, BA, Zon, LI, Cochran, MK, Paul, SR, Chauhan, D, Mohrbacher, A, Fingeroth, J, Anderson, KC. *Leuk Res* 16: 951–959, 1992.
20. Brox, LW, Belch, A, Pollock, E, He, XX, De Braekeleer, M, Lin, CC. *Cancer Genet Cytogenet* 27: 135–144, 1987.
21. Tagawa, S, Doi, S, Taniwaki, M, Abe, T, Kanayama, Y, Nojima, J, Matsubara, K, Kitani, T. *Leukemia* 4: 600–605, 1990.
22. Shimizu, S, Takiguchi, T, Fukutoku, M, Yoshioka, R, Hirose, Y, Fukuhara, S, Ohno, H, Isobe, Y, Konda, S. *Leukemia* 7: 274–280, 1993.
23. Jelinek, DF, Ahmann, GJ, Greipp, PR, Jalal, SM, Westendorf, JJ, Katzmann, JA, Kyle, RA, Lust, JA. *Cancer Res* 53: 5320–5327, 1993.
24. Ishii, K, Yamato, K, Kubonishi, I, Taguchi, H, Ohtsuki, Y, Miyoshi, I. *Am J Hematol* 41: 218–224, 1992.
25. Ohmori, M, Nagai, M, Fujita, M, Dobashi, H, Tasaka, T, Yamaoka, G, Kawanishi, K, Taniwaki, M, Takahara, J. *Br J Haematol* 101: 688–693, 1998.
26. Westendorf, JJ, Ahmann, GJ, Greipp, PR, Witzig, TE, Lust, JA, Jelinek, DF. *Leukemia* 10: 866–876, 1996a.
27. Hamilton, M, Ball, J, Bromidge, E, Lowe, J, Franklin, I. *Br J* 75: 378–384, 1990.

28. Kubonishi, I, Seto, M, Shimamura, T, Enzan, H, Miyoshi, I. *Cancer* 70: 1528–1535, 1992.
29. Goldstein, M, Hoxie, J, Zembryki, D, Matthews, D, Levinson, AI. *Blood* 66: 444–446, 1985.
30. Barker, HF, Hamilton, MS, Ball, J, Drew, M & Franklin, IM. In: Radl, J, van Camp, B. (eds.), *EURAGE Monoclonal Gammopathies III: Clinical Significance and Basic Mechanisms*, EURAGE, Leiden, the Netherlands), pp 155–158, 1991.
31. Nacheva, E, Fischer, PE, Sherrington, PD, Labastide, W, Lawlor, E, Conneally, E, Blaney, C, Hayhoe, FG, Karpas, A. *Br J Haematol* 74: 70–76, 1990.
32. Karpas, A, Fischer, P, Swirsky, D. *Science* 216: 997–999, 1982.
33. Matsuzaki, H, Hata, H, Takeya, M, Takatsuki, K. *Blood* 72: 978–982, 1988.
34. Hata, H, Matsuzaki, H, Sonoki, T, Takemoto, S, Kuribayashi, N, Nagasaki, A, Takatsuki, K. *Leukemia* 8: 1768–1773, 1994.
35. Togawa, A, Inoue, N, Miyamoto, K, Hyodo, H, Namba, M. *Int J Cancer* 29: 495–500, 1982.
36. Namba, M, Ohtsuki, T, Mori, M, Togawa, A, Wada, H, Sugihara, T, Yawata, Y, Kimoto, T. *In Vitro Cell Dev Biol* 25: 723–729, 1989.
37. Otsuki, T, Nakazawa, N, Taniwaki, M, Yamada, O, Sakaguchi, H, Wada, H, Yawata, Y, Ueki, A. *Int J Oncol* 12: 545–552, 1998.
38. Goto, H, Shimazaki, C, Tatsumi, T, Yamagata, N, Fujita, N, Tsuchiya, M, Koishihara, Y, Ohsugi, Y, Nakagawa, M. *Leukemia* 9: 711–718, 1995.
39. Diehl, V, Schaadt, M, Kirchner, H, Hellriegel, KP, Gudat, F, Fonatsch, C, Laskewitz, E, Guggenheim, R. *Blut* 36: 331–338, 1978.
40. Durie, BG, Vela, E, Baum, V, Leibovitz, A, Payne, CM, Richter, LC, Grogan, TM, Trent, JM. *Blood* 66: 548–555, 1985.
41. Lohmeyer, J, Hadam, M, Santoso, S, Forster, W, Schulz, A, Pralle, H. *Br J Haematol* 69: 335–343, 1988.
42. Corradini, P, Ladetto, M, Voena, C, Palumbo, A, Inghirami, G, Knowles, DM, Boccadoro, M, Pileri, A. *Blood* 81: 2708–2713, 1993.
43. Pegoraro, L, Malavasi, F, Belloni, G, Massaia, M, Boccadoro, M, Saglio, G, Guerrasio, A, Benetton, G, Lombardi, L, Coda, R, Avanzi, G. *Blood* 73: 1020–1027, 1989.
44. Yufu, Y, Goto, T, Choi, I, Uike, N, Kozuru, M, Ohshima, K, Taniguchi, T, Motokura, T, Yatabe, Y, Nakamura, S. *Cancer* 85: 1750–1757, 1999.
45. Goldman-Leikin, RE, Salwen, HR, Herst, CV, Variakojis, D, Bian, ML, Le Beau, MM, Selvanayagan, P, Marder, R, Anderson, R, Weitzman, S, et al. *J Lab Clin Med* 113: 335–345, 1989.
46. Van Riet, I, De Greef, C, Aharchi, F, Woischwill, C, De Waele, M, Bakkus, M, Lacor, P, Schots, R, van Camp, B. *Leukemia* 11: 284–293, 1997.
47. Suzuki, A, Takahashi, T, Okuno, Y, Fukumoto, M, Fukui, H, Koishihara, Y, Ohsugi, Y, Ohno, Y, Imura, H. *Leuk Res* 15: 1043–1050, 1991.
48. Okuno, Y, Takahashi, T, Suzuki, A, Fukumoto, M, Nakamura, K, Fukui, H, Koishihara, Y, Ohsugi, Y, Imura, H. *Exp Hematol* 20: 395–400, 1992.
49. Donelli, A, Narni, F, Tabilio, A, Emilia, G, Selleri, L, Colo, A, Zucchini, P, Montagnani, G, Torelli, G, Torelli, U. *Int J Cancer* 40: 383–388, 1987.
50. Gazdar, AF, Oie, HK, Kirsch, IR, Hollis, GF. *Blood* 67: 1542–1549, 1986.
51. Nagai, T, Ogura, M, Morishita, Y, Okumura, M, Kato, Y, Morishima, Y, Hirabayashi, N, Ohno, R, Saito, H. *Int J Hematol* 54: 141–149, 1991.
52. Hitzler, JK, Martinez-Valdez, H, Bergsagel, DB, Minden, MD, Messner, HA. *Blood* 78, 1996–2004, 1991.

53. Wandl, U, Hoang, T, Minden, M, Messner, HA. *J Cell Physiol* 136: 384–388, 1988.
54. Takahashi, T, Lim, B, Jamal, N, Tritchler, D, Lockwood, G, McKinney, S, Bergsagel, DE, Messner, HA. *J Clin Oncol* 3: 1613–1623, 1985.
55. Borset, M, Waage, A, Brekke, OL, Helseth, E. *Eur J Haematol* 53: 31–37, 1994.
56. Katagiri, S, Yonezawa, T, Kuyama, J, Kanayama, Y, Nishida, K, Abe, T, Tamaki, T, Ohnishi, M, Tarui, S. *Int J Cancer* 36: 241–246, 1985.
57. Takahira, H, Kozuru, M, Hirata, J, Obama, K, Uike, N, Iguchi, H, Miyamura, T, Yamashita, S, Kono, A, Umemura, T. *Exp Hematol* 22: 261–266, 1994.
58. Bellamy, WT, Dalton, WS, Gleason, MC, Grogan, TM, Trent, JM. *Cancer Res* 51: 995–1002, 1991.
59. Eton, O, Scheinberg, DA, Houghton, AN. *Leukemia* 3: 729–735, 1989.
60. Weinreich, SS, von dem Borne, AE, van Lier, RA, Feltkamp, CA, Slater, RM, Wester, MR, Zeijlemaker, WP. *Br J Haematol* 79: 226–234, 1991.
61. Jernberg, H, Nilsson, K, Zech, L, Lutz, D, Nowotny, H, Scheirer, W. *Blood* 69: 1605–1612, 1987a.
62. Jernberg, H, Bjorklund, G, Nilsson, K. *Int J Cancer* 39: 745–751, 1987c.
63. Scibienski, RJ, Paglieroni, TG, MacKenzie, MR. *Leukemia* 4: 775–780, 1990.
64. Farnen, JP, Tyrkus, M, Hanson, CA, Cody, RL, Emerson, SG. *Leukemia* 5: 574–584, 1991.
65. Ozaki, S, Wolfenbarger, D, deBram-Hart, M, Kanangat, S, Weiss, DT, Solomon, A. *Leukemia* 8: 2207–2213, 1994.
66. Nakamura, M, Merchav, S, Carter, A, Ernst, TJ, Demetri, GD, Furukawa, Y, Anderson, K, Freedman, AS, Griffin, JD. *J Immunol* 143: 3543–3547, 1989.
67. Burk, KH, Drewinko, B, Trujillo, JM, Ahearn, MJ. *Cancer Res* 36: 2508–13, 1978.
68. Urashima, M, Chauhan, D, Uchiyama, H, Freeman, GJ, Anderson, KC. *Blood* 85: 1903–1912, 1995.
69. Van Riet, I, De Waele, M, Remels, L, Lacor, P, Schots, R, van Camp, B. *Br J Haematol* 79: 421–427, 1991.
70. Miller, CH, Carbonell, A, Peng, R, Paglieroni, T, MacKenzie, MR. *Cancer* 49: 2091–2096, 1982.
71. Duperray, C, Klein, B, Durie, BG, Zhang, X, Jourdan, M, Poncelet, P, Favier, F, Vincent, C, Brochier, J, Lenoir, G, et al. *Blood* 73: 566–572, 1989.
72. Pellat-Deceunynck, C, Bataille, R, Robillard, N, Harousseau, JL, Rapp, MJ, Juge-Morineau, N, Wijdenes, J, Amiot, M. *Blood* 84: 2597–2603, 1994.
73. Ritts, RE, Jr, Ruiz-Arguelles, A, Weyl, KG, Bradley, AL, Weihmeir, B, Jacobsen, DJ, Strehlo, BL. *Int J Cancer* 31: 133–141, 1983.
74. Van Riet, I. *Pathol Biol (Paris)* 47: 98–108, 1999.
75. Zhang, XG, Olive, D, Devos, J, Rebouissou, C, Ghiotto-Ragueneau, M, Ferlin, M, Klein, B. *Leukemia* 12: 610–618, 1998.
76. Westendorf, JJ, Ahmann, GJ, Lust, JA, Tschumper, RC, Greipp, PR, Katzmann, JA, Jelinek, DF. *Curr Top Microbiol Immunol* 194: 63–72, 1995.
77. Drew, M, Barker, HF, Ball, J, Pearson, C, Cook, G, Franklin, I. *Leuk Res* 20: 619–624, 1996.
78. Kawano, MM, Huang, N, Tanaka, H, Ishikawa, H, Sakai, A, Tanabe, O, Nobuyoshi, M, Kuramoto, A. *Br J Haematol* 79: 583–858, 1991.
79. Durie, BG, Grogan, TM, Spier, C, Vela, E, Baum, V, Rodriquez, MA, Frutiger, Y. *Blood* 73: 770–776, 1989.
80. Westendorf, JJ, Ahmann, GJ, Armitage, RJ, Spriggs, MK, Lust, JA, Greipp, PR, Katzmann, JA, Jelinek, DF. *J Immunol* 152 117–128, 1994.

81. Jourdan, M, Ferlin, M, Legouffe, E, Horvathova, M, Liautard, J, Rossi, JF, Wijdenes, J, Brochier, J, Klein, B. *Br J Haematol* 100: 637–646, 1998.
82. Ridley, RC, Xiao, H, Hata, H, Woodliff, J, Epstein, J, Sanderson, RD. *Blood* 81: 767–774, 1993.
83. Uchiyama, H, Barut, BA, Mohrbacher, AF, Chauhan, D, Anderson, KC. *Blood* 82: 3712–3720, 1993.
84. Kawano, MM, Huang, N, Harada, H, Harada, Y, Sakai, A, Tanaka, H, Iwato, K, Kuramoto, A. *Blood* 82: 564–570, 1993.
85. Kim, I, Uchiyama, H, Chauhan, D, Anderson, KC. *Br J Haematol* 87: 483–493, 1994.
86. Ahsmann, EJ, Lokhorst, HM, Dekker, AW, Bloem, AC. *Blood* 79: 2068–2075, 1992.
87. Caligaris-Cappio, F, Gregoretti, MG, Nilsson, K. In: Caligaris-Cappio, F. (ed.), *Human B Cell Populations*, Vol 67, Karger, Basel, pp 102–113, 1997.
88. De Greef, C, van Riet, I, Bakkus, MH, van Camp, B. *Leukemia* 12: 86–93, 1998.
89. Barker, HF, Hamilton, MS, Ball, J, Drew, M, Franklin, IM. *Br J Haematol* 81: 331–335, 1992.
90. Caligaris-Cappio, F, Gregoretti, MG, Merico, F, Gottardi, D, Ghia, P, Parvis, G, Bergui, L. *Leuk Lymphoma* 8: 15–22, 1992.
91. Hallek, M, Bergsagel, P, Anderson, K. *Blood* 91: 3–21, 1998.
92. Klein, B. *Curr Opin Hematol* 5: 186–191, 1998.
93. Hellman, L, Josephson, S, Jernberg, H, Nilsson, K, Pettersson, U. *Eur J Immunol* 18: 905–910, 1988.
94. Nilsson, K, Jernberg, H, Pettersson, M. *Curr Top Microbiol Immunol* 166: 3–12, 1990.
95. Jernberg Wiklund, H, Pettersson, M, Carlsson, M, Nilsson, K. *Leukemia* 6: 310–318, 1992a.
96. Portier, M, Rajzbaum, G, Zhang, XG, Attal, M, Rusalen, C, Wijdenes, J, Mannoni, P, Maraninchi, D, Piechaczyk, M, Bataille, R, et al. *Eur J Immunol* 21: 1759–1762, 1991.
97. Hata, H, Xiao, H, Petrucci, MT, Woodliff, J, Chang, R, Epstein, J. *Blood* 81: 3357–3364, 1993.
98. Sati, HI, Apperley, JF, Greaves, M, Lawry, J, Gooding, R, Russell, RG, Croucher, PI. *Br J Haematol* 101: 287–295, 1998.
99. Freeman, GJ, Freedman, AS, Rabinowe, SN, Segil, JM, Horowitz, J, Rosen, K, Whitman, JF, Nadler, LM. *J Clin Invest* 83: 1512–1518, 1989.
100. Schwab, G, Siegall, CB, Aarden, LA, Neckers, LM, Nordan, RP. *Blood* 77: 587–593, 1991.
101. Jelinek, DF, Aagaard Tillery, KM, Arendt, BK, Arora, T, Tschumper, RC, Westendorf, JJ. *J Clin Invest* 99: 447–456, 1997a.
102. Jelinek, DF, Witzig, TE, Arendt, BK. *J Immunol* 159: 487–496, 1997b.
103. Westendorf, JJ, Jelinek, DF. *J Immunol* 157: 3081–3088, 1996b.
104. Costes, V, Portier, M, Lu, ZY, Rossi, JF, Bataille, R, Klein, B. *Br J Haematol* 103: 1152–1160, 1998.
105. Georgii-Hemming, P, Wiklund, HJ, Ljunggren, O, Nilsson, K. *Blood* 88: 2250–2258, 1996.
106. Georgii-Hemming, P, Stromberg, T, Janson, ET, Stridsberg, M, Wiklund, HJ, Nilsson, K. *Blood* 93: 1724–1731, 1999.
107. Jernberg-Wiklund, H, Pettersson, M, Nilsson, K. *Eur J Haematol* 46: 231–239, 1991b.
108. Hata, H, Matsuzaki, H, Takatsuki, K. *Acta Haematol* 83: 133–136, 1990.
109. Diamant, M, Hansen, MB, Rieneck, K, Svenson, M, Yasukawa, K, Bendtzen, K. *Leuk Res* 20: 291–301, 1996.

110. Neumann, C, Zehentmaier, G, Danhauser-Riedl, S, Emmerich, B, Hallek, M. *Eur J Immunol* 26: 379–384, 1996.
111. Okuno, Y, Takahashi, T, Suzuki, A, Ichiba, S, Nakamura, K, Hitomi, K, Sasaki, R, Imura, H. *Biochem Biophys Res Commun* 170: 1128–1134, 1990.
112. Johnson, BH, Gomi, M, Jakowlew, SB, Moriwaki, K, Thompson, EB. *Cell Growth Differ* 4: 25–30, 1993.
113. Borset, M, Medvedev, AE, Sundan, A, Espevik, T. *Cytokine* 8: 430–438, 1996.
114. Salmon, SE, Soehnlen, B, Dalton, WS, Meltzer, P, Scuderi, P. *Blood* 74: 1723–1727, 1989.
115. Freund, GG, Kulas, DT, Mooney, RA. *J Immunol* 151: 1811–1820, 1993.
116. Freund, GG, Kulas, DT, Way, BA, Mooney, RA. *Cancer Res* 54: 3179–3185, 1994.
117. Villunger, A, Egle, A, Kos, M, Egle, D, Tinhofer, I, Henn, T, Uberall, F, Maly, K, Greil, R. *Br J Haematol* 102, 1069–1080, 1998.
118. Paul, SR, Barut, BA, Bennett, F, Cochran, MA, Anderson, KC. *Leuk Res* 16: 247–252, 1992.
119. Zhou, L, Peng, S, Duan, J, Zhou, J, Wang, L, Wang, J. *Biochem Mol Biol Int* 45: 1113–1119, 1998.
120. Jernberg-Wiklund, H, Pettersson, M, Larsson, LG, Anton, R, Nilsson, K. *Int J Cancer* 51: 116–123, 1992b.
121. Schwabe, M, Brini, AT, Bosco, MC, Rubboli, F, Egawa, M, Zhao, J, Princler, GL, Kung, HF. *J Clin Invest* 94: 2317–2325, 1994.
122. Zhang, XG, Gu, JJ, Lu, ZY, Yasukawa, K, Yancopoulos, GD, Turner, K, Shoyab, M, Taga, T, Kishimoto, T, Bataille, R, et al. *J Exp Med* 179: 1337–1342, 1994b.
123. Petrucci, MT, Ricciardi, MR, Ariola, C, Gregorj, C, Ribersani, M, Savino, R, Ciliberto, G, Tafuri, A. *Ann Hematol* 78: 13–18, 1999.
124. Gu, ZJ, Costes, V, Lu, ZY, Zhang, XG, Pitard, V, Moreau, JF, Bataille, R, Wijdenes, J, Rossi, JF, Klein, B. *Blood* 88: 3972–3986, 1996a.
125. Gu, ZJ, Zhang, XG, Hallet, MM, Lu, ZY, Wijdenes, J, Rossi, JF, Klein, B. *Exp Hematol* 24: 1195–1200,1996b.
126. Jourdan, M, Zhang, XG, Portier, M, Boiron, JM, Bataille, R, Klein, B. *J Immunol* 147: 4402–4407, 1991.
127. Lu, ZY, Zhang, XG, Rodriguez, C, Wijdenes, J, Gu, ZJ, Morel-Fournier, B, Harousseau, JL, Bataille, R, Rossi, JF, Klein, B. *Blood* 85: 2521–2527, 1995a.
128. Lu, ZY, Gu, ZJ, Zhang, XG, Wijdenes, J, Neddermann, P, Rossi, JF, Klein, B. *FEBS Lett* 377:515–518, 1995b.
129. Levy, Y, Tsapis, A, Brouet, JC. *J Clin Invest* 88: 696–699, 1991.
130. Hawley, RG, Berger, LC. *Leuk Lymphoma* 29: 465–475, 1998.
131. Nishimoto, N, Ogata, A, Shima, Y, Tani, Y, Ogawa, H, Nakagawa, M, Sugiyama, H, Yoshizaki, K, Kishimoto, T. *J Exp Med* 179: 1343–1347, 1994.
132. Spets, H, Jernberg-Wiklund, H, Sambade, C, Soderberg, O, Nilsson, K. *Br J Haematol* 98: 126–133, 1997.
133. Harrington, EA, Bennett, MR, Fanidi, A, Evan, GI. *EMBO J* 13: 3286–3295, 1994.
134. Datta, SR, Dudek, H, Tao, X, Masters, S, Fu, H, Gotoh, Y, Greenberg, ME. *Cell* 91: 231–241, 1997.
135. Vanderkerken, K, Asosingh, K, Braet, F, van Riet, I, van Camp, B. *Blood* 93: 235–241, 1999.
136. Anderson, KC, Jones, RM, Morimoto, C, Leavitt, P, Barut, BA. *Blood* 73: 1915–1924, 1989.
137. Carter, A, Merchav, S, Silvian-Draxler, I, Tatarsky, I. *Br J Haematol* 74: 424–431, 1990.

138. Urashima, M, Ogata, A, Chauhan, D, Hatziyanni, M, Vidriales, MB, Dedera, DA, Schlossman, RL, Anderson, KC. *Blood* 87: 1928–1938, 1996.
139. Kobayashi, M, Miyagishima, T, Imamura, M, Maeda, S, Gotohda, Y, Iwasaki, H, Sakurada, K, Miyazaki, T. *Br J Haematol* 78: 217–221, 1991.
140. Kobayashi, M, Tanaka, J, Imamura, M, Maeda, S, Iwasaki, H, Tanaka, M, Tsudu, Y, Sakurada, K, Miyazaki, T. *Br J Haematol* 83: 535–538, 1993.
141. Borset, M, Helseth, E, Naume, B, Waage, A. *Br J Haematol* 85: 446–451, 1993.
142. Idestrom, K, Cantell, K, Killander, D, Nilsson, K, Strander, H, Willems, J. *Acta Med Scand* 205: 149–154, 1979.
143. Brenning, G. *Scand J Haematol* 35: 178–185, 1985.
144. Brenning, G, Jernberg, H, Gidlund, M, Sjoberg, O, Nilsson, K. *Scand J Haematol* 37: 280–288, 1986.
145. Einhorn, S, Fernberg, JO, Grander, D, Lewensohn, R. *Eur J Cancer Clin Oncol* 24: 1505–1510,1988.
146. Portier, M, Zhang, XG, Caron, E, Lu, ZY, Bataille, R, Klein, B. *Blood* 81: 3076–3082, 1993.
147. Palumbo, A, Battaglio, S, Napoli, P, Omede, P, Fusaro, A, Bruno, B, Boccadoro, M, Pileri, A. *Br J Haematol* 86: 726–732, 1994.
148. Dewald, GW, Kyle, RA, Hicks, GA, Greipp, PR. *Blood* 66: 380–390, 1985.
149. Ferti, A, Panani, A, Arapakis, G, Raptis, S. *Cancer Genet Cytogenet* 12: 247–253, 1984.
150. Sawyer, JR, Waldron, JA, Jagannath, S, Barlogie, B. *Cancer Genet Cytogenet* 82: 41–49, 1995.
151. Drach, J, Angerler, J, Schuster, J, Rothermundt, C, Thalhammer, R, Haas, OA, Jager, U, Fiegl, M, Geissler, K, Ludwig, H, et al. *Blood* 86: 3915–3921, 1995.
152. Tabernero, D, San Miguel, JF, Garcia-Sanz, M, Najera, L, Garcia-Isidoro, M, Perez-Simon, JA, Gonzalez, M, Wiegant, J, Raap, AK, Orfao, A. *Am J Pathol* 149: 153–161, 1996.
153. Bergsagel, PL, Chesi, M, Nardini, E, Brents, LA, Kirby, SL, Kuehl, WM. *Proc Natl Acad Sci USA* 93: 13931–13936, 1996.
154. Bergsagel, PL, Nardini, E, Brents, L, Chesi, M, Kuehl, WM. *Curr Top Microbiol Immunol* 224: 283–287, 1997.
155. Fourney, R, Palmer, M, Ng, A, Dietrich, K, Belch, A, Paterson, M, Brox, L. *Dis Markers* 8: 117–124, 1990.
156. Chesi, M, Bergsagel, PL, Brents, LA, Smith, CM, Gerhard, DS, Kuehl, WM. *Blood* 88: 674–681, 1996.
157. Kuehl, WM, Brents, LA, Chesi, M, Huppi, K, Bergsagel, PL. *Curr Top Microbiol Immunol* 224: 277–282, 1997.
158. Mazars, GR, Portier, M, Zhang, XG, Jourdan, M, Bataille, R, Theillet, C, Klein, B. *Oncogene* 7: 1015–1018, 1992.
159. Pettersson, M, Jernberg-Wiklund, H, Larsson, LG, Sundstrom, C, Givol, I, Tsujimoto, Y, Nilsson, K. *Blood* 79: 495–502, 1992.
160. Sonoki, T, Nakazawa, N, Hata, H, Taniwaki, M, Nagasaki, A, Seto, M, Yoshida, M, Kuribayashi, N, Kimura, T, Harada, N, Mitsuya, H, Matsuzaki, H. *Int J Hematol* 68: 459–461, 1998.
161. Ohtsuki, T, Nishitani, K, Hatamochi, A, Yawata, Y, Namba, M. *Br J Haematol* 77: 172–179, 1991.
162. Teoh, G, Urashima, M, Ogata, A, Chauhan, D, DeCaprio, JA, Treon, SP, Schlossman, RL, Anderson, KC. *Blood* 90: 1982–1992, 1997.

163. Urashima, M, Teoh, G, Ogata, A, Chauhan, D, Treon, SP, Sugimoto, Y, Kaihara, C, Matsuzaki, M, Hoshi, Y, DeCaprio, JA, Anderson, KC. *Clin Cancer Res* 3: 2173–2179, 1997.
164. Portier, M, Moles, JP, Mazars, GR, Jeanteur, P, Bataille, R, Klein, B, Theillet, C. *Oncogene* 7: 2539–2543, 1992.
165. Iida, S, Rao, PH, Butler, M, Corradini, P, Boccadoro, M, Klein, B, Chaganti, RS, Dalla-Favera, R. *Nat Genet* 17: 226–230, 1997.
166. Jernberg, H, Zech, L, Nilsson, K. *Int J Cancer* 40: 811–817, 1987b.
167. Corradini, P, Inghirami, G, Astolfi, M, Ladetto, M, Voena, C, Ballerini, P, Gu, W, Nilsson, K, Knowles, DM, Boccadoro, M, et al. *Leukemia* 8: 758–767, 1994.
168. Chesi, M, Nardini, E, Brents, LA, Schrock, E, Ried, T, Kuehl, WM, Bergsagel, PL. *Nat Genet* 16: 260–264, 1997.
169. Chesi, M, Nardini, E, Lim, RS, Smith, KD, Kuehl, WM, Bergsagel, PL. *Blood* 92: 3025–3034, 1998b.
170. Chesi, M, Bergsagel, PL, Shonukan, OO, Martelli, ML, Brents, LA, Chen, T, Schrock, E, Ried, T, Kuehl, WM. *Blood* 91: 4457–4463, 1998a.
171. Klein, G. *Cell* 32: 311–315, 1983.
172. Sumegi, J, Hedberg, T, Bjorkholm, M, Godal, T, Mellstedt, H, Nilsson, MG, Perlman, C, Klein, G. *Int J Cancer* 36: 367–371, 1985.
173. Selvanayagam, P, Blick, M, Narni, F, van Tuinen, P, Ledbetter, DH, Alexanian, R, Saunders, GF, Barlogie, B. *Blood* 71: 30–35, 1988.
174. Neri, A, Murphy, JP, Cro, L, Ferrero, D, Tarella, C, Baldini, L, Dalla-Favera, R. *J Exp Med* 170: 1715–1725, 1989.
175. Paquette, RL, Berenson, J, Lichtenstein, A, McCormick, F, Koeffler, HP. *Oncogene* 5: 1659–1663,1990.
176. Ladanyi, M, Wang, S, Niesvizky, R, Feiner, H, Michaeli, J. *Am J Pathol* 141: 949–953, 1992.
177. Hollis, GF, Gazdar, AF, Bertness, V, Kirsch, IR. *Mol Cell Biol* 8: 124–129, 1988.
178. Palumbo, AP, Pileri, A, Dianzani, U, Massaia, M, Boccadoro, M, Calabretta, B. *Cancer Res* 49: 4701–4704, 1989.
179. Nobuyoshi, M, Kawano, M, Tanaka, H, Ishikawa, H, Tanabe, O, Iwato, K, Asaoku, H, Sakai, A, Kuramoto, A. *Br J Haematol* 77: 523–528, 1991.
180. Bakkus, MH, Brakel-van Peer, KM, Michiels, JJ, van't Veer, MB, Benner, R. *Oncogene* 5: 1359–1364, 1990.
181. Palumbo, AP, Boccadoro, M, Battaglio, S, Corradini, P, Tsichlis, PN, Huebner, K, Pileri, A, Croce, CM. *Cancer Res* 50: 6478–6482, 1990.
182. Paulin, FE, West, MJ, Sullivan, NF, Whitney, RL, Lyne, L, Willis, AE. *Oncogene* 13: 505–513, 1996.
183. Tsujimoto, Y, Gorham, J, Cossman, J, Jaffe, E, Croce, CM. *Science* 229: 1390–1393, 1985.
184. Hamilton, MS, Barker, HF, Ball, J, Drew, M, Abbot, SD, Franklin, IM. *Leukemia* 5: 768–771, 1991.
185. Sangfelt, O, Osterborg, A, Grander, D, Anderbring, E, Ost, A, Mellstedt, H, Einhorn, S. *Int J Cancer* 63: 190–192, 1995.
186. Tu, Y, Xu, FH, Liu, J, Vescio, R, Berenson, J, Fady, C, Lichtenstein, A. *Blood* 88: 1805–1812, 1996.
187. Harada, N, Hata, H, Yoshida, M, Soniki, T, Nagasaki, A, Kuribayashi, N, Kimura, T, Matsuzaki, H, Mitsuya, H. *Leukemia* 12: 1817–1820, 1998.

188. Dao, DD, Sawyer, JR, Epstein, J, Hoover, RG, Barlogie, B, Tricot, G. *Leukemia* 8: 1280–1284,1994.
189. Billadeau, D, Jelinek, DF, Shah, N, LeBien, TW, van Ness, B. *Cancer Res* 55: 3640–3646, 1995.
190. Preudhomme, C, Facon, T, Zandecki, M, Vanrumbeke, M, Lai, JL, Nataf, E, Loucheux-Lefebvre, MH, Kerckaert, JP, Fenaux, P. *Br J Haematol* 81: 440–443, 1992.
191. Ollikainen, H, Syrjanen, S, Koskela, K, Pelliniemi, TT, Pulkki, K. *Scand J Clin Lab Invest* 57: 281–289, 1997.
192. Tasaka, T, Asou, H, Munker, R, Said, JW, Berenson, J, Vescio, RA, Nagai, M, Takahara, J, Koeffler, HP. *Br J Haematol* 101: 558–564, 1998.
193. Kawano, MM, Mahmoud, MS, Ishikawa, H. *Br J Haematol* 99: 131–138, 1997.
194. Nilsson, K. *Int J Cancer* 7: 380–396, 1971.
195. Hardin, J, MacLeod, S, Grigorieva, I, Chang, R, Barlogie, B, Xiao, H, Epstein, J. *Blood* 84:3063–3070, 1994.
196. Gazitt, Y, Fey, V, Thomas, C, Alvarez, R. *Int J Oncol* 13: 397–405, 1998.
197. Prosser, E, Carroll, K, O'Kennedy, R, Clark, K, Grogan, L, Otridge, B. *Biochem Soc Trans* 17: 1055–1056, 1989.
198. Niho, Y, Shibuya, T, Yamasaki, K, Kimura, N. *Int J Cell Cloning* 2: 161–172, 1984.
199. Jobin, ME, Fahey, JL, Price, Z. *J Exp Med* 140: 494–507, 1974.
200. Ishihara, N, Kiyofuzi, T, Oboshi, S. *Proc Jap Cancer Assoc, 36th Annual Meeting* 120, 1977.
201. Xu, FH, Sharma, S, Gardner, A, Tu, Y, Raitano, A, Sawyers, C, Lichtenstein, A. *Blood* 92: 241–251, 1998.

Chapter 5

T-Cell Acute Lymphoblastic Leukemia and Natural Killer Cell Lines

Harry W. Findley, Heather L. Johnson and Stephen D. Smith
Department of Pediatrics, Emory University School of Medicine, 2040 Ridgewood Drive NE, Atlanta, GA 30322 and Department of Pediatrics, The University of Kansas Medical Center, 3901 Rainbow Boulevard, 3032 Delp Pavilion, Kansas City, KS 66160-7357, USA. Tel: +1-913-588-6340; Fax: +1-913-588-2245; E-mail:ssmith8@kumc.edu

1. INTRODUCTION

This chapter reports the characterization of forty-four cell lines that were established from patients with T-cell acute lymphoblastic leukemia (T-ALL) and five natural killer (NK) cell lines established from patients with leukemia with features of NK cells. Several subclones (variant cell lines derived from a parental line) and sister cell lines (cell lines derived independently from the same patient but from a different specimen or at a different time during the clinical course) are also described.

Acute lymphoblastic leukemia with a T-lymphocyte phenotype (T-ALL) has unique clinical, immunophenotypic, biochemical, and karyotypic features. Approximately 15% of children with leukemia have a thymic (T) cell phenotype as characterized by the ability to rosette with sheep RBCs or react with monoclonal antibodies associated with the T-lymphocyte lineage. Children with T-ALL characteristically are male, commonly present during adolescence, possess an anterior mediastinal mass and have a high white count. Immunophenotypically, T-ALL cells are characterized as immature T lymphocytes that possess pan-T cell antigens as detected by monoclonal antibodies. Early in thymocyte development, there is rearrangement of the T-cell receptor (*TCR*) α, β, γ or δ genes associated with expression of CD7. Many T-ALL cells express CD2 and CD5 and cytoplasmic CD3. With T-cell maturation, *TCR* (α/β, γ/δ) is expressed on the cell surface.

T-ALL cells have a high adenosine deaminase (ADA) level and a low nucleoside phosphorylase (NP) level compared to normal T-lymphocytes. The T-ALL cell lines are generally terminal deoxynucleotidyl transferase (TdT) positive [165]. While the retrovirus human T-cell leukemia/lymphoma virus

J.R. W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol. Ill, 157–206.

type 1 is commonly associated with adult T-cell leukemia/lymphoma, T-ALL is not associated with the human T-cell virus type 1 or 2.

T-ALL is characterized by a lack of hyperdiploidy (>50 chromosomes) and a high incidence of chromosome translocations involving the T-cell receptor (*TCR*) α/δ or β locus. About 40% of T-ALL cases have nonrandom breakpoints and translocations within the 14q11, 7q34, or 7p15 region, which contain the *TCR* α/δ, *TCR* β, and *TCR* γ genes, respectively. At least ten recurring chromosome translocations have been observed in T-ALL. These translocations commonly result in fusion of genes that encode a chimeric protein [133]. The most frequent recurring abnormality in childhood T-ALL is t(11;14)(pl3;q11), which accounts for about 7% of cases [138]. The t(11;14)(pl5;ql1) is found in about 1% of T-ALL cases. In both translocations, the breakpoint on 14q occurs within the *TCR* α/δ locus and the breakpoint on 11pl5 occurs within a rhombotin related gene (*RBTN1*). The gene on 11pl3 (*RBTN2*) has extensive homology with *RBTN1* and both genes are normally involved in erythroid differentiation. Ectopic production of RBTN is thought to be a major factor in T-cell transformation.

Newly established cell lines should be compared to the patient's leukemia cells by an analysis of morphology, karyotype, immunophenotype, Southern blot, Northern blot, and protein expression. Two T-ALL cell lines with NK activity (SPI 801, SPI 802) were demonstrated to be contaminated with the K-562 cell line by analysis of the gene rearrangement pattern on Southern blot analysis [48,90]. Using karyotype analysis, it was found that KE-37 was contaminated with SKW3 cells, because both shared the t(8:14)(q24:q11) which was lacking in the original KE-37 cells [93]. Evaluation of established cell lines by DNA fingerprinting, immunophenotyping, karyotyping, and isoenzyme analysis has helped to identify cell line cross contamination and mislabeling [61]. At least one stock of the cell line EU-7 has been shown to be cross-contaminated with CCRF-CEM, emphasizing the need to screen stocks of EU-7 by a method such as DNA profiling.

2. CLINICAL CHARACTERISTICS

The first leukemia cell line was established by Dr. George Foley in 1964 [45]. In vitro cell cultures were produced from the peripheral blood of a two-year-old girl with leukemia. The resultant CCRF-CEM cell line was subsequently found to have a T-cell phenotype. A series of T-ALL cell lines (the MOLT lines) has been established by Dr. Jun Minowada [106]. The availability of the CEM and MOLT lines has been critical to progress in understanding the cellular and molecular biology of T-ALL, and the pioneering work of these investigators in establishing and characterizing those cell lines has greatly

contributed to leukemia research. The RPMI 8402 cell line [113], the Jurkat cell line [146], and the KARPAS 45 cell line [76] have also been widely distributed. The MOLT 1-4 lines were established simultaneously from the same patient (a 19-year-old male) at first relapse [106]. While numerous studies of MOLT 3 and 4 have been reported, MOLT 1 and 2 have been less well characterized.

The clinical characteristics of the 44 T-ALL cell lines are summarized in Table la. One cell line was established in the 1960s, 9 cell lines in the 1970s, 26 in the 1980s, and 8 in the 1990s. Corresponding to the low incidence of T-ALL in adults, most of the cell lines were derived from children (median age 11 years) and the sex distribution was predominantly male. Interestingly, most of the cell lines were established at relapse and only eleven cell lines were established at the time of diagnosis. Twenty cell lines were cultured from bone marrow samples, eighteen from peripheral blood and one from pleural effusion. The origin of the cell line from the patient was verified in a relatively high proportion of cases. Generally this was accomplished by a direct comparison between the patient's leukemia cells and the established cell line (growing continuously for >12 months) by comparing the monoclonal antibody profile, karyotype or the Southern blot *TCR* rearrangement profile. The medium used was predominantly RPMI -1640 with 10–20% fetal calf serum. While McCoy's 5a medium (and a hypoxic environment) was used for the initial growth of SUP-T cells, the cells grew in RPMI -1640 with 10% fetal calf serum once established [154]. Many T-ALL cell lines (e.g. CCRF-CEM, Jurkat, MOLT-4, etc.) are available from the American Type Cell Culture (ATCC) Rockville, MD and international cell banks.

The clinical characteristics of the NK cell lines are summarized in Table Ib. Natural killer (NK) cells represent a small proportion of the peripheral blood lymphocytes that frequently display azurophilic granules and thus have been named large granular lymphocytes (LGLs). Detailed phenotypic and genotypic studies of LGLs demonstrate that there are two distinct cell populations: LGLs which are CD3–, CD16+, CD56+ and lack rearrangement of the *TCR* genes and LGLs which are CD3+, CD 16+, CD56+ and possess rearrangement of the *TCR* genes [96]. Patients with CD3+ LGL leukemias often exhibit specific clinical manifestations such as rheumatoid arthritis, recurrent bacterial infections, chronic neutropenia and pure red cell aplasia. Patients with CD3– LGLleukemias commonly present with hepatosplenomegaly, lymphadenopathy, skin involvement, and bone marrow infiltration [96]. The first cell line with natural killer (NK) activity (YT cells) was established in 1983 [172]. Five NK cell lines have been reported during the past decade, established from peripheral blood from adults with features of leukemia. Interleukin-2 (or conditioned media) was added to the initial culture

Table 1a. T-ALL cell lines: clinical characteristics

Cell line[a]	Patient age/sex[b]	Diagnosis[c]	Treatment status	Specimen site[d]	Authenti-cation[e]	Year est.	Culture medium[f]	Availability[g]	Primary ref.
BE-13	11 F	T-ALL	Relapse	BM		1981	RPMI 1640 + 20% FCS	DSMZ	46
CCRF-CEM[h]	2 F	T-ALL	Relapse	PB		1964	Eagle MEM + 10% FCS	ATCC, CCR, JCRB	45
DND-41	13 M	T-ALL	Not stated	Not stated		1977	RPMI 1640 + 12% FCS	Author	36,98
DU.528	16 M	T-ALL	Relapse	PB	Yes	1985	RPMI 1640 + 10% HS + 10% FCS	Author	91
EU-7[9]	16 F	T-ALL	Relapse	BM	Yes	1991	RPMI 1640 + 20% FBS	Author	175
EU-9	8 M	T-ALL	Relapse	BM	Yes	1993	RPMI 1640 + 20% FBS	Author	175
HPB-ALL	14 M	T-ALL	Diagnosis	PB		1973	RPMI 1640 + 20% FBS	Author	114
JM/JURKAT	14 M	T-ALL	Relapse	PB		1974	RPMI 1640 +10% FCS	ATCC, ECACC	146 36
KARPAS 45	2 M	T-ALL	Not stated	BM	Yes	1977	RPMI 1640 + 10% FCS	Author	76
KE-37	27 M	T-ALL	Not stated	Not stated		1985	RPMI 1640 + 5% FCS	DSMZ	36
KH-1	9 M	T-ALL	Relapse	PB		1982	RPMI 1640 + 20% FCS	Author	115
K-T1	16 M	T-ALL	Relapse	BM	Yes	1982	McCoy 5A + 15% FCS	Author	152
L-KAW	6 M	T-ALL	Relapse	PB	Yes	1990	RPMI 1640 + 10% FCS	Author	104
Loucy	38 F	T-ALL	Relapse	PB	Yes	1990	RPMI 1640 + 30% FBS	DSMZ	11
MOLT-1[i]	19 M	T-ALL	Relapse	PB		1971	RPMI 1640 + 10% FBS		106

Continued on next page

Table 1a. (continued)

Cell line[a]	Patient age/sex[b]	Diagnosis[c]	Treatment status	Specimen site[d]	Authenti-cation[e]	Year est.	Culture medium[f]	Availability[g]	Primary ref.
MOLT-2-4								ATCC, DSMZ, ECACC, JCRB, RGB	
MOLT-12[j]	2 F	T-ALL	Diagnosis	BM	Yes	1983	RPMI 1640 + 10% FBS	Author	39,110
MOLT-13	2 F	T-ALL	Relapse	BM	Yes	1983	RPMI 1640 + 10% FBS	Author	39,110
MOLT-14	2 F	T-ALL	Relapse	BM	Yes	1983	RPMI 1640 + 10% FBS	ATCC	39,110
MOLT-16[k]	5 F	T-ALL	Relapse	PB	Yes	1985	RPMI 1640 +10% FCS	DSMZ	39,110
MOLT-17	5 F	T-ALL	Relapse	PB	Yes	1985	RPMI 1640 + 10% FCS	DSMZ	39,110
MT-ALL	15 M	T-ALL	Relapse	PB	Yes	1989	IMDM with 20% FCS	Author	56,57
P12/Ichikawa	7	T-ALL	Not stated	PB		1978	Nude Mice	DSMZ	168
P30/Ohkubo	11 F	T-ALL	Relapse	BM	Yes	1982	RPMI 1640 + 10% FCS	JCRB	66
PEER	4 F	T-ALL	Relapse	PB	Yes	1977	RPMI 1640 + 20% FCS	DSMZ, JCRB	136
PER 117	1 M	T-ALL	Relapse	BM	Yes	1987	RPMI 1640 + 20% FCS	Author	77
PER 255	5 M	T-ALL	Diagnosis	BM	Yes	1989	RPMI 1640 + Human Serum	Author	79
PER 423	5 M	T-ALL	Diagnosis	BM	Yes	1993	RPMI-1640 + FCS + IL-2	Author	80
PF-382[l]	6 F	T-ALL	Relapse	PE		1985	RPMI 1640 + 10% FCS	DSMZ	130

Continued on next page

Table 1a. (continued)

Cell line[a]	Patient age/sex[b]	Diagnosis[c]	Treatment status	Specimen site[d]	Authentication[e]	Year est.	Culture medium[f]	Availability[g]	Primary ref.
RPMI 8402	16 F	T-ALL	Relapse	PB		1972	RPMI 1640 + 20% FCS	DSMZ, CCR	71,113
SUP-T2	52 F	T-ALL	Diagnosis	BM	Yes	1984	McCoy 5A + 15% FCS	Author	152
SUP-T3	12 M	T-ALL	Relapse	PB	Yes	1984	McCoy 5A + 15% FCS	Author	152
SUP-T6	7 M	T-ALL	Diagnosis	BM	Yes	1986	McCoy 5A + 15% FCS	Author	153
SUP-T7	19 M	T-ALL	Diagnosis	BM	Yes	1985	McCoy 5A + 15% FCS	Author	153
SUP-T8	8 F	T-ALL	Relapse	BM	Yes	1987	McCoy 5A + 15% FCS	Author	154
SUP-T9	10 F	T-ALL	Relapse	BM	Yes	1987	McCoy 5A + 15% FCS	Author	154
SUP-T10	8 M	T-ALL	Relapse	BM	Yes	1987	McCoy 5A + 15% FCS	Author	154
SUP-T12	17 M	T-ALL	Diagnosis	PB	Yes	1987	McCoy 5A + 15% FCS	Author	154
SUP-T13	3 F	T-ALL[m]	Relapse	PB	Yes	1987	McCoy 5A + 15% FCS	Author	154
SUP-T14	6 M	T-ALL	Diagnosis	BM	Yes	1987	McCoy 5A + 15% FCS	Author	154
TALL-1 (a)	28 M	T-ALL	Relapse	BM		1976	RPMI 1640 + 20% FCS	Author	111
TALL-1 (b)	4 M	T-ALL	Relapse	Not stated	Yes	1987	IMDM + 20% FBS	Author	92
TALL-101	9 M	T-ALL	Relapse	Not stated	Yes	1987	IMDM + 20% FBS + GM-CSF	Author	92
TALL-103/2[n]	6 M	LL[o]	Relapse	BM	Yes	1988	IMDM + 10% FBS + IL-2	Author	121
TALL-104	2 M	T-ALL	Relapse	PB	Yes	1991	IMDM + 10% FBS + IL-2	ATCC	122
TALL-105	9 F	T-ALL	Diagnosis	BM	Yes	1991	IMDM + 10% FBS	Author	122

Continued on next page

Table 1a. (continued)

Cell line[a]	Patient age/sex[b]	Diagnosis[c]	Treatment status	Specimen site[d]	Authentication[e]	Year est.	Culture medium[f]	Availability[g]	Primary ref.
TALL-106	15 M	T-ALL	Diagnosis	BM	Yes	1991	IMDM + 10% FBS + IL-3	Author	122
UHKT-42	12 M	AUL[p]	Relapse	PB	Yes	1988	RPMI 1640 + 20% FCS	Author	157

[a] Names of cell lines are indicated as given in the original literature. Subclones (variant cell lines derived from a parental cell line) and sister cell lines (derived independently from the same patient from different specimens or at different time points) are indicated for each cell line.
[b] Age at the time of establishment of cell line.
[c] Diagnosis is indicated as given in the original literature.
[d] BM – bone marrow; PB – peripheral blood; PE – pleural effusion.
[e] Evidence (eg. cytogenetic marker chromosomes, immunophenotype, others) that the cell line was derived from the patient indicated.
[f] Culture medium as indicated in the original literature; cell line may grow with other media and/or supplements.
[g] Available from cell banks (American Type Culture Collection ATCC; Coriell Cell Repository CCR; Deutsche Sammlung von Mikroorganismen und Zelkulturen DSMZ; European Collection of Animal Cell Cultures ECACC; Japanese Collection of Research Bioresources JCRB; and Riken Gene Bank RGB), or from the original investigator.
[h] CCRF-CEM C1 and CCRF-CEM-C7 are sublines of CCRF-CEM.
[i] MOLT 2-4 are subclones of MOLT 1.
[j] MOLT 13-14 are sister cell lines which were established from the same patient as MOLT 12.
[k] MOLT 17 is a sister cell line which was established from the same patient as MOLT 16.
[l] GI-CO-T-9 is a PF-382 subclone which was selected because of the elaboration of factors which suppress T-cell proliferation [131].
[m] Patient originally incorrectly reported as lymphoblastic lymphoma.
[n] TALL-103/2 is a T-lymphoid subclone of TALL 103/3. TALL 103/3 is an IL-3 dependent cell line which has lost T-cell specific markers and acquired a myeloid phenotype (CD15+, CD33+) [121].
[o] LL – lymphoblastic lymphoma, cell line established from BM in leukemic phase at relapse.
[p] AUL – acute undifferentiated leukemia.
[q] At least one stock of EU-7 cells has been found to be in fact CCRF-CEM.

Table 1b. NK cell lines: clinical characterization

Cell line[a]	Patient age/sex[b]	Diagnosis[c]	Treatment status	Specimen Site[d]	Authenti-cation[e]	Year est.	Culture medium[f]	Availability[g]	Primary ref.
NK-92	50 M	NK-NHL	Not stated	PB	Yes	1994	αMEM + 15% FCS+ 12.5% HS + IL-2+ Hydrocortisone	Author	51
NKL	62 M	Chronic leukemia LGL	Relapse	PB	Yes	1994	RPMI 1640 + 15% AB Serum + IL-2	Author	139
NOI 90	45 M	NK-NHL	Diagnosis	PB	Yes	1990	RPMI 1640 + 10% FCS	Author	142
TKS-1	21 M	NK cell leukemia	Not stated	PB	Yes	1992	RPMI 1640 + 10% FCS + IL-2	Author	89
YT[h]	15 M	LL[i]	Not stated	Pericardial effusion	Yes	1983	RPMI 1640 + 10% FCS	Author	172
YT2C2						1985	RPMI 1640 + 10% FCS	Author	160,173
YT3C						1984	RPMI 1640 + 10% FCS	Author	172,173

[a] Names of cell lines are indicated as given in the original literature. Subclones (variant cell lines derived from a parental cell line) and sister cell lines (derived independently from the same patient from different specimens or at different time points) are indicated for each cell line.
[b] Age at the time of establishment of cell line.
[c] Diagnosis is indicated as given in the original literature.
[d] BM: bone marrow; PB: peripheral blood.
[e] Evidence (eg. cytogenetic marker chromosomes, immunophenotype, others) that the cell line was derived from the patient indicated.
[f] Culture medium as indicated in the original literature; cell line may grow with other media and/or supplements.
[g] Available from the original investigator.
[h] YT2C2 and YT3C are subclones of YT.
[i] LL – lymphoblastic leukemia.

media in the NK cell cultures and the NK cell line (NOI-90) was found to have an IL-2 autocrine loop.

3. IMMUNOPHENOTYPE

T-ALL cases have distinctive immunophenotypes that generally reflect their origin from cells at different stages of intrathymic differentiation. Reinherz and others have proposed models of thymic maturation based on expression of surface antigens, which have been useful for classification of T-ALL [137,107]. This classification was subsequently modified after the introduction of additional Leukocyte Workshop antibodies and Cluster Designation (CD) categories [30,134]. T-ALL/thymocyte-maturation stages include the early thymocyte phenotype (stage I), characterized by expression of CD2, 5 and 7 without CD1, 3, 4 or 8; the intermediate thymocyte phenotype (stage II), with expression of CD1, 2, 5 and 7 and variable expression of CD3, 4 and 8; and the mature thymocyte phenotype (stage III), expressing CD2, 3, 5 and 7 and in most cases either CD4 or 8 (the latter antigens generally being expressed singly rather than in combination). CD1 (the common thymocyte antigen) is negative in stage I and III cases, and therefore its presence is diagnostic for the stage II intermediate (or common) thymocyte group. CD3 may be weakly expressed on the cell surface in some stage II cases, or may be present only in the cytoplasm. Also, stage II cases may express both CD4 and CD8. However, some T-ALL cell lines and primary T-ALL cells do not fit into any one category due to their heterogeneity in antigen expression.

During T-cell differentiation, TCR genes rearrange to permit receptor expression and diversity. Two types of *TCR* molecules have been identified on the surface of T-ALL and normal T-cells, both of which consist of a polymorphic heterodimer of two polypeptide chains $\alpha\beta$ or γ/δ. In normal T-cells, the *TCR* is linked to a monomorphic glycoprotein complex (CD3). In T-ALL, however, CD3 is commonly expressed in the cytoplasm but not on the cell surface, indicating transformation at an immature stage of development [167]. T-ALL cell lines lacking surface CD3 are generally also negative for surface *TCR* [16].

MOLT-3 and -4 belong to the intermediate thymocyte category (Stage II), based on expression of CD1, 2, 5 and 7 on >50% of the cells. CD4 and 8 are expressed on a minority of cells, and CD3 is negative [110]. MOLT-12 also has the intermediate thymocyte phenotype (Stage II thymocyte), characterized by expression of CD1, 2, 5, and 7 [39,110]. Unlike MOLT-3 and -4, MOLT-12 also expresses surface CD3 on >50% of the cells. MOLT-13 and -14 differ from MOLT-12 in having lost expression of CD1 and 2; since MOLT-13 and -14 were established from the same patient as MOLT-

12 after relapse, this antigen loss may represent the appearance of a less mature phenotype at relapse [39]. Based on the absence of CD1, 2, 4 and 8, MOLT-13 and -14 appear to have an immature thymocyte phenotype (Stage I). However, both MOLT-13 and -14 express surface CD3 on at least 50% of cells, which is associated with a more differentiated phenotype (Stage II or III); therefore, it is difficult to assign these lines to a precise category. MOLT-16 appears to represent a similar stage of differentiation (Stage I) based on absence of CD1, 4, and 8, and the presence of CD2, 5 and 7 [39]. However, as in the case of MOLT-13 and -14, the presence of surface CD3 on >50% of these cells complicates the assignment, and this line has features of Stage II (intermediate thymocyte), except for a lack of CD1.

The immunophenotypes of the T-ALL cell lines are summarized in Table 2a. Most of the T-ALL cell lines express both CD5 (31/37) and CD7 (37/39) and many express CD1 (19/43), CD3 (23/44), or CD4 (25/43). Several T-ALL cell lines express CD2 (29/41) and about 70% express CD8 (25/40). Of the 25 cell lines that express CD8, most also express CD2 (21/25) and dual expression of CD2/CD8 is associated with a mid-thymocyte stage of T-cell development [137]. When evaluated, T-ALL cell lines have rearrangements of the *TCR* genes ($\alpha/\beta/\gamma/\delta$) and many express the *TCR* α/β or γ/δ on the cell surface. While CD10 expression was detected on 20% (7/34) of the T-ALL cell lines tested, other B lymphocyte markers are not expressed, with the exception of MOLT-3 and -4 which express the B-lineage marker CD9. Myelomonocytic markers are uniformly negative.

T-ALL cells are generally TdT positive, a feature shared with normal cortical thymocytes but not mature lymphocytes [37]. Most of the cell lines express TdT (25/34) and are HLA-DR negative (31/33). Only 2 cell lines express the interleukin-2 receptor CD25 (TALL-101, TALL- 103/2).

The immunophenotypic characteristics of the NK cells are summarized in Table 2b. Two populations of normal LGLs have been described: CD3–, CD 16+, CD56+, TCR– germline and CD3+, CD 16+, CD56+, *TCR*– rearranged. The YT and NK92 cells are CD3–, CD16–, CD56+; NKL cells are CD3–, CD16–, CD56–; TKS1 cells are CD3–, CD16+, CD56– and NOI90 cells are CD3+, CD16–, CD56+. All of the NK cell lines (except YT subclones YT2C2) express CD25 and proliferate in response to IL-2 or conditioned media. The YT2C2 cell line was specifically subcloned to provide cells that lack CD25 for comparative analysis with CD25+ YT cells [173]. While YT cells possess EBV, it is interesting to note that the cells lack CD21 which is the putative EBV receptor. All the NK cell lines are CD4– but express the pan-T cell antibodies CD7, while lacking B-lymphoid and myelomonocytic markers. Only the TKS1 cells express CD16.

Table 2a. T-ALL cell lines: immunophenotype characteristics

Cell line	T-/NK-cell marker[a]	T-cell receptor[b]	B-cell marker	Myelomonocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
BE-13	CD1+ CD8− CD3+ CD4+		sIg−				46
CCRF-CEM[c]	CD1− CD5+ CD2+ CD7+ CD3− CD8− CD4+	TCRα R/G TCRβ G/R TCRγ R/R TCRδ D/D TCRα, β, γ expressed on northern blot	CD9− cIg− CD10+ sIg− CD19− CD20− CD21− CD24−		CD18+ CD25− CD28+ CD71+ HLA-DR− TdT+		112, 54, 94, 16
DND-41	CD1+ CD5+ CD2+ CD7+ CD3+ CD8− CD4+	TCRα G/G TCRβ G/R TCRγ G/R TCRδ G/R TCRβ, γ, δ expressed by northern blot	CD9− CD10+ CD24−		CD18+ CD25− CD28+ HLA-DR− TdT+		4, 98, 94
DU.528[d]	CD1− CD7+ CD2− CD8− CD3− CD4−	TCRα R TCRβ R TCRγ R TCRδ R		CD14−	CD25+ CD34(+) CD71+ HLA-DR− TdT−		91, 8, 112

Continued on next page

Table 2a. (continued)

Cell line	T-/NK-cell marker[a]	T-cell receptor[b]	B-cell marker	Myelomonocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
EU-7[f]	CD1+ CD2− CD5+ CD3+ CD7+ CD4+ CD8+		CD10+ CD19− CD20− CD24−	CD13− CD15+ CD33−	CD34− CD38+ HLA-DR− TdT+		175
EU-9	CD1+ CD2+ CD5+ CD3− CD7+ CD4− CD8+		CD10− CD19− CD20− CD24−	CD13− CD15+ CD33−	CD34− CD38+ HLA-DR− TdT+		175
HPB-ALL	CD1+ CD5+ CD2+ CD7+ CD3+ CD8+ CD4+	TCRγ R	CD9− cIg− CD10+ sIg− CD19− CD20− CD21− CD24−		HLA-DR− TdT+		84, 12
JM/JURKAT	CD1+ CD5+ CD2+ CD7+ CD3+ CD8+ CD4+	TCRγ R	CD9+ CD10− CD24−		HLA-DR− TdT+		36, 84, 94
KARPAS 45	CD1− CD5(+) CD3− CD8+ CD4+		CD10− sIg−				76, 26

Continued on next page

Table 2a. (continued)

Cell line	T-/NK-cell marker[a]	T-cell receptor[b]	B-cell marker	Myelomonocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
KE-37	CD1− CD5+ CD2− CD7+ CD3− CD8− CD4+	TCRγ R	CD9− CD10− CD24−		CD30+ HLA-DR− TdT−		36, 84, 94, 58
KH-1	CD1+ CD5+ CD2+ CD8+ CD3+ CD4+		sIg−		CD71−		115
K-T1	CD1− CD5− CD2+ CD7+ CD3+ CD8+ CD44		cIg− sIg−		CD38+ CD71+ HLA-DR− TdT+		152
L-KAW	CD1− CD5+ CD2+ CD7+ CD3+ CD8+ CD4− CD56− CD57−	TCRα E TCRβ E	CD10− CD19− CD20− CD21−	CD13− CD14− CD16− CD33−	CD18+ CD25− CD28+ CD34+ CD45 RA− CD45 RO+ CD71+ HLA-DR−	CD11a+	104
LOUCY	CD1− CD4+ CD2− CD8+ CD3+		CD10+ cIg− CD19− sIg−		CD25− TdT−	CD11b−	11

Continued on next page

Table 2a. (continued)

Cell line	T-/NK-cell marker[a]	T-cell receptor[b]	B-cell marker	Myelomonocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
MOLT-3[e]	CD1+ CD5+ CD2+ CD7+ CD3− CD8(+) CD4+ CD57(+)	TCRβ R TCRγ G	CD9+ CD10− sIg− CD19−	CD13−	HLA-DR− TdT+	CD11a+ CD11b−	55, 108, 109, 156, 16
MOLT-4	CD1+ CD5+ CD2− CD7+ CD3− CD8+ CD4+ CD57+	TCRβ R TCRγ G	CD9+ CD10− sIg− CD19− CD24−	CD13−	HLA-DR− TdT+	CD11a−	55, 84, 108, 109, 156, 144
MOLT-12	CD1+ CD5+ CD2+ CD7+ CD3+ CD8+ CD4−		CD10− CD19− sIg− CD20−	CD13− CD15−	CD25− CD38+ HLA-DR− TdT+	CD11b−	39, 110
MOLT-13	CD1− CD5+ CD2− CD7+ CD3+ CD8 CD4−	TCRγ E TCRδ E	CD10− CD19− sIg− CD20−	CD13− CD15−	CD25− CD38+ CD147+ HLA-DR− TdT+	CD11b−	39, 110, 88, 85, 31, 32
MOLT-14	CD1− CD5+ CD2− CD7+ CD3+ CD8− CD4−	TCRγ E TCRδ E	CD10− sIg− CD19− CD20−	CD13− CD15−	CD25+ CD38+ HLA-DR− TdT+	CD11b−	39, 110, 88, 31, 32

Continued on next page

Table 2a. (continued)

Cell line	T-/NK-cell marker[a]	T-cell receptor[b]	B-cell marker	Myelomonocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
MOLT-16	CD1− CD5+ CD2+ CD6+ CD3+ CD7+ CD4− CD8−	TCRα E TCRβ E	CD10− sIg− CD19− CD20−	CD13− CD15− CD16−	CD25− CD28− CD38− HLA-DR− TdT+	CD11b−	39, 110, 88, 16, 32
MOLT-17	CD1- CD5+ CD2+ CD6+ CD3+ CD7+ CD4+ CD8−	TCRα E TCRβ E	CD10− CD19− CD20−	CD13− CD15−	CD25− CD38+ HLA-DR− TdT+	CD11b−	39, 88, 16
MT-ALL	CD5− CD2+ CD7+ CD3+ CD8− CD4−	TCRα E TCRβ E		CD13+ CD14− CD33−	CD25−		57, 56, 74
P12/Ichikawa	CD1+ CD5+ CD2+ CD7+ CD3− CD8+ CD4+		CD9− CD10− CD24−		CD30+ HLA-DR− TdT+		168, 58
P30/Ohkubo	CD1− CD5+ CD3− CD8− CD4−	TCRβ G TCRγ G	CD10+ cIg− sIg−		HLA-DR+ TdT+		66, 84

Continued on next page

Table 2a. (continued)

Cell line	T-/NK-cell marker[a]	T-cell receptor[b]	B-cell marker	Myelomonocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
PEER	CD1− CD5+ CD2− CD7+ CD3+ CD8− CD4+	TCRα G/G TCRβ G/R TCRγ G/R TCRδ R/G TCRβ, γ, δ expressed on northern blot	CD9+ CD10− CD24−		CD18+ CD25− CD28+ HLA-DR+ TdT−		4, 94
PER 117	CD1− CD7+ CD2− CD8− CD3− CD4−			CD15−	CD38+ CD71− HLA-DR− TdT−		77
PER 255	CD1+ CD5− CD2+ CD6− CD3− CD7− CD4+ CD8(+)	TCRβ− G/R	CD10− CD19− CD20−		CD38+ CD71− TdT+	CD11b−	79
PER 423	CD1− CD5− CD2− CD6− CD3− CD7+ CD4− CD8+ CD57+	TCRβ− R	CD10− CD19−	CD16−	CD25− CD122+ HLA-DR−	CD11b(+)	80

Continued on next page

Table 2a. (continued)

Cell line	T-/NK-cell marker[a]	T-cell receptor[b]	B-cell marker	Myelomonocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
PF-382	CD1+ CD5+ CD2+ CD7+ CD3− CD4− CD8+		CD9+ CD10− CD24−		TdT−		130
RPMI 8402	CD1− CD5+ CD2+ CD7+ CD3− CD8− CD4+	TCRγ R	CD10+		HLA-DR− TdT+		71, 84
SUP-T2	CD1− CD5+ CD2+ CD7+ CD3− CD8+ CD4−		CD10− sIg−	CD13− CD33−	CD38− CD71+ HLA-DR− TdT+		151
SUP-T3	CD1+ CD5+ CD2+ CD7+ CD3− CD8+ CD4+		CD10− sIg−	CD13− CD33−	CD25− CD38+ CD71− HLA-DR− TdT+		151
SUP-T6	CD1− CD5+ CD2+ CD7+ CD3− CD8+ CD4−	TCRβ R CRγ R	CD10− sIg−	CD13− CD33−	CD25− HLA-DR− TdT+		154

Continued on next page

Table 2a. (continued)

Cell line	T-/NK-cell marker[a]	T-cell receptor[b]	B-cell marker	Myelomonocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
SUP-T7	CD1− CD5+ CD2− CD7+ CD3+ CD4+ CD9+	TCRβ R	CD10− sIg−	CD13− CD33−	CD25− CD38+ CD71+ HLA-DR− TdT+		153
SUP-T8	CD1− CD5+ CD2− CD7+ CD3− CD8− CD4+	TCRβ G/G TCRγ G/G	CD10− sIg−	CD13− CD33+	CD25− HLA-DR− TdT−		154
SUP-T9	CD1+ CD5+ CD2+ CD7+ CD3− CD8+ CD4+	TCRβ R TCRγ R	CD10− sIg−	CD13− CD33−	CD25− HLA-DR− TdT+		154
SUP-T10	CD1− CD5− CD2− CD7+ CD3− CD8− CD4−	TCRβ G/G TCRγ G/G	CD10− sIg−	CD13− CD33−	CD25− HLA-DR− TdT+		154
SUP-T12	CD1+ CD5+ CD2+ CD7+ CD3− CD8+ CD4+	TCRβ R TCRγ R	CD10− sIg−	CD13− CD33−	CD25− HLA-DR− TdT+		154

Continued on next page

Table 2a. (continued)

Cell line	T-/NK-cell marker[a]	T-cell receptor[b]	B-cell marker	Myelomonocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
SUP-T13	CD1− CD5+ CD2+ CD7+ CD3+ CD8+ CD4+	TCRβ G/G TCRγ R	CD10− sIg−	CD13− CD33−	CD25− HLA-DR− TdT+		154
SUP-T14	CD1− CD5+ CD2+ CD7+ CD3+ CD8− CD4+	TCRβ G/G TCRγ R	CD10− sIg−	CD13− CD33−	CD25− HLA-DR− TdT+		154
TALL-1(a)	CD1+ CD5+ CD2+ CD7+ CD3+ CD8+ CD4+		CD9− CD10− CD24−		HLA-DR− TdT+		36, 156, 94
TALL-1(b)	CD1+ CD5+ CD2+ CD7+ CD3+ CD8+ CD4+		CD10−		HLA-DR− TdT+		92
TALL-101	CD1− CD5− CD2− CD7− CD3−		CD10− CD19−	CD16+ CD38+	CD25+ CD71− TdT−	CD11b+	164
TALL-103/2	CD1− CD2+ CD7+ CD3+ CD8+ CD4− CD56+	TCRγ E TCRδ E	CD34− CD38+	CD14− CD15− CD33+	CD71+ HLA-DR− IL-2α+ IL-2β+	CD11b− CD16− CD64−	121, 122

Continued on next page

Table 2a. (continued)

Cell line	T-/NK-cell marker[a]	T-cell receptor[b]	B-cell marker	Myelomonocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
TALL-104	CD2+ CD7+ CD3+ CD8+ CD4− CD56+	TCRα E TCRβ E		CD33− CD45−	CD34−	CD11b−	122
TALL-105	CD1+ CD2+ CD7+ CD3+ CD56− CD4−	TCRα E TCRβ E		CD15− CD33−	IL-2α− IL-2β− CD34+	CD11b−	122
TALL-106	CD1+ CD2+ CD7+ CD3+ CD56− CD4(+)	TCRα E TCRβ E		CD15− CD33−	IL-2α− IL-2β− CD34+	CD11b+	122
UHKT-42	CD1a− CD5+ CD1b− CD7+ CD2− CD8− CD3− CD56− CD4− CD57−	TCRβ R TCRδ R	CD10− sIg− CD19− CD20− CD21− CD24−	CD13− CD14− CD15− CD16− CD33− CD41a− CD61−	CD25− CD28+ CD34+ CD38+ CD45 RO+ CD71+ HLA-DR− TdT−		157

[a] + – strong, definite protein expression (more than 10% of cells positive); (+) – weak protein expression qualitatively and quantitatively, less than 10% cells positive; (−) – no protein expression; all immunophenotyping is for surface expression. [b] TCR – T cell receptor, R – rearranged, G – germ line, D – deleted, E – expressed on cell surface. [c] CCRF-CEM cells are cytoplasmic CD3+ and surface CD3− [16]. [d] DU.528 is a stem cell line with the capacity to produce cells of the T-lymphoid, granulocyte/monocyte and erythroid lineage. [e] MOLT 2-4 are subclones of MOLT-1. Comprehensive immunophenotype and other data have not been reported for MOLT 1 and 2. [f] At least one stock of EU-7 cells has been found to be in fact CCRF-CEM, and this cell line should not be used unless the stocks available are shown to be distinct from CCRF-CEM.

Table 2b. NK cell lines immunophenotypic characteristics

Cell line	T-/NK-cell marker[a]	T-cell receptor[b]	B-cell marker	Myelo-monocytic marker	Progenitor/ activation marker	Adhesion marker	Ref.
NK92	CD1− CD7+ CD2+ CD8− CD3− CD28+ CD4− CD56+ CD5−	TCRβ G TCRγ G	CD10− CD19− CD20− CD23−	CD14− CD16−	CD25+ CD34− CD45+ CD54+ CD122+ HLA-DR−	CD11a+	51
NKL	CD1− CD7− CD2+ CD8− CD3− CD26+ CD4− CD27+ CD5− CD28− CD6+ CD56− CD57−	TCRα/β not expressed TCRγ/δ not expressed	CD19− CD20−	CD14− CD16− CD29+	CD25+ CD38+ CD69− CD71+ CD81+ CD94+ CD95+ HLA-DR+	CD11a+ CD11b− CD43+	139
NOI 90	CD1 (+) CD7+ CD2− CD8− CD3+ CD56+ CD4 (+) CD57+ CD5−	TCRβ G TCRγ G	CD10−	CD13− CD14− CD16−	CD25 (+) CD34−		142
TKS1	CD2+ CD5− CD3− CD8− CD4− CD56− CD57−	TCRβ G TCRγ G	CD10− CD19− CD20−	CD16+	CD25+ CD122−		89
YT	CD1− CD5− CD2− CD6− CD3− CD7+ CD4− CD8− CD5− CD28+ CD56+ CD57−	TCRβ G TCRγ G TCRδ G	CD10− CD19− CD20− CD21− CD23− CD24−	CD13− CD15− CD16−	CD25+ CD30+ CD33− CD38− CD45RA− CD45RO+ HLA-DR+	CD11b−	172,173
YT2C2	CD1− CD2− CD6− CD3− CD7+ CD4− CD8− CD5− CD28+ CD56+ CD57−	TCRβ G TCRγ G TCRδ G	CD10− CD19− CD20− CD21− CD23− CD24−	CD13− CD15− CD16−	CD25− CD30+ CD33− CD38− CD45RA− CD45RO+ HLA-DR+	CD11b−	173,160
YT3C	CD1− CD2− CD6− CD3− CD7+ CD4− CD8− CD5− CD28+ CD56+ CD57−	TCRβ G TCRγ G TCRδ G	CD10− CD19− CD20− CD21− CD23− CD24−	CD13− CD15− CD16−	CD25+ CD30+ CD33− CD38− CD45RA− CD45RO+ HLA-DR+	CD11b−	173,172,160

[a] + – strong definite protein expression (more than 10% of cell positive); (+) – weak protein expression qualitatively and quantitatively, less than 10% cells positive; − – no protein expression.

[b] TCR = T cell receptor, R – rearranged, G – germ line, D – deleted, E = expressed on cell surface.

4. CYTOKINE-RELATED CHARACTERIZATION

Cytokine receptor expression, cytokine production, cytokine induced proliferation and differentiation and cytokine dependency of T-ALL cell lines are summarized in Table 3a. Of note is that 39 of the 44 T-ALL cell lines were established without the addition of exogenous cytokines. Five T-ALL cell lines show increased proliferation in response to IL-2 and three cell lines are dependent on IL-2 for cellular proliferation. Phorbol ester induces IL-2R in some T-ALL cell lines and supplemental IL-2 could induce NK-like activity against K562 cells. PER 117, PER 255 and PER 423 express c-kit receptors and PER 423 cells proliferate synergistically when cultured with human stem cell factor (SCF) and IL-2 [82].

MOLT-3 expresses the hemopexin receptor and proliferation is stimulated by heme-hemopexin [155]. PHA induces MOLT-4 cells to secrete IL-2 and IL-4 [31]. MOLT-13, -14, and -16 constitutively produce and secrete IL-2 at low levels (<1 U/ml), and production is increased after stimulation with PHA, interferon (IFN) γ (MOLT-13, -14), IFN α (MOLT-16), and TNF α (MOLT-13, -16) [31,32]). Interestingly, IFN α and γ have reciprocal effects on MOLT-13, -14 compared to MOLT-16: IFN α inhibits IL-2 production by MOLT-13 and -14, while IFN γ augments IL-2 production by MOLT-16. Like MOLT-4, MOLT-13, -14, and -16 also produce low levels (< 1 ng/ml) of IL-4 upon stimulation with PHA [32]. Furthermore, anti-CD7 but not PHA induces expression of GM-CSF by MOLT-13 [18].

MOLT-16 cells express IL-2R, and since these cells also secrete IL-2, the possibility for autocrine growth exists, although this has not been demonstrated [4]. MOLT-14 and -16 also express mRNA for the cholecystokinin (CCK)-B/gastrin receptor (specific for hormones CCK and gastrin) as well as gastrin mRNA. Although the presence of the respective proteins was not examined, proliferation of MOLT-16 is inhibited by a receptor-specific antagonist in the absence of added ligand, suggesting a possible gastrin-mediated autocrine loop [73]. Although differentiation of MOLT cell lines in response to cytokines has not been reported, MOLT-13 acquires NK-like activity against K562 cells following incubation with IL-2 or phorbol ester (PMA)[4].

Dr. Lange and colleagues established several T-ALL cell lines that are dependent on cytokines for continuous cell proliferation. One T-ALL cell line (TALL-101) responds to GM-CSF and IL-3 and is GM-CSF dependent for *in vitro* growth. TALL-103/2 is IL-2 dependent and responds to the cytokines IL-2 and IL-1. Under the influence of IL-2, TALL-103 cells have a T-lymphoid monoclonal antibody profile while TALL-103 cells cultured with IL-3 have a myeloid immunophenotype [20, 145]. TALL-104 cells proliferate in response to IL-2 and are IL-2 dependent. TALL-105 cells proliferate in

Table 3a. T-ALL cell lines: cytokine-related characterization

Cell line	Cytokine receptor expression	Cytokine production	Proliferation response to cytokines	Differentiation response to cytokines	Dependency on cytokines	Ref.
DND-41				IL-2 induced NK like activity to K562 cells.		4
HPB-ALL				Forskolin augmented PMA induced expression of IL-2R		120
MOLT-13	IL-2R induced by treatment with IL-2 or PMA.	IL-2 production in unstimulated and PHA stimulated cultures; increased by addition hIFN- γ and hTNF- α, inhibited by IFNα. Anti CD7 induced transcription of GM-CSF gene.		IL-2 induced NK like activity against K562 cells and induced expression of the β sub-unit of IL-2R.		4,31,32,18
MOLT-14	IL-2R induced by treatment with IL-2 or PMA. Cholecystokinin-B/gastrin receptor expressed; cells demonstrated ligand dependent proliferation in serum- free media.	IL-2 production in unstimulated and PHA stimulated cultures increased by addition of IFNγ, inhibited by IFNα. Cholecystokinin- B/gastrin may act as autocrine factor. IL-4 production followed PHA stimulation.		Treatment with IL-2 or PMA induced NK like activity against K562 cells, expression of IL- 2R.		4,73,31,32,69

Continued on next page

Table 3a. (continued)

Cell line	Cytokine receptor expression	Cytokine production	Proliferation response to cytokines	Differentiation response to cytokines	Dependency on cytokines	Ref.
MOLT-16	IL-2R induced by treatment with IL-2 or PMA. Cholecystokinin-B/gastrin receptor expressed. 68-kD GTP-binding protein immunoprecipitated with TcR/CD-3 complex.	IL-2 production following stimulation with PHA was increased by IL-1, INFα, or TNFα. INFα increased apoptosis following PHA stimulation. Cholecystokinin-B/gastrin may act as an autocrine factor.		Treatment with IL-2 or PMA induced NK-like activity against K562 cells, expression of IL-2 receptor.		4,32,33,68,69, 73,127
PEER				PHA and PMA increased expression of IL-4R, IL-2Rβ and IL-2Rα.		59
PER 117	Expressed low level of c-kit receptor. PMA, IL-1 induced expression of IL-2R.	IL-2. IL-1 required for optimal IL-2 secretion.	IL-2			82,143,78,81
PER 255	Expressed low level of c-kit receptor.					82
PER 423	Tac-Mikβ1+ Expresses c-kit receptor.		Cells proliferated in response to IL-2. Human steel factor acts in synergy with IL-2 to promote cellular proliferation.		IL-2	82,80

Continued on next page

Table 3a. (continued)

Cell line	Cytokine receptor expression	Cytokine production	Proliferation response to cytokines	Differentiation response to cytokines	Dependency on cytokines	Ref.
TALL-101			GM-CSF, IL-3		GM-CSF	92
TALL-103/2		Activation signals induced production of IFNγ, TNF-α, and GM-CSF.	IL-2, IL-1		IL-2	145,20
TALL-104		Activation signals induced production of IFNγ, TNF- α, and GM-CSF.	IL-2		IL-2	20,122
TALL-105			IL-1, IL-7		None	122
TALL-106			IL-2, IL-3, IL-4, IL-6		IL-3	122
UHKT-42	CD25– IL-2Rβ–	PHA and TPA induced IL-2 production.		TPA induced CD-25 expression.		157

IFN – interferon; TNF – tumor necrosis factor; GM-CSF – granulocyte- macrophage colony stimulating factor.

response to IL-1 and IL-7 but continuous cell line growth is growth factor independent. TALL-106 cells respond to IL-2, IL-3, IL-4, and IL-6 and require IL-3 for continuous growth *in vitro* [122].

Cytokine receptor expression, cytokine production, cytokine-induced proliferation and differentiation, and cytokine dependency of NK cell lines are summarized in Table 3b. The NK cell lines generally possess IL-2R and respond to exogenous IL-2. NK92 cells proliferate in response to IL-2 and IL-7 and are dependent on IL-2 for continuous cell proliferation *in vitro* [51]. NOI-90 cells have an autocrine loop with IL-2, and anti-IL-2 antibodies block cell proliferation [142]. The NK92 and TKS1 cells are IL-2 dependent, and YT cells are dependent on conditioned media for continuous cell growth. The YT2C2 cells were subcloned from YT to be IL-2 independent, and YT2C2 cells do not respond to exogenous IL-2.

5. GENETIC CHARACTERIZATION

The genetic characteristics of the T-ALL cell lines are summarized in Table 4a. No established T-ALL cell line has a normal karyotype. While most T-ALL cell lines have complex karyotypes with multiple abnormalities, a few have a single chromosome translocation (EU-9, KH-1, SUP-T7, TALL-101, TALL-104, TALL-105) or certain chromosome deletions (Jurkat, PF-382, SUP-T2, SUP-T14). Many of the 40 karyotyped T-ALL cell lines have common recurring chromosome breakpoints which occur at or near the *TCR* β gene (7q34, N = 6) or the *TCR* α/δ gene (14q11.2, N = 7) or both (N = 1). The t(8;14)(q24;q11) is observed in approximately 2% of T-ALL cases and breakpoints occur within the MYC protooncogene and the *TCR* α gene. The t(8;14)(q24;q11) was demonstrated in MOLT-16, TALL-101, TALL-103, TALL-105, and TALL-106 cell lines. In MOLT-16 cells, c-myc is juxtaposed with the *TCR* α loci [44,101]. Specifically, the MOLT-16 breakpoint at 14(q11) occurs within the joining (J) region of the *TCR* α gene. The *TCR* α constant region and part of the J region are then translocated to the 3′ side of c-myc on chromosome 8 [149]. Cytogenetic analysis has not been reported for MOLT-12, -13 and -14.

The most frequently recurring abnormality in childhood T-ALL is t(11;14)(p13;q11), which accounts for about 7% of cases and this translocation was observed in the TALL-104 cell line [138]. The t(l;14)(q32;q11) occurs in about 3% of T-ALL cases. The breakpoint at 1p32 occurs in TAL1 whereas the breakpoint on 14q11 occurs at a TCR α/δ site, as has been reported in the DU.528 cell line [8,12,44]. The Karpas 45 cell line has a t(X;11)(q13;q23.3) which results in a fusion of the *AFX* gene on chromosome X band q13 to the *MLL* gene [100].

Table 3b. NK cell lines: cytokine-related characterization

Cell line	Cytokine receptor expression	Cytokine production	Proliferation response to cytokines	Differentiation response to cytokines	Dependency on cytokines	Ref.
NK92	IL-2 p55 positive IL-2 p75 positive		IL-2, IL-7 induced cellular proliferation. No proliferation with IL-1, IL-6, TNFα, IFNα, IFNγ		IL-2	51
NKL	IL-2Rα expressed		IL-2		IL-2	139
NOI 90	Low number of high affinity IL-2R	IL-2	No response to exogenous IL-2		Autocrine IL-2 Expressed a low number of high affinity IL-2R with autocrine IL-2 loop.	142
TKS-1	IL-2Rαpositive IL-2Rβ initially positive but negative after 6 months in culture		IL-2		IL-2	89
YT	Inducible high affinity IL-2R IL-2Rβ expressed			PHA/PMA reduced IL-2Rβ expression and induced IL-2Rα. IL-1α promoted expression of IL-2Rα. Forskolin induced both high and low affinity IL-2R	Conditioned media.	172,59,120
YT2C2	IL-2Rα not expressed IL-2Rβ expressed		No response to exogenous IL-2			173,160

Table 4a. T-ALL cell lines: genetic characterization

Cell line	Cytogenetic karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements[a]	Ref.
BE-13	Not reported		No p53 protein, p53 wt, Rb protein expressed	23–25
CCRF-CEM	90–91, XXY, −5, −6, −9, −9, iso (9q) x2, +14, +14, −16, −17, −18, −19, +20, +20, +22, +22, +4 mar 46, X, −X, −9, −18, +20, + der(18)t(X;18)(q12;p11.3), del(8)(p12), del(9)(p21), + der(9)t(9;?)(p23;?)		p53M, Rb protein expressed, p15 P, p16D/D, Rb-wt	23, 65, 176, 40, 14, 99
DND-41	Not reported		p15G/D, p16D/M	14
DU.528	46, XY, del(1)(p33), t(1;14)(p33;q11), del(13)(q14), der(14), 19q+	t(1;14)(p32;q11) results in truncated SCL gene SCL – TCR-δ	Rb protein expressed	23, 12, 64, 8, 44
EU-7	47, XX, +20, t(8;9)(p11.2;p24), del(9)(p22), inv(9)		p16D, p15D, p53M	176, 43, 177
EU-9	46, XY, t(1;7)(p34; q34)	β-TCR-B constant region and LCK gene	p16D, p15P, p53 not expressed	176, 43, 177
HPB-ALL	94-96, XXYY, t(1;5)(q23;q25)x2, del(2)(p22)x2, −3, −3, −9, add(14)(q32), −16, −16, −18, +19, +del(20)(q11)x2, +del(22)(q21)x2, +4mar		p15P, p16P	65, 14

Continued on next page

Table 4a. (continued)

Cell line	Cytogenetic karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements[a]	Ref.
JM/JURKAT	45, X, −Y, del(2)(p23), del(8)(q23)		p53M, p15D/D p16D/D, Rb protein expressed	23, 65, 40, 14
KARPAS 45	84, −Y, −Y, t(X;11)(q13;q23.3), der(X)t(X;11)(q13;q23.3)t(1;5)(q25;q13.1) x2, −2, −3, −4, del(4)(q21.1q31.1), +6, −9, der(11)t(14;11)(11;X)(q11;p13q23.3;q13), −13, −14, +19, −20, −21	AFX-MLL fusion		87, 100, 13
KE-37FN	46, XY, der(9)t(9;?)(p24;?)/45, X, −Y, der(9)t(9;?)(p24;?), +variation			93
KH-1	46, XY, t(8q+;15q−)(8pter→8qter::15q15→15qter)			115
K-T1	46, XY, t(1;11)(p36.2;p13) del(2)(p16.3p22.1), del(9)(p12p21.1)		p16D, p15D, p53 – wt	152, 176
L-KAW	46, XY, −1, −2, +6, −7, −9, −14, −18, del(3)(p23), + der(2)t(2;?)(p23;?), + der(7)t(7;7)(q12;q35), + der(9)t(9;?)(q34;?), + der(14)t(14;?)(q11;?), + mar			104
LOUCY	45, X, −X, del(5)(pter→q15::q35→qter), t(16;20)(p12;q13)		p53M	11, 132

Continued on next page

Table 4a. (continued)

Cell line	Cytogenetic karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements[a]	Ref.
MOLT-3	Hypertetraploid (modal n = 97) XXYY, +4, +6, −7, −7, +8, +8, −9, +11, −14, +15, −18, −18, +20, del(1)(q21;q42), der(2)t(2;2)(p15;q11), del(6)(q13;q21)x2, i(17q), +der(7)x2, t(7;7)(p15;q11)		Expressed alternately spliced P53, p15DG/GG, p16DD/DR, Rb-wt	55, 27, 63, 176, 40
MOLT-4	Hypertetraploid (modal n = 98) XXYY, +4, −7, −7, +8, +11, −14, +15, −18, −18, +20, +20, del(1)(q21;q42), del(6)(q13q21), i(17q), +der(7)x2, t(7;7)(p15;q11)		p16D/R, Expressed alternately spliced p53, Rb protein expressed	23, 55, 123, 27, 63
MOLT-13			p15D, p16D	14
MOLT-14			p16D/R, p15D/R, p53M	123, 23, 14
MOLT-16	45, XX, −7, −9, t(3;11)(q26;p14), t(8;14)(q24;q11), dic(9;15)(p11;p11), + der(7)t(7;7)(7qter→7p15::7q11→7qter), + der(9)t(9;9)(p24;p11)	MYC-TCRα	p53 MRb, protein expressed, p16D/R, p15D/R	14, 39, 149, 101, 123, 23, 75
MOLT-17			p15D, p16D	14
MT-ALL	47, XY, +19, del(6)(q15q25)t(1;10;12)(q25;p13;p13)			56, 57
P12/Ichikawa	Hypotetraploid with an increased number of chromosomes in groups A, C, D, F, and G.		p16D, p15P	168, 14

Continued on next page

Table 4a. (continued)

Cell line	Cytogenetic karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements[a]	Ref.
P30/Ohkubo	46 (45), X (X), del(2)(qter→p23), del(9)(p12→q31), t(11;12)(11pter→11q25::12q13→12qter; 12pter→ 12q13::11q25→11qter)			66
PEER	46, XX, −4, + der(4) rea (4), del(5)(q21q23), del(6)(q14q22), del(9)(p12p21), i(9p)		p16 D, p15 P	99, 14
PER 117	46, XY, t(1;11)(p13; p15), −16, +18			77
PER 255	46, XY, t(7;10)(q32-34;q24), t(9;12)(p22;p12–13)	HOX11 – TCRβ		79, 83
PF-382	46, X, Xq−, 15p+			130
RPMI 8402	100% of the cells possessed 1 or 2 minute chromosomes and 1 subtelocentric chromosome		P53G, Rb protein expressed	23, 71
SUP-T2	46, XX, del(6)(q21q27)			152
SUP-T3	46, XY, del(4)(q31q35), t(7;9)(q34;q32)			153,170, 169
SUP-T6	46, XY, t(7;9)(q34;q32), del(6)(q21)			154
SUP-T7	46, XY, t(7;19)(q34;p13.1)	LYL1 – TCRβ		154, 102, 28

Continued on next page

Table 4a. (continued)

Cell line	Cytogenetic karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements[a]	Ref.
SUP-T8	44, XX, del(1)(p32p35), 1p+, +1p+, 2q+, −4, t(4;19)(q21;p13), 4q−, 7p+, +8, −9, 10p+, del(11)(q21q25), −12, −16, −17, der(17)t(12;17)(p13;p13), 19p+, −22, +mar			154
SUP-T9	46, XX, t(6;14;21)(q23;q11.2;q22), del(11)(q23q25), t(15;21)(q15q22)			154
SUP-T10	47, XY, del(5)(q31), t(7;11)(p13;p13), t(8;12)(q13;p13), t(9;16)(p22;p13), t(17;18)(q11.2;q23), +mar			154
SUP-T12	60, XY, +Y, t(1;7)(p34;q34), +17, +der(1)t(1;7)(p34;q34), +4, +6, +7, +8, +8, +10 +13, +16, +18, +19, +19	LCK – TCRβ		154, 162, 17
SUP-T13	46, XX, t(1;8)(q32;q24), t(1;5)(q41;p11), del(9)(q24q34), t(11;19)(q24;p13)			154
SUP-T14	46, XY, del(6)(q15), del(9)(p22)			154
TALL-1(a)	Hypertetraploid with chromosome numbers ranging from 95 to 101		p15 P, p16 P	111, 14
TALL-1(b)	46, XY, del(6)(q23), t(1;8)(q32;q24)			92

Continued on next page

Table 4a. (continued)

Cell line	Cytogenetic karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements[a]	Ref.
TALL-101	46, XY, t(8;14)(q24;q11), inv(9)			92
TALL-103	47, XY, −9, −13, t(8;14)(q24;q11) der(12)t(12;13)(p11-13;q11-14), +12, +17, +mar			122
TALL104	46, XY, t(11;14)(p13;q11)			122
TALL105	46, XX, t(8:14)(q24;q11)			122
TALL-106	46, t(8;14)(q24;q11), inv(14)(q11q32)			122

[a] G – germ line, M – mutated, D – deleted, wt – wild type, P – DNA present on Southern blot, R – rearranged.

[b] At least one stock of EU-7 cells has been found to be in fact CCRF-CEM, and this cell line should not be used unless the stocks available are shown to be distinct from CCRF-CEM.

Table 4b. NK cell lines: genetic characterization

Cell line	Cytogenetic karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements[a]	Ref.
NK92	Near-tetraploid with multiple aneuploidies and structural rearrangements.			51
NKL	47, XY, add(1)(q42), +6, del(6)(q15q23), del(17)(p11)			139
NOI 90	47, XY, +8, 17p−			142
TKS1	46, XY, 17p+, 21p+			89
YT	Tetraploid (83–95 with mode = 92), duplicated marker of 4q+ consistently found.			172

T-ALL cases often have translocations with a breakpoint involving the β T-cell receptor (*TCR*) at chromosome 7 band q34-35 and several other partner genes [133]. The t(7;19)(q34;p13) as seen in the SUP-T7 cell line involves a fusion of LYL (19p13) to *TCR* β [102]. The t(7;9)(q34;q32) as seen in the SUP-T3 and SUP-T6 cell lines involves fusion of the TAL2 gene (9q32) and *TCR* β [170], and the t(1;7)(p34;q34) as seen in the EU-9 and SUP-T12 cell lines involves the LCK gene (1p34) and *TCR* β [17,162].

Both MOLT-3 and -4 are hypertetraploid (modal 97 and 98 chromosomes, respectively) with shared chromosomal gains, losses and deletions. In particular, both lines have an intrachromosomal translocation t(7:7)(p15;q11) and a rearrangement of *TCR*γ gene. However, the breakpoint at 7p15 does not involve the *TCR* γ joining or constant regions [55].

T-ALL is commonly associated with a deletion in chromosome 9p21, which occurs in approximately 60% of patients. The 9p21 region is the locus for the Ink4 family of Cdk4 inhibitors, including $p15^{ink}4b$ and $p16^{ink}4a$, and T-ALL cells frequently have a deletion of one or both of these tumor-suppressor genes [6,124,135]. Deletion of p15/p16 is thought to deregulate cell-cycle in tumor cells by abrogating inhibition of cyclin-dependent kinase 4 (Cdk4). The 9p21 region also contains the methylthioadenosine phosphorylase (*MTAP*) gene, and T-ALL cells with 9p21 deletions are typically deficient in *MTAP*, an enzyme important in the salvage pathway for adenine and methionine [5]. Five cell lines have a deletion of the short arm of chromosome 9 with the resultant loss of p15, p 16 and *MTAP*.

Another important tumor suppressor gene and regulator of cell cycle progression, the p53 gene, is frequently mutated in T-ALL cells at relapse, with an incidence of approximately 25% [23,171]. MOLT-3, -4, -14, and -16 as well as EU-7 express a mutant or alternately-spliced p53; EU-9 does not express p53.

The karyotype of the NK cell lines is summarized in Table 4b. No established NK cell line has a normal karyotype. Two cell lines (NK92, YT) have near tetraploidy and three NK cell lines have abnormalities involving the short arm of chromosome 17. No evaluation of p53, p16 or p15 has been reported in the NK cell lines.

6. SPECIAL FUNCTIONS AND CYTOCHEMISTRY

The functional characterizations of the T-ALL cell lines are listed in Table 5a. Biochemically, T-ALL cells have aberrant expression of certain enzymes involved in purine biosynthesis. Specifically, T-ALL cells have high levels of adenosine deaminase and low levels of purine nucleoside phosphorylase and 5′-nucleotidase drelative to normal T-cells and non-T-ALL lymphoid

malignancies. Additionally, T-ALL cells may show a deficiency in methylthioadenosine phosphorylase (*MTAP*) activity due to a 9p21 deletion, as discussed earlier.

Leukemia cells often appear to be altered in their responses to apoptotic stimuli, and this may be due to aberrant expression or regulation of certain anti-apoptosis genes. The MDM2 (Murine-Double-Minute 2) gene codes for a protein that inhibits p53 function, thereby making the cell more resistant to factors that activate p53-mediated apoptosis. MDM2 is frequently overexpressed in ALL cells that express a wild-type p53 [15,175]. Similarly, members of the Bcl-2 family having anti-apoptotic activity (Bcl-2 and Bcl-X1) may be expressed at high levels (or show abnormal regulation following exposure to DNA-damaging agents) in both T-lineage and B-lineage ALL [29,43]. T-ALL cells may also express high levels of Fas (CD95), a member of the tumor-necrosis-factor receptor superfamily which is capable of inducing apoptosis upon binding of its ligand (FasL). However, the sensitivity of Fas+ T-ALL cells to FasL is heterogeneous and may be affected by other anti-apoptosis proteins [34, 177].

Several of the MOLT lines show abnormal regulation of purine and pyrimidine pathway enzymes commonly associated with T-ALL, and these lines have been widely used in studies of the effects of antimetabolites on T-ALL cells [5, 166, 174]. The HPB-ALL and PEER lines have been used to evaluate the effects of deoxyadenosine and deoxycoformycin on T-ALL cells expressing high levels of adenosine deaminase [41].

Several T-ALL cell lines, including MOLT-3, Jurkat and CCRF-CEM, have been shown to be induced by phorbol esters (such as TPA) to differentiate into cells with more mature phenotypes. TPA induces MOLT-3 and Jurkat cells to acquire increased sheep RBC rosette-forming ability (corresponding to increased expression of CD2) and lose TdT activity. Jurkat cells respond to TPA and differentiate but CCRF-CEM cells do not [116]. When MOLT-3 and Jurkat cells are treated with TPA, CD3 cells increase but the proportions of CD4, 1, and 8 cells decrease [117]. Phorbol dibutyrate (PDB) induces a rapid and reversible loss of the expression of CD4 antigen and a slower increase in CD2 antigen on the Jurkat cells [19]. When treated with thymosin fraction 5, MOLT-3 cells increase 5′-ectonucleotidase activity, but decrease adenosine deaminase activity, proliferating activity, and percent of cells that are positive for CD1. TPA induces purine nucleoside phosphorylase and increases the expression of CD2 with suppression of TdT activity but does not affect 5′-ectonucleotidase or adenosine deaminase activity [67]. CEM cells were induced with TPA to express a cell surface antigen pattern of both suppressor and cytotoxic T-lymphocytes [140]. HPB-ALL cells, when exposed to TPA, differentiate into a more mature T-cell phenotype as judged by morphologic changes, loss of TdT activity, and increased sheep red blood cell rosetting

Table 5a. T-cell lines: functional characterization

Cell line	Doubling time	EBV status	Cytochemistry	Differentiation	Heterotransplant-ability into mice	Special functional features	Ref.
BE-13						Dexamethasone sensitive Inhibited by haptoglobulin related antisense cDNA	47,147
CCRF-CEM			Oil Red O– PAS+ ALP– ACP– ANAE+	TPA induced ↑CD3/CD8		Cyclosporin induced apoptosis Ectopic p16 expression resulted in growth arrest TPA induced carboxylic esterase, LDH and acid phosphatase. Subclone has defective methotrexate polyglutamate synthesis.	140,45,72 40,38,105
DU. 528	48–96 hrs		NBT(+), Perox–, NSE(+)	PMA induced myeloid differentiation with ↑NBT, ↑MPO, ↑NSE			91
EU-7*	45–55 hrs	Neg	NSE+ SB– SE– Perox–		SCID mice	Colony formation in methylcellulose Bcl-2 family gene expression: Bcl-2–, Bcl-xl+, Bax+ (by W/Blot) MDM2+ by W/Blot Fas+ (high level expression by FC)	175,43,177

Continued on next page

Table 5a. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Differentiation	Heterotransplant-ability into mice	Special functional features	Ref.
EU-9	45–55 hrs	Neg	NSE+ Perox− SB− SE−		SCID mice	Colony formation in methylcellulose Bcl-2 family gene expression: Bcl-2−, Bcl-xl+, Bax+ (by W/Blot) MDM2− by W/Blot Fas+ (low level expression by FC)	175,43,177
HPB-ALL		EBNA−		TPA induced ↓TdT, ↓CD1, ↑SRBC		HPB-ALL cells are inhibited by deoxyadenosine and deoxycoformycin HPB-ALL cells are infectable by EBV. 1, 25 dihydroxy vitamin D3 did not inhibit HPB-ALL cells	118,114,41, 128,119
JM/JURKAT		EBNA−	PAS− ACP+ NSE− Perox−	TPA induced ↑CD2, ↑CD3, ↓TdT PDB induced ↓CD4		Ectopic p16 expression resulted in growth arrest TPA induced acid phosphatase	117,19,146, 40,38
KARPAS 45		EBNA−	SB−, Perox−, ANAE−, ANBE−, PAS(+) ACP+ ALP−				76
K-T1	68 hrs						152

Continued on next page

Table 5a. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Differentiation	Heterotransplant-ability into mice	Special functional features	Ref.
L-KAW	36 hrs		NSE(+) PAS+ Perox− N AS-D CE−			PHA induced growth inhibition and apoptosis of L-KAW cells.	104
LOUCY	36–48 hrs		ACP+ PAS− Perox− SB− Esterase−				11
MOLT-3	3–5 days	Neg	AP+, Perox- SB−	In SCID mice, ↑CD5, ↑CD8, ↓CD45RA TPA induced ↑CD2, ↑CD3, ↑PNP, ↓TdT TF5 induced ↓ADA and ↓CD1 PMA and A23187 induced calpain secretion.	Nude and SCID mice	Colony formation in agar Overexpressed H-ras c-myc, v-myb, v-Hras, N-Ras, v-vef, v-fos by N Blot Sublines resistant to MTX overexpressed DHFR; some with ↓ expression of folate carrier gene. Sensitive to anti-CD-7-PAP in SCID mice. Subline resistant to trimetrexate with increased DHFR and MDR1. Increased CTP synthetase activity.	106,60,163, 125,126,95, 49,50,3, 117,166,67, 35,86,53

Continued on next page

Table 5a. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Differentiation	Heterotransplant-ability into mice	Special functional features	Ref.
MOLT-4	3–5 days	Neg	AP+ Perox− SB−	In SCID mice, ↑CD5, ↑CD8, ↓CD45RA TPA induced selective loss of CD-4 TPA induced resistance to deoxyguanosine.	Nude and SCID mice	Overexpressed H-ras c-myc Expressed TALLA-1 Ly6 gene overexpressed Expressed methylthioadenosine phosphorylase Cyclosporin induced apoptosis Expressed specific 1, 25 dihydroxy vitamin D_3 receptors and vitamin D_3 inhibits cellular proliferation. Cells overexpressed cyclin D3 TPA induced carboxylic esterase and acid phosphatase	106,174,163, 70,49,50, 158,159,141, 72,161,5, 53,38
MOLT-13		Neg				Anti-CD7 induced increase in cytoplasmic free calcium.	39,18
MOLT-16	Neg	Neg			Growth in SCID mice inhibited by anti-CD-7 antibody.	Expressed methylthioadenosine phosphorylase and is resistant to AMP-synthesis inhibitor alanosine.	39,7,5
MT ALL					Growth in SCID mice inhibited by anti-CD-7 antibody.		74
P12/Ichikawa						1, 25 dihydroxy vitamin D3 did not inhibit P12/Ichikawa cells.	119

Continued on next page

Table 5a. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Differentiation	Heterotransplant-ability into mice	Special functional features	Ref.
P30/Ohkubo	30 hrs	Neg	Perox− PAS+ ACP+ ALP− ANAE−				66
PEER	36 hrs	Neg	SB− Oil Red O− Perox− NS+ PAS+ Muramidase− ACP+			PEER cells strongly inhibited by deoxyadenosine plus deoxycoformycin	136,41
PER 117	55–65 hrs		ACP+ PAS+ Perox− NSE−				77
PER 255		Neg	ACP+ PAS+ Perox− NSE− SB−				79

Continued on next page

Table 5a. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Differentiation	Heterotransplant-ability into mice	Special functional features	Ref.
PF-382		Neg	ACP+ PAS− Perox− ANAE−			Cell line released a factor which inhibits CFU-GM and BFU-E growth *in vitro*. Cell line released potent enhancers of monocyte effect on BFU-E growth	9,10,130
RPMI 8402				TPA induced a more mature phenotype. TPA induced TdT loss and resistance to deoxyguanosine.		Alpha and beta interferon inhibits cellular proliferation. Cells expressed 1, 25 dihydroxy vitamin D3 receptors.	141,148,150, 161
SUP-T2	130 hrs	Neg					152
SUP-T3	61 hrs	Neg					152
SUP-T6		Neg					152
SUP-T7		Neg					153
SUP-T8		Neg					154
SUP-T9		Neg					154
SUP-T10		Neg					154
SUP-T12		Neg					154
SUP-T13		Neg				Anti-CD3 inhibits DNA synthesis and triggers cell death.	154,178
SUP-T14		Neg					154
TALL-1 (a)	60–90 hrs						111
TALL-103/2						Lyses only NK targets.	21

Continued on next page

Table 5a. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Differentiation	Heterotransplant-ability into mice	Special functional features	Ref.
TALL-104						Lyses a broad range of tumor targets.	21,22
UHKT-42			ACP+ PAS+ Perox− SB− NSE−	TPA induced IL-2R expression.			157

ACP – acid phosphatase; ALP – alkaline phosphatase; ANAE – alpha naphthol acetate esterase; ANBE – alpha naphthol butyrate esterase; FC – flow cytometry; N AS-D CE – naphthol AS-D Chloroacetate Esterase; NBT – nitro blue tetrazolium; NSE – non-specific esterase; PAS – periodic acid-schiff; Perox – peroxidase; PMA – phorbol myristate acetate; SB – sudan black; TF5 – thymosin fraction 5; TPA – 12-O-tetradecamoylphorbol 13 – acetate.
* At least one stock of EU-7 cells has been found to be in fact CCRF-CEM, and this cell line should not be used unless the stocks available are shown to be distinct from CCRF-CEM.

Table 5b. NK cell lines: functional characterization

Cell line	Doubling time	EBV status	Cytochemistry	Differentiation	Heterotransplant-ability into mice	Special functional features	Ref.
NK92	24 hrs	EBV−				NK activity against both K562 and Daudi	51
NKL	24–48 hrs +(IL2)					NKL cells lysed K562, JURKAT, Daudi cells and mediated antibody-dependent cellular cytotoxicity.	139
NOI90	48 hrs		Perox− NSE− ACP+			Lacks NK or LAK activity Expressed a low number of high affinity IL-2R with autocrine IL-2 loop.	142
TKS-1		Not stated	ACP+ NSE+ (inhibited by sodium fluoride)			High cytotoxic activity against K562 Raji and Daudi target cells.	89
YT		EBV infected	Perox− PAS+			YT cells exhibit NK and antibody-dependent cellular cytotoxicity	173
YT2C2		EBV infected				YT2C2 cells do not exhibit NK or antibody-dependent cellular cytotoxicity.	173
YTC3		EBV infected				YT3C cells do not exhibit NK or antibody-dependent cellular cytotoxicity.	173

ACP – acid phosphatase; NSE – non-specific esterase; PAS – periodic acid- schiff; Perox – peroxidase.

Table 6. Lymphoblast cell lines: unconfirmed cell lines (not fully characterized, not verified, other)

Cell line	Patient	Features	Ref.	Remarks
ALL-Sil		t(10;14)(q24;q11) HOX 11 – TCR delta	62,97	Provided by Dr. Jun Minowada, Fujisaki Cell Center
K3P		t(10;14)(q24;q11) HOX 11 – disregulated	42	
MOLT-10	T-cell lymphoma	T-ALL cell lines established by Dr. Jun Minowada, Fujisaki Cell Center	110,109	Full characterization not reported.
MOLT-11		T-ALL cell lines established by Dr. Jun Minowada, Fujisaki Cell Center	110,109	Full characterization not reported.
REX	From a patient with lymphoblastic leukemia	CD1– CD2+ CD3+ CD4+ CD5+ CD6+ CD8+	1,2	Used to dissect human T-Cell receptor.
SUP-T11	From BM from 74 y/o M with leukemia	CD1– CD2+ CD3+ CD4– CD5+ CD7+ CD8– 40, X, –Y, t(7;15;?)(q34;q22?)-1, 1q–, 2p+, –4, t(5;9;?), der(5q), 6q+, –9, 9q–, –10, 12p+, –13, t(14;14)(q11.2;q32), –15, –16, 16q+, –17, 19p+, –21, 21q+, –22, +5mar	154	While the patient was originally reported to have T-ALL, the patient's age and the presence of t(14;14) supports the diagnosis of T-CLL.

ACP – acid phosphatase; NSE – non-specific esterase; PAS – periodic acid- schiff; Perox – peroxidase.

(CD2) activity and the disappearance of CD1 [118]. RPMI 8402 cells, when treated with TPA, acquire a more differentiated phenotype [141].

The functional characterization of the NK cell lines are listed in Table 5b. The NK cell lines YT and the sub-clones YT2C2 and YTC3 are EBNA positive and are the first reported EBV+ NK cell lines. While NOI-90 cells lack NK or LAK activity, cell lines NK-92, YT, and TSK were toxic against NK, ADCC, K562, Daudi, and Raji targets.

Cell lines which have not been fully characterized are reported in Table 6. The ALL-Sil and K3P cell lines carry the t(10;14)(q24;q11) and have been used to evaluate the HOX 11 gene. The T-ALL cell lines MOLT-10 and MOLT-11 have not been fully reported. The Rex cell line, a T-ALL cell line, has been used for identification of α and β subunits of the human T-cell receptor. The Rex cells have also been used to identify and study ion channels which are activated by Ti or T3 antibodies [1,2,129]. While the SUP-T11 cell line was originally reported as T-ALL, a review of the clinical and laboratory characterization revealed that this patient most likely had T-CLL. This impression was supported by the presence of the t(14;14)(q11;q32) which is a characteristic marker of T-CLL [103,154].

References

1. Acuto O, Fabbi M, Smart J et al. *Proc Natl Acad Sci USA* 81: 3851–3855, 1984.
2. Acuto O, Hussey RE, Fitzgerald KA et al. *Cell* 34: 717–726, 1983.
3. Arkin H, Ohnuma T, Kamen BA et al. *Cancer Res* 42: 1655–1660, 1989.
4. Ariyasu T, Kimura N, Kikuchi M et al. *Leukemia* 5: 807–812, 1991.
5. Batova A, Diccianni MB, Nobori T et al. *Blood* 88: 3083–3090, 1996.
6. Batova A, Diccianni MB, Yu JC et al. *Cancer Res* 57: 832–836, 1997.
7. Baum W, Steininger H, Bair JH et al. *Br J Haematol* 95: 327–338, 1996.
8. Begley CG, Aplan PD, Davey MP et al. *Proc Natl Acad Sci USA* 86: 2031–2035, 1989.
9. Bellone G, Foa R, Fierro MT et al. *Leukemia* 1: 603–608, 1987.
10. Bellone G, Avanzi GC, Lista P et al. *J Cell Phys* 135: 127–132, 1988.
11. Ben-Bassat H, Shlomai Z, Kohn G et al. *Cancer Genet Cytogenet* 49: 241–248, 1990.
12. Bernard O, Guglielmi P, Jonveaux P et al. *Genes Chromosomes & Cancer* 1: 194–208, 1990.
13. Borkhardt A, Repp R, Haas OA et al. *Oncogene* 14: 195–202, 1997.
14. Brandter LB, Heyman M, Rasool O et al. *Eur J Haematol* 56: 313–318, 1996.
15. Bueso-Ramos CE, Yang Y, deLeon E et al. *Blood* 82: 2617–2623, 1993.
16. Burger R, Hansen-Hagge T, Drexler HG et al. *Leukemia* Research 23: 19–27, 1999.
17. Burnett RC, Thirman MJ, Rowley JD et al. *Blood* 84: 1232–1236, 1994.
18. Carrel S, Salvi S, Rafti F et al. *Eur J Immunol* 21: 1195–1200, 1991.
19. Cassel DL, Hoxie JA, Cooper RA. *Cancer Res* 43: 4582–4586, 1983.
20. Cesano A, Santoli D. In Vitro *Cell Dev Biol* 28a: 657–662, 1992a.
21. Cesano A, Santoli D. In Vitro *Cell Dev Biol* 28a: 648–656, 1992b.
22. Cesano A, Visonneau S, Santoli D. *Anticancer Res* 18: 2289–2295, 1998.
23. Cheng J, Haas M. *Mol Cell Biol* 10: 5502–5509, 1990a.

24. Cheng J, Scully P, Shew J-Y et al. *Blood* 75: 730–735, 1990b.
25. Cheng J, Yee JK, Yeangin J et al. *Cancer Res* 52: 222–226, 1992.
26. Chou JL, Barker CR, Prospero TD. *Leukemia Res* 10: 211–220, 1986.
27. Chow VT, Quek HH, Tock EP. *Cancer Lett* 73: 141–148, 1993.
28. Cleary ML, Mellentin JD, Spies J et al. *J Exp Med* 167: 682–687, 1988.
29. Conroy LA, Alexander DR. *Leukemia* 10: 1422–1435, 1996.
30. Crist WM, Shuster JJ, Falletta J et al. *Blood* 72: 1891–1897, 1988.
31. Dao T, Holan V, Minowada J. *Cell Immunol* 151: 451–459, 1993a.
32. Dao T, Holan V, Minowada J. *Int. J. Hematol* 57: 139–146, 1993b.
33. Dao T, Ariyasu T, Holan V. *Cell Immunol* 155: 304–311, 1994.
34. Debatin KM, Krammer PH. *Leukemia* 9: 815–820, 1995.
35. Deshpande RV, Goust JM, Hogan EL et al. *J Neurosci Res* 42: 259–265, 1995.
36. Drexler HG, Gaedicke G, Minowada J. *Leukemia Res* 9: 549–559, 1985.
37. Drexler H, Messmore H, Menon M et al. *Acta Haematol* 75: 12–17, 1986.
38. Drexler HG. *Blut* 54: 79–87, 1987.
39. Drexler HG, Minowada J. *Hematol Oncol* 7: 115–125, 1989.
40. Drexler HG. *Leukemia* 12: 845–859, 1998.
41. Duan DS, Smith W, Sadee W et al. *Thymus* 19: 1–10, 1992.
42. Dube ID, Kamel-Reid S, Yang CC et al. *Blood* 78: 2996–3003, 1991.
43. Findley HW, Gu L, Yeager AM et al. *Blood* 89: 2986–2993, 1997.
44. Finger LR, Kaga J, Christopher G et al. *Proc Natl Acad Sci USA* 86: 5039–5043, 1989.
45. Foley GE, Lazarus H, Farber S et al. *Cancer* 18: 522529, 1965.
46. Galili N, Galili U, Ravid Z et al. *Human Lymph Diff* 1 123–130, 1981.
47. Galili U, Peleg A, Milner Y, Galili N. *Cancer Res* 44: 4594–4601, 1984.
48. Gignac SM, Steube K, Schleithoff L et al. *Leukemia and Lymphoma* 10: 359–368, 1993.
49. Goldman J, McGuire WA. *Pediatric Hematol Oncol* 9: 357–363, 1992a.
50. Goldman J, McGuire WA. *Pediatric Hematol Oncol* 9: 309–316, 1992b.
51. Gong J-H, Maki G, Klingemann H-G. *Leukemia* 8: 652–658, 1994.
52. Gong J, Bhatia U, Traganos F et al. *Leukemia* 9: 893–899, 1995.
53. Gong M, Yess J, Connolly T et al. *Blood* 89: 2494–2499, 1997.
54. Greaves MF, Rao J, Hariri G, Verbi W et al. *Leukemia Res* 5: 281–299, 1981.
55. Greenberg JM, Gonzalez-Sarmiento R, Arthur D et al. *Blood* 1755–1760, 1988.
56. Griesinger F, Arthur DC, Drunning R et al. *J Exp Med* 169: 1101–1120, 1989a.
57. Griesinger F, Greenberg JM, Arthur DC et al. Hem *Blut* 32: 214–219, 1989b.
58. Grass H-J, DaSilva N, Hu Z-B et al. *Leukemia* 8: 2083–2094, 1994.
59. Guizani L, Lang P, Stancou R, Bertoglio J. *Lymphokine and Cytokine Res* 12: 25–31, 1993.
60. Gunther R, Chelstrom LM, Finnegan D et al. *Leukemia* 7: 298–309, 1993.
61. Hane B, Tummler M, Jager K et al. *Leukemia* 6: 1129–1133, 1992.
62. Hatano M, Roberts CWM, Minden M et al. *Science* 253: 79–82, 1991.
63. Hayata M, Oshimura J, Minowada J et al. *In Vitro* 11: 361–368, 1975.
64. Hershfield MS, Kurtzberg J, Harden E et al. *Proc Natl Acad Sci USA* 81: 253–257 1984.
65. Heyman M, Grander D, Brondum-Nielsen K et al. *Leukemia* 8: 425–434, 1994.
66. Hirose M, Minato K, Tobinai K et al. *Gann* 73: 600–605, 1982.
67. Ho AD, Ma DDF, Price G et al. *Leukemia* Res 7: 779–86, 1983.
68. Holan V, Minowada J. *Folia Biol* 39: 1–13, 1993.
69. Holan V, Dao T, Kosarova M. *Cell Immunol* 157: 549–555, 1994.
70. Horie M, Okutomi K, Taniguchi Y. *Genomics* 53: 365–368, 1998.
71. Huang CC, Hou Y, Woods LK et al. *JNCI* 53: 655–660 1974.

72. Ito C, Ribeiro RC, Behm FG et al. *Blood* 91: 1001–1007, 1998.
73. Iwata, N Murayama T, Matsumori Y et al. *Blood* 88: 2863–2869, 1996.
74. Jansen B, Vallera DA, Jaszcz WB et al. *Cancer Res* 52: 1314–1321, 1992.
75. Kawamura M, Ohnishi H, Guo S et al. *Leukemia Res* 23: 115–126, 1999.
76. Karpas A, Hayhoe FGJ, Greenberger JS et al. *Leukemia Res* 1: 35–49, 1977.
77. Kees UR, Ford J, Price PJ et al. *Leukemia Res* 11: 489–498, 1987.
78. Kees UR. *Blood* 72: 1524–1529, 1988.
79. Kees UR, Lukeis R, Ford J et al. *Blood* 74: 369–373, 1989.
80. Kees UR, Ford J, Peroni SE et al. *Leukemia* 17: 51–59, 1993.
81. Kees UR, Peroni SE, Ranford PR et al. *J Immunol* 168: 1–8, 1994.
82. Kees UR, Ashman LK. *Leukemia* 9: 1046–1050, 1995.
83. Kennedy MA, Gonzalez-Sarmiento R, Kees UR et al. *Proc Natl Acad Sci USA* 88: 8900–8904, 1991.
84. Kimura N, Du T-P, Mak TW. European *J Immunol* 17: 1653–1656, 1987.
85. Kirsch AH, Diaz LA, Bonish B et al. *Tissue Antigens* 50: 147–152, 1997.
86. Kobayashi H, Dorai T, Holland JF et al. *Cancer Res* 54: 1271–1275, 1994.
87. Kobayashi, H Espinosa III, R Thirman J et al. *Blood* 81: 3027–3033, 1993.
88. KohnoK, ShibataY, MatsuoY et al. *Cellular Immunol* 131: 1–10, 1990.
89. Kojima H, Suzukawa K, Yatabe Y et al. *Leukemia* 8: 1999–2004, 1994.
90. Komiyama A, Kawai H, Miyagawa Y et al. *Blood* 6: 1429–1436, 1982.
91. Kurtzberg J, Bigner SH, Hershfield MS. *J Exp Med* 162: 1561–1578, 1985.
92. Lange BM, Valtieri D, Santoli D et al. *Blood* 70: 192–199, 1987.
93. Larsen CJ, Mathieu-Mahul D, Bernard O et al. *Leukemia* 2: 247–248, 1988.
94. LeBien T, Kersey J, Nakazawa S et al. *Leukemia Res* 6: 299–305, 1982.
95. Li XX, Kobayashi H, Holland JF et al. *Leukemia Res* 17: 483–490, 1993.
96. Loughran TP. *Blood* 82: 1–14, 1993.
97. Lu M, Gong Z, Shen W et al. *EMBO J* 10: 2905–2910, 1991.
98. Martin PJ, Giblett ER, Hansen JA. *Immunogenetics* 15: 385–398, 1982.
99. Massaad L, Venuat A, Luccioni, C et al. *Cancer Genet Cytogenet* 53: 23–24, 1991.
100. McCabe NK, Kipiniak M, Kobayashi H et al. *Genes, Chromosomes & Cancer* 9: 211–224, 1994.
101. McKeithan TW, Shima-Rich EA, Le Beau MM et al. *Proc Natl Acad Sci USA* 83: 6636–6640, 1986.
102. Mellentin JD, Smith SD, Cleary ML. *Cell* 58: 77–83, 1989.
103. Mengle-Gaw L, Willard HF, Smith CIE et al. *EMBO J* 6: 2273–2280, 1987.
104. Minegishi M, Minegishi N, Yanagisawa T et al. *Leukemia Res* 19: 433–442, 1995.
105. Mini E, Stanyon R, Coronnello M et al. *Tumori* 77: 95–99, 1991.
106. Minowada J, Ohnuma T, Moore GE. *J Natl Cancer Inst* 49: 891–895, 1972.
107. Minowada J, Koshiba H, Sagawa K et al. *J Cancer Res Clin Oncol* 101: 91–100, 1981.
108. Minowada J, Minato K, Srivastava BIS et al. In: Serrou B and Rosenfield H. (eds.) 1982a *Current Concepts in Human Imm & Cancer Imm*, North Holland, Amsterdam, pp 75–83.
109. Minowada J, Sagawa K, Trowbridge IS et al. 1982b In: Rosenberg SA and Kaplan HS (eds.), *Malignant Lymphomas, Etiology, Immunology, Pathology, Treatment*, Academic Press, New York, pp 53–74.
110. Minowada J, Drexler HG, Menon M. *Hematol-Bluttransfus* 29: 426–429, 1985.
111. Miyoshi I, Hiraki S, Tsubota T et al. *Nature* 267: 843–845, 1977.
112. Mohammad RM, Vistisen K, Al-Katib A. *Electrophoresis* 15: 1218–1224, 1994.
113. Moore GE, Woods LK, Minowada J et al. *In Vitro* 8: 434–439, 1973.

114. Morikawa S, Tatsumi E, Baba M et al. *Int J Cancer* 21: 166–170, 1978.
115. Nagasaka M, Maeda S, Mabuchi O et al. *Int J Cancer* 30: 173–180, 1982.
116. Nagasawa K, Howatson A, Mak TW. *J Cell Physiol* 109: 181–192, 1981.
117. Nagasawa K, Mak TW. *Cell Immunol* 71: 396–403, 1982.
118. Nakao Y, Matsuda S, Fujita T et al. *Cancer Res* 42: 3843–3850, 1982.
119. Nakao Y, Koizumi T, Matsui T et al. *JNCI* 78: 1079–1086, 1987.
120. Narumiya S, Hirata M, Nanba T et al. *Biochem & Blophys Res Comm* 143: 753–760, 1987.
121. O'Connor R, Cesano A, Kreider BL et al. *J Immunol* 145: 3779–3787, 1990.
122. O'Connor R, Cesano A, Lange B et al. *Blood* 77: 1534–1545, 1991.
123. Ogawa S, Hirano N, Sato N et al. *Blood* 84: 2431–2435, 1994.
124. Ohnishi H, Kawamura M, Ida K et al. *Blood* 86: 1269–1275, 1995.
125. Ohnoshi T, Ohnuma T, Takahashi I et al. *Cancer Res* 42: 1655–1660, 1982.
126. Ohnuma T, Arkin H, Takahashi I et al. *Mol Inter Nut & Cancer* 105–121, 1985.
127. Ohmura T, Sakata A, Onoue *K. J Exp Med* 176: 887–891, 1992.
128. Paterson RLK, Kelleher C, Amankonah TD et al. *Blood* 85: 456–464, 1995.
129. Pecht I, Corcia A, Pia M et al. *EMBO J* 6: 1935–1939, 1987.
130. Pegoraro L, Fierro MT, Lusso P et al. *J Natl Cancer Inst* 75: 285–290, 1985.
131. Ponzoni M, Montaldo PG, Lanciotti M et al. *Biochem and Biophys Res Comm* 150: 702–710, 1988.
132. Prokocimer M, Peller S, Ben-Bassat H. *Leukemia Lymphoma* 29: 607–611, 1998.
133. Rabbitts TH. Nature 372: 143–149, 1994.
134. Raimondi SC, Behm FG, Roberson PK et al. *Blood* 72: 1560–1566, 1988.
135. Rasool O, Heyman M, Brandter LB et al. *Blood* 85: 3431–3436, 1995.
136. Ravid Z, Goldblum N, Zaizou R et al. *Int J Cancer* 25: 705–710, 1980.
137. Reinherz EL, Kung PC, Goldstein G et al. *Proc Natl Acad Sci USA* 77: 1588–1592, 1980.
138. Ribeiro RC, Raimondi SC, Behm FG et al. *Blood* 78: 466–471, 1991.
139. Robertson MJ, Cochran KJ, Cameron C et al. *Exp Hematol* 24: 406–415, 1996.
140. Ryffel B, Henning CB, Huberman E. *Proc Natl Acad Sci USA* 79: 7336–7340, 1982.
141. Sacchi N, Fiorini G, Plevani P et al. *J Immunol* 130: 1622–1626, 1983.
142. Sahraoui Y, Perraki M, Theodoropoulou M et al. *Leukemia* 11: 245–252, 1997.
143. Salvati MD, Peroni SE, Ranford PR et al. *Immunology* 78: 449–454, 1993.
144. San Jose E, Sahuquillo AG, Bragado R et al. *Eur J Immunol* 28: 12–21, 1998.
145. Santoli D, O'Connor R, Cesano A et al. *J Immunol* 144: 4703–711, 1990.
146. Schneider U, Schwenk H-U, Bornkamm G. *Int J Cancer* 19: 621–626, 1977.
147. Shalitin C, Valansi C, Lev A et al. *Anticancer Res* 18: 145–150, 1998.
148. Shibata T, Shimada Y, Shimoyama M. *Jpn J Clin Oncol* 15: 67–75, 1985.
149. Shima-Rich EA, Harden AM, McKeithan TW et al. *Genes Chromosomes and Cancer* 10: 363–371, 1997.
150. Shimada Y, Shimoyama M *Gann* 75: 1116–1124, 1984.
151. Smith SD, Shatsky M, Cohen PS et al. *Cancer Res* 44: 5657–5660, 1984.
152. Smith SD, Morgan R, Link MP et al. *Blood* 67: 650–656, 1986.
153. Smith SD, Morgan R, Gemmell R et al. *Blood* 71: 395–02, 1988.
154. Smith SD, McFall P, Morgan R et al. *Blood* 73: 2182–2187, 1989.
155. Smith A, Eskew JD, Borza CM et al. Exp *Cell* Res 232: 246–254, 1997.
156. Srivastava BI, Minowada J. *Leukemia Res* 7: 331–338, 1983.
157. Stockbauer P, Ariyasu T, Stary J et al. *Human Cell* 7: 40–46, 1994.
158. Takagi S, Fujikawa K, Imai T et al. *Int J Cancer* 61: 706–715, 1995.

159. Tatsumi E, Piontek C, Sugimoto T et al. *Am J Hematol* 17: 287–294, 1984.
160. Teshigawara K, Wang HM, Kato K et al. *J Exp Med* 165: 223–238, 1987.
161. Thomas GA, Simpson RU. *Cancer Biochem Biophys* 8: 221–234, 1986.
162. Tycko B, Smith SD, Sklar J. *J Exp Med* 174: 867–873, 1991.
163. Uittenbogaart CH, Anisman DJ, Tary-Lehman M et al. *Int J Cancer* 56: 546–551, 1994.
164. Valtieri M, Santoli D, Caracciolo BL. *J Immunol* 138: 4042–4050, 1987.
165. van de Griend RJ, van der Reijden HJ, Bolhuis RLH et al. *Blood* 62: 669–674, 1983.
166. van den Berg AA, van Lenthe H, Kipp JB et al. Eur J *Cancer* 31 A: 108–112, 1995.
167. van-Dongen JJ, Krissansen GW, Wolvers-Tettero IL et al. *Blood* 71: 603–612, 1988.
168. Watanabe S, Shimosato Y, Kameya T et al. *Cancer Res* 38: 3494–3498, 1978.
169. Westbrook CA, Rubin CM, Le Beau MM et al. *Proc Natl Acad Sci USA* 84: 251–255, 1987.
170. Xia Y, Brown L, Yang CYetal. *Proc Natl Acad Sci USA* 88: 11416–11420, 1991.
171. Yeargin J, Cheng J, Haas M. *Leukemia* 6(Suppl): 85–91, 1992.
172. Yodoi J, Teshigawara K, Nikaido T et al. *J Immunol* 134: 1623–1630, 1985.
173. Yoneda N, Tatsumi E, Kawano S et al. *Leukemia* 6: 136–141, 1992.
174. Yu J, Batova A, Shao L et al. Clin *Cancer Res* 3: 433–138, 1997.
175. Zhou M, Gu L, James CD et al. *Blood* 85: 1608–1614, 1995a.
176. Zhou M, Gu L, James CD et al. *Leukemia* 9: 1159–1161, 1995b.
177. Zhou M, Yeager AM, Findley HW. *Leukemia* 12: 1756–1763, 1998.
178. Zhu L, Anasetti C. *J Immunol* 154: 192–200, 1995.

Chapter 6

Myelocytic Cell Lines

Hans G. Drexler
DSMZ-German Collection of Microorganisms & Cell Cultures, Dept. of Human and Animal Cell Cultures, Braunschweig, Germany. Tel: +49-531-2616-160; Fax: +49-531-2616-150; E-mail: hdr@dsmz.de

1. INTRODUCTION

The fully differentiated end stage cell of the myelocytic/granulocytic series is the mature granulocyte. The mature granulocyte has a polylobed nucleus, hence the designation "polymorphonuclear leukocyte" [1]. In the normal adult, granulocytes are produced in the bone marrow. Granulocytes pass through three compartments during their life span: maturation in the bone marrow, transit in the blood, and function in the tissues [1]. All cells of this group play an essential role in acute inflammation in protection against microorganisms, although they do not show any inherent specificity for antigens. Their predominant function is phagocytosis [2]. The three types of granulocytes (neutrophils, eosinophils and basophils) have a parallel development [3].

The earliest morphologically recognizable cell of the granulocytic series is the myeloblast, which gives rise sequentially to the promyelocyte, myelocyte, metamyelocyte, stab cell and polymorph [4]. From promyelocyte onwards, specific granules (neutrophilic, eosinophilic or basophilic) become increasingly conspicuous in the cytoplasm and they distinguish the common neutrophilic granulocytes from the much less common eosinophils and the normally rare basophils [5]. Mitoses may occur in immature cells up to the metamyelocyte stage. Between the pluripotent stem cell and the first morphologically recognizable granulocyte precursor are committed stem cells and progenitor cells of lesser or greater restriction, with an inverse relationship between commitment to differentiation and self-renewal capacity [6]. Normal clonogenic progenitors common to granulocytes, erythrocytes, monocytes and megakaryocytes are denoted as CFU-GEMM. These progenitors give rise to granulocyte- monocyte (CFU-GM) colonies.

J.R.W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol. III, 207–236.

Neutrophils constitute over 90% of the circulating polymorphs and possess two main types of granules: the primary (azurophilic) granules are lysosomes containing acid hydrolases, myeloperoxidase and lysozyme; the secondary (specific) granules contain lactoferrin in addition to lysozyme [2,5]. Eosinophils comprise between 2–5% of blood leukocytes [7,8]. The granules of eosinophils contain specific products, eventually released by degranulation, which are not produced by neutrophils or basophils [9]. Morphocytochemically, the eosinophilic and basophilic granules can be distinguished by their typical size and color. Eosinophilic granules are stained with Luxol fast blue which is specific for eosinophils, but are not metachromatic when stained with toluidine blue, a characteristic which is commonly used to identify basophils.

Basophils are found in small numbers in the circulation (commonly < 1% of leukocytes). A "relative" of the basophil, the mast cell, is not seen at all in the peripheral blood and is only resident in body tissues. The relationship of the mast cell to the basophil is controversial. There are similarities, but also distinct differences between these two types of cells, and the interrelationship of the basophil-mast cell system requires further study [10–13]. While the basophil clearly arises in the bone marrow, the origin of mast cells has been more difficult to establish [14]. There may be a common progenitor for mast cells and basophils, because mast cell and basophil precursors have been found among the blast cells in CML. Various analyses of single mixed hematopoietic colonies (CFU-GEMM) also show human "basophil/mast cells" to have a clonal origin from circulating multipotent hematopoietic progenitors. Similarly, "basophils/mast cells" can be grown in suspension culture from umbilical cord mononuclear cells or the peripheral blood of individuals with allergies [15].

Malignancies involving the myelocytic cells are of acute or chronic nature. Clinicians distinguish the acute myeloid leukemias (AML) from the myeloproliferative disorders (most prominently CML) and the "preleukemic" myelodysplastic syndromes (MDS). While monocytic, erythroid and megakaryocytic acute leukemias have been assigned to the morphological subtypes FAB M4/M5, M6 and M7, respectively, the myelocytic subtypes of AML have been designated FAB M0, Ml, M2 and M3, depending on the degree of apparent morphological maturation and cytochemical differentiation [16,17]. With regard to diagnosis, prognosis and treatment, the unique subtypes AML M2 and AML M3 are of particular interest [18–20]. Cytogenetically, AML M2 is associated with the t(8;21) [21,22], whereas the t(15;17) is specific for AML M3 [23]. Cell lines are available for these clinically and scientifically interesting AMLs (Table 1b). Equally important is the availability of cell lines as model systems for the rare basophilic, mast cell and eosinophilic leukemias [10] (Table 1b). MDS with its different entit-

ies may terminate in various subtypes of AML [24–26]. The most common chronic form of myelocytic leukemia is CML [27–29], which ends invariably in a blast crisis. The predominant cell type during this blast crisis has myelocytic features.

The establishment of human myelocytic cell lines has always been difficult [30,31]. However, the number of myelocytic cell lines has increased dramatically [32–34], and now more than 40 well-characterized and mostly authenticated myelocytic cell lines have been described (Tables 1–5). The first continuous human myelocytic leukemia cell line was HL-60, which was established in 1976 [47]. Due to its widespread availability, this cell line has become the quintessential *in vitro* paradigm for myeloid leukemia cells [111]. Another widely used, early myelocytic cell line is KG-1 [62]. Myelocytic cell lines have also been established from specific AML subtypes, CML in myeloid blast crisis, MDS and rare myeloid leukemias. Of particular importance are several unique promyelocytic, basophilic, mast cell and eosinophilic cell lines [10,100] (Table 1b).

The data listed in Tables 1–6 provide detailed characteristics of individual myelocytic cell lines. The lines differ greatly, despite their origin from the same or similar types of leukemia. This multiplicity of markers reflects the heterogeneity inherent in human myeloid leukemias. A number of the myelocytic cell lines described below are available from major cell banks (Appendix 1, p. 284).

2. CLINICAL CHARACTERIZATION

Fifty-one well-characterized myelocytic cell lines are listed in Table 1a. These lines were established from 34 patients with various forms of AML (1 AML M0, 3 AML M1, 14 AML M2, 5 AML M3, 6 AML M4, 1 eosinophilic AML, 4 unspecified AML), 13 patients with CML in blast crisis, and one patient each with CMML, MDS, mast cell leukemia or T-ALL. In the latter case, a lymphoid cell line was not established, but myelocytic cells grew in culture. Corresponding to the relatively low incidence of AML and CML in childhood, 46 myelocytic cell lines were derived from adults and only five from children; the sex distribution is 29 from male and 22 from female patients. Only 15 (29%) lines were established at the diagnosis of the disease (i.e. prior to therapy), whereas 36 (71%) lines were derived from cases at relapse, in blast crisis or resistant to therapy. Thirty-three (64%) and fifteen (29%) cell lines were developed from samples taken from peripheral blood and bone marrow, respectively. The origin of the cell lines from the presumed patient was confirmed in a relatively high percentage of cell lines; 29/51 (57%). Apart from Iscove's MEM (n = 3) and α-MEM (n = 6), the

Table 1a. Myelocytic cell lines: clinical characterization

Cell line[a]	Patient[b] age/sex	Diagnosis[c]	Treatment status[d]	Specimen site[e]	Authentication[f]	Year of est.	Culture medium[g]	Availability[h]	Primary ref.
AML14[i]	68 M	AML M2	refractory	PB	no	1991	RPMI 1640 + FBS		35,36
AR230	52 F	CML-my BC	BC	PB	yes	1991	RPMI 1640 + FBS	Author	37
CML-C-1[j]	42 F	CML-BC	BC	PB	yes	1990	RPMI 1640 + FBS + mouse BM stromal feeder layer		38
CTS	13 F	AML M1 (post BMT)	R	PB	no	1992	RPMI 1640 + FBS	Author	39
Ei501[k]	17 F	AML M3	D	BM	no	1994	RPMI 1640 + FBS	Author	40
EM-2[l]	5 F	CML-my BC (post BMT)	2nd R	BM	yes	1982	RPMI 1640 + FBS	DSMZ	41
EoL-1[m]	33 M	hypereosinophilic syndrome → eosinophilic leukemia	D	PB	yes	1984	RPMI 1640 + FBS	DSMZ, RIKEN	42
FKH-1	61 M	Ph-neg CML with trilineage myelodysplasia → AML M4	refractory	PB	yes	1993	RPMI 1640 + FBS + GM-CSF		43
GDM-1	66 F	MPD (CML-like) → AML M4	refractory	PB	yes	1979	RPMI 1640 + FBS	DSMZ	44
GF-D8	82 M	AML M1	D	PB	no	1989	RPMI 1640 + FBS (or serum-free) + GM-CSF	Author	45
GM/SO	50 F	CML-my BC	BC	BM	yes	1988	RPMI 1640 + FBS + GM-CSF	Author	46
HL-60	35 F	initially AML M3 – later changed to AML M2	D	PB	yes	1976	RPMI 1640 + FBS	ATCC, DSMZ, IFO, JCRB, RIKEN	47–49
HMC-1	52 F	mast cell leukemia	refractory	PB	yes		IMDM + FBS	Author	15
HNT-34	47 F	MDS (CMML) → AML M4	refractory	PB	yes	1994	RPMI 1640 + FBS	Riken	50
HT93	66 M	AML M3	R	PB	no	1993	RPMI 1640 + FBS		51
IRTA17[n]	25 F	AML M2	R	BM	yes	1993	IMDM + FBS + G-CSF, GM-CSF or SCF		52
K051[o]	46 M	AML M2	D	BM	yes	1991	α-MEM + FBS	Author	53
Kasumi-1	7 M	AML M2 (post-BMT)	2nd R	PB	yes	1989	RPMI 1640 + FBS	DSMZ	54
Kasumi-3	57 M	AML M0	D	BM	yes	1990	RPMI 1640 + FBS	Author	55
Kasumi-4	6 F	CML-my BC	BC	PB	yes	1993	RPMI 1640 + FBS	Author	56
KBM-7[p]	39 M	CML-my BC (post BMT)	2nd BC	BM	yes	1984	IMDM + FBS	Author	57,58
KCL-22	32 F	CML-BC	BC	PE	no	1981	RPMI 1640 + FBS		59,60
KF-19[q]	35 M	AML M2	R	Pericardial effusion	no		α-MEM + FBS		61

Continued on next page

Table 1a. (continued)

Cell line[a]	Patient[b] age/sex	Diagnosis[c]	Treatment status[d]	Specimen site[e]	Authentication[f]	Year of est.	Culture medium[g]	Availability[h]	Primary ref.
KG-1[r]	59 M	erythroleukemia → AML	R	BM	yes	1977	α-MEM or RPMI 1640 + FBS	ATCC, DSMZ, JCRB, RIKEN	62,63
KOPM-28	64 F	CML-my BC	BC	PB	no	1982	RPMI 1640 + FBS	Author	64
KT-1	32 M	CML-BC	BC	PB	yes	1991	RPMI 1640 + FBS	Author	65
KU-812[s]	38 M	CML-BC	BC	PB	no	1981	McCoy's 5A or RPMI 1640 + FBS	DSMZ, IFO, JCRB, RIKEN	66,67
KY821[t]	28 M	AML M2	R	meninges	no	1982	RPMI 1640 + FBS	JCRB	68
KYO-1	22 M	CML-my BC	BC	PB	no	1981	RPMI 1640 + FBS	Author	69
LW/SO	60 F	AML M4 → AML M2	R	BM	no		RPMI 1640 + FBS		70
M20	10 F	AML M2	D	PB	no		RPMI 1640 + FBS		71
Marimo	68 F	essential thrombocythemia → t-AML M2	terminal	BM	no	1993	RPMI 1640 + FBS	Author	72
MDS92	52 M	MDS (RARS/RAEB) (prior leukemic transformation)	D	BM	no	1991	RPMI 1640 + FBS + IL-3	Author	73
MOLM-6[u]	44 M	CML-my BC	BC	PB	no	1992	RPMI 1640 + FBS	Author	74
MUTZ-2	62 M	AML M2	D	PB	yes	1993	α-MEM + FBS + SCF or 5637 CM	DSMZ	75
NB4[v]	23 F	AML M3	2nd R	BM	yes	1989	RPMI 1640 + FBS	DSMZ	76–78
NKM-1	33 M	AML M2	D	PB	no	1981	RPMI 1640 + FBS or serum-free		79
OCI/AML-1[w]	73 F	AML M4	D	PB	no	1987	α-MEM + FBS + G-CSF or 5637 CM	Author	80,81
OCI/AML-4	35 F	Hodgkin's disease + AML M4	D	PB	yes	1987	α-MEM + FBS (+ 5637 CM)	Author	82
OCI/AML-5	77 M	AML M4	R	PB	yes	1990	α-MEM + FBS + GM-CSF or 5637 CM	DSMZ	83
OHN-GM	60 M	Hodgkin's disease → t-MDS (RA/RAEB) (t-AML)	refractory	BM	yes	1995	RPMI 1640 + FBS + GM-CSF	Author	84
OIH-1	72 M	MDS (RAEB) → AML	D	PB	yes	1993	RPMI 1640 + FBS + G-CSF or GM-CSF		85
PL-21	24 M	(mediastinal) granulocytic sarcoma → AML M3	refractory	PB	no	1981	RPMI 1640 + FBS		59,86
SKNO-1	22 M	AML M2	2nd R	BM	yes	1990	RPMI 1640 + FBS + GM-CSF	Author	87
SR-91	22 M	T-ALL (post-BMT)	R	PB	yes	1991	RPMI 1640 + FBS	Author	88
TI-1	42 M	AML M2	D	PB	no	1988	RPMI 1640 + FBS	Author	89
UCSD/AML1	73 F	AML	R	BM	yes	1989	RPMI 1640 + FBS + GM-CSF	Author	90

Continued on next page

Table 1a. (continued)

Cell line[a]	Patient[b] age/sex	Diagnosis[c]	Treatment status[d]	Specimen site[e]	Authenti-cation[f]	Year of est.	Culture medium[g]	Availability[h]	Primary ref.
UF-1	33 F	AML M3	2nd R	PB	no	1994	RPMI 1640 + FBS	Author	91
YJ	69 M	CMML (with eosinophilia)	BC	PB	no	1994	RPMI 1640 + FBS	Author	92
YNH-1	46 M	AML M1	D	PB	yes	1994	RPMI 1640 + FBS + GM-CSF	Riken	93
YOS-M[x]	77 M	CML-my BC	BC	PB	yes	1990	RPMI 1640 + FBS	Author	94

[a] Names of cell lines are as given in the original literature; subclones (variant cell lines derived from a parental cell line) and sister cell lines (derived independently from the same patient from different specimens or at different time points) are indicated. [b] Age at the time of establishment of cell line. [c] Diagnoses are indicated as given in the original literature; →: disease progressed from a pre-malignancy/first malignancy into the final malignancy. [d] BC: at blast crisis; CP: in the chronic phase; D: at diagnosis (prior to therapy); R: at relapse; T: during therapy. [e] BM: bone marrow; PB: peripheral blood; PE: pleural effusion; Tu: tumor. [f] Evidence (e.g. cytogenetic marker chromosomes, immunoprofile, others) that this cell line was derived from the patient indicated. [g] Culture media as indicated in the original literature; cell line might also grow with other medium and/or supplements. [h] Availability from cell banks (ATCC; DSMZ; IFO; JCRB; RIKEN) or from the original investigator (author). [i] Subclone AML14.3D10 differentiates spontaneously to eosinophils. [j] CML-C-1 is the *in vitro* variant of cell line CML-N-1 (which was established in a nude mice and is serially transplantable *in vivo* , but failed to proliferate *in vitro* under standard liquid culture conditions). [k] Ei501 seems to be lost entirely due to yeast contamination. [l] The sister cell line EM-3 was established independently (EM-2 at day 28 and EM-3 at day 47 post- BMT) showing similar features. [m] The two sister subclones EoL-2 and EoL-3 show similar immunophenotypical and functional features; Eo-B is an EBV+ B-LCL from the same patient. [n] Sister cell line IRTA21 is immunophenotypically and cytogenetically different from IRTA17; both IRTA17 and IRTA21 are lost. [o] Sister cell line K052 was established at relapse showing different immunophenotypic, cytogenetic and oncogene point mutation features. [p] The subclone KBM-7/B5 is near-haploid, but has similar immunological and functional features. [q] Subclones KF-10AraC, KF-10ADR and KF-19VCR are constitutively resistant to cytosine arabinoside, adriamycin and vincristine. [r] Subclone KG-1a established between passages 15 and 35 of the original KG-1 is morphologically, cytochemically, immunologically and functionally less mature than KG-1. [s] Subclones KU-812-E and KU-812-F were isolated by semi-solid culture; these subclones possess similar properties as KU-812. [t] Subclone KY821A3 is methotrexate-resistant. [u] Seven cell lines from a single sample from the same patient were established: MOLM-7 and MOLM-11 represent the megakaryocytic lineage; MOLM-6, -8, -9, -10, -12 represent the myelocytic lineage. [v] Retinoic acid-resistant subclones NB4.306 and NB4-RAr were established. [w] Subclone OCI/AML-1a has similar features as the parental line OCI/AML-1. [x] YOS-B is a sister cell line, an EBV+ B-LCL carrying also the Philadelphia chromosome.

Table 1b. Myelocytic cell lines: cell lines with promyelocytic, eosinophil, basophil or mast cell features

Promyelocytic	Eosinophil	Basophil	Mast cell
Ei501[a]	AML14[b]	GRW[b]	HMC-1
HL-60[c]	EoL-1	HL-60[b]	
HT93[a]	HL-60[b]	KU-812	
NB4[a]	ME-1[b]	LAMA-84[b]	
UF-1[a]	OMA-AML-1[b]		
	YJ		

[a] Cell line carries the AML M3-specific t(15;17)(q22;q21) translocation with the PML-RARA fusion gene.
[b] Only weak constitutive expression of eosinophil or basophil features which, however, can be up-regulated by differentiation-inducing agents.
[c] HL-60 does not carry the specific t(15;17)(q22;q21) translocation, but has promyelocytic morphology and responds to retinoic acid.
Cell lines ME-1, OMA-AML-1, GRW, and LAMA-84 are described in the chapters on monocytic and erythroid-megakaryocytic cell lines, respectively.

preferred choice of medium is RPMI 1640 (n = 41) supplemented with FBS; only two cell lines (GF-D8, NKM-1) were reported to also grow under serum-free conditions (Appendix 2, p. 286). Thirteen of these cell lines (25%) can be obtained from cell banks (Appendix 1, p. 284).

Of note are the sister cell lines which were established from the same patient (simultaneously or at different time points: EM-3, K052, MOLM 7–12), subclones with more or less significant phenotypic differences (derived from AML14, EoL-1, KBM-7, KF-10, KG-1, KU-812, KY821, NB4, OCI/AML-1) and complementary EBV+ B-LCLs from the same patient (Eo-B, YOS-B).

Several cell lines deserve special mention as they represent the *in vitro* counterparts of rare primary leukemia subtypes (Table 1b). While HL-60 has been the *in vitro* model cell line for AML M3 (or acute promyelocytic leukemia), these cells do not carry the AML M3-specific t(15;17) resulting in the *PML-RARA* fusion gene (Table 4). Nevertheless, HL-60 can be induced by a variety of reagents to undergo differentiation to a promyelocytic stage and beyond [31,114]. Several other authentic AML M3 promyelocytic cell lines have now been established: Ei501, HT93, NB4, and UF-1 (Table 1b). Some cell lines display features of eosinophils constitutively or upon induced differentiation. The best-known and most widely used cell line is EoL-1 (and its subclones EoL-2/-3). The KU-812 cell line was generated from a CML patient. In line with the biology of CML, KU-812 exhibits a multilineage differentiation capacity. However, the basophil differentation potential is pre-

Table 2. Myelocytic cell lines: immunophenotypical characterization

Cell line	T-/NK-cell marker[a]	B-cell marker	Myelomonocytic marker	Erythroid-megakaryocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
AML14	CD2−	CD20−	CD13+ CD14(+) CD33+		CD34−	CD11b+	35
AR230	CD2− CD3− CD4+ CD5− CD7− CD8−	CD10− CD19− CD20− CD22− CD24−	CD13+ CD33+ CD35+		CD34− HLA-DR−	CD11b(+)	37
CML-C-1	CD3−	CD19−	CD13+ CD33−	CD41a−	CD34+ CD38− HLA-DR+	CD11b−	38
CTS	CD1− CD2− CD3− CD4− CD5− CD7+ CD8−	CD10− CD19− CD20−	CD13+ CD14− CD33+	CD41− GlyA−	CD34+ HLA-DR+		39
Ei501			CD13+ CD33+		CD34− CD38+ HLA-DR+	CD11a+ CD18+ CD54+	40
EM-2		CD10− CD19−	CD13+ CD14+ CD15+ CD33+	CD41− CD42b− vWF−	CD34− HLA-DR− TdT−		41,95,96
EoL-1	CD2− CD3− CD4+ CD5− CD7− CD8− CD28− CD57−	CD9− CD10− CD19− CD20− CD21− CD23− cy/sIg−	CD13− CD14− CD15+ CD16− CD32+ CD33+ CD64−		CD34− CD71− HLA-DR+ TdT−	CD11a+ CD11b− CD54(+)	42,95,97
FKH-1			CD13+ CD33+		CD34− HLA-DR+	CD11a+ CD11b+ CD11c+	43
GDM-1	CD1b− CD2− CD3− CD4+ CD5− CD7− CD8− CD28− TCR$\alpha\beta$−	CD9+ CD10− CD19− CD20− CD21− cy/sIg−	CD13− CD14− CD15+ CD33+	CD41a− CD61−	CD34+ CD71+ HLA-DR+ TdT−	CD11b+	44,95
GF-D8	CD2− CD3− CD4− CD5− CD7− CD8−	CD10− CD19− CD20− CD21− CD22−	CD13+ CD14− CD15− CD33+	CD41−	CD34− HLA-DR− TdT−	CD11a+ CD11b+CD11c− CD49d− CD54−	45
GM/SO	CD3− CD7−	CD10− CD20−	CD13+ CD14− CD16−	CD36+ CD42b−	CD34+ HLA-DR+	CD11b−	46
HL-60	CD1− CD2− CD3− CD4+ CD57− TCR$\alpha\beta$− TCRδ−	CD9+ CD10− CD19− CD20− CD22− CD24+ CD80− CD86− sIg−	CD13+ CD14− CD15+ CD33+	CD41a− CD42b− CD61− vWF−	CD34− CD38+ CD71+ HLA-DR− TdT−	CD11b− CD44+ CD54−	48,95
HMC-1[b]	CD1− CD2+ CD3− CD4− CD5− CD7− CD8− CD56− CD57−	CD9+ CD10− CD19− CD20− CD21− CD22− CD23− CD24− CD37+ CD40+ CD74−	CD12− CD13+ CD14− CD15− CD16− CD17+ CD32+ CD33− CD35− CD65− CD68− CD88+	CD31− CD41− CD61+ CD63+	CD34− CD38− CD69− CD71− HLA-DR− TdT−	CD11a+ CD11b− CD11c(+) CD18+ CD43+ CD44+ CD54+	15,98
HNT-34	CD1− CD2− CD3− CD4+ CD5− CD7− CD8−	CD10− CD19− CD20−	CD13+ CD14− CD33+	CD41a− CD42b− GlyA−	CD34+ HLA-DR−		50
HT93	CD1− CD2− CD3− CD4− CD7− CD8− CD56+	CD10− CD19− CD20− CD21− CD22− CD23+	CD13− CD14− CD15(+) CD33+	CD36− CD41− GlyA−	CD34+ CD38− HLA-DR−	CD11b−	51,99
IRTA17	CD2− CD3+ CD4+ CD7(+) CD8−	CD10− CD19− CD20−	CD13+ CD14+ CD33−	CD36−	CD34+ HLA-DR(+)		52
K051	CD2− CD3− CD5− CD7−	CD10− CD19− CD20−	CD13+ CD14− CD33+	CD41+ GlyA+	CD34− HLA-DR−		53
Kasumi-1	CD2− CD3− CD4+ CD5− CD7− CD8−	CD10− CD19− CD20− CD21−	CD13+ CD14− CD15+ CD33+ CD68−		CD34+ CD38+ CD71+ HLA-DR+	CD11b− CD11c−	54,95

Continued on next page

Table 2. (continued)

Cell line	T-/NK-cell marker[a]	B-cell marker	Myelomonocytic marker	Erythroid-megakaryocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
Kasumi-3	CD2− CD3− CD4+ CD5− CD7+ CD8−	CD10− CD19− CD20−	CD13+ CD14− CD15− CD33+	CD36− CD41− CD42−	CD34+ HLA-DR+	CD11a+ CD11b(+) CD11c(+) CD54+	55
Kasumi-4	CD2− CD3− CD4− CD7− CD8−	CD10− CD19− CD20−	CD13+ CD14− CD33+	CD36− CD41a− CD42b− GlyA− PPO−	CD34+ HLA-DR+	CD11b−	56
KBM-7	CD4+	CD20(+)	CD13+ CD14(+) CD33+		CD34+ HLA-DR(+) TdT−	CD11b(+)	58
KCL-22	CD1b− CD2− CD4(+) CD28−	CD20− CD80− CD86− cy/sIg−	CD13+ CD14− CD15+ CD33+ CD65+	CD9+ CD41a+ CD61+ GlyA−	CD34+ HLA-DR− TdT−	CD11b− CD54+	60,100
KF-19	CD3− CD5−	CD19−	CD13+ CD14− CD33+			CD11b−	61
KG-1	CD1− CD2− CD3− CD4− CD5− CD7− CD8− CD28− CD56− CD57− TCR$\alpha\beta$− TCRδ−	CD9− CD10− CD19− CD20− CD21− CD22− CD24+ CD80+ CD86− sIg−	CD13+ CD14− CD15+ CD33+ CD65+	CD36− CD41− CD42b− CD61+ GlyA− vWF−	CD34+ CD38+ CD71+ HLA DR+ TdT−	CD11b+ CD44+ CD54+	30,95,99
KOPM-28	CD1a− CD2− CD3− CD4+ CD8−	CD10− CD19− CD20− CD22− cy/sIg−	CD13+ CD15- CD33+	CD41+ PPO-	CD38+ CD71+ HLA-DR+ TdT−	CD11b+	64,101
KT-1	CD2− CD3− CD4+ CD5− CD7− CD8−	CD10− CD19− CD20−	CD13− CD14− CD33+	CD41a−	CD34− HLA-DR− TdT−	CD11b−	65
KU-812[b]	CD2− CD3− CD5− CD7−	CD9+ CD10− CD19− CD20− CD21− CD22− CD23− CD24+ CD37− CD39− CD40+ CD80− CD86+ cy/sIg−	CD13+ CD14− CD15− CD16− CD17+CD32+ CD33+ CD35+ CD65− CD68+ CD88+	CD31+ CD41+ CD42+ CD61+ CD63+ GlyA(+)	CD34− CD38− CD71+ HLA-DR− TdT−	CD11a− CD11b(+) CD11c− CD18(+) CD43+ CD44+ CD54+ CD69−	66,95,98
KY821	CD2− CD3−	CD19−	CD13+ CD14− CD15+ CD33+		CD34− HLA-DR−		68
KYO-1	CD2−	sIg−		GlyA−	HLA-DR− TdT−	CD11b+	69
LW/SO	CD2− CD3(+) CD4+ CD5+ CD7+ CD8− CD56−	CD10−	CD15+	CD41− CD42−	CD34(+) CD38(+) CD71+ HLA-DR− TdT−		70
M20	CD7+	sIg−			HLA-DR−		71
Marimo	T-marker neg	B-marker neg	CD13+ CD14− CD15+ CD33−	ery-meg marker neg	CD34− HLA-DR−		72
MDS92	CD2− CD3− CD7−	CD19− CD20(+)	CD13+ CD14(+) CD33+	CD41(+) CD61− GlyA−	CD34+ HLA-DR+	CD11b+	73
MOLM-6	CD3− CD4+ CD7+ CD8−	CD9(+) CD10− CD19− CD20− CD24−	CD13+ CD14− CD15+ CD33+	CD41a− CD61−	CD34(+) CD71+ HLA-DR+ TdT−	CD11b+	74
MUTZ-2	CD1− CD3− CD4+ CD5− CD7− CD8− CD56−	CD10− CD19− CD20−	CD13+ CD14− CD15+ CD16− CD33+ CD65+ CD68+	CD41a− CD42b− CD61− GlyA−	CD30+ CD34+ CD38+ CD71+ HLA-DR+ TdT−	CD11b−	75,95

Continued on next page

Table 2. (continued)

Cell line	T-/NK-cell marker[a]	B-cell marker	Myelomonocytic marker	Erythroid-megakaryocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
NB4	CD2+ CD3− CD4+ CD5− CD6− CD7− CD8− TCRαβ- TCRγδ−	CD9+ CD10− CD19− CD20− CD23−	CD13+ CD14− CD15+ CD33+ CD68−	CD36− CD41− CD42− GlyA−	CD34− CD38+ HLA-DR−	CD11b+ CD11c−	76,91,95
NKM-1	CD2− CD3− CD5− CD7−	CD10− CD19− CD20−	CD13− CD14− CD15+ CD16− CD32+ CD33−	CD41− GlyA−	HLA-DR+	CD11b− CD18+	79
OCI/AML-1	CD7− CD8−	CD19− CD20−	CD13+ CD14(+) CD33+	CD36+ CD41a− GlyA−	CD34+ CD38+ CD71+ HLA-DR+	CD11b+	81,83,102
OCI/AML-4	CD1− CD2− CD3− CD7+	CD10− CD19+ CD20−	CD13+ CD14+ CD33+	CD41− GlyA−	CD34− HLA-DR+ TdT+	CD11+	82
OCI/AML-5	CD3− CD7− CD8+	CD19−	CD13+ CD14− CD15+ CD33+ CD68+		CD34+ HLA-DR+ TdT+		83,95
OHN-GM	CD3− CD4− CD5− CD7+ CD8−	CD10− CD19− CD20−	CD13+ CD33+	CD41− GlyA−	CD34+ CD38+ CD71+ HLA-DR+		84
OIH-1	CD1− CD2− CD3− CD4+ CD5− CD7+ CD8−	CD10− CD19(+) CD20−	CD13+ CD14− CD33+	CD41a− CD42b− GlyA−	CD34+ HLA-DR+		85
PL-21	CD2− CD4+	CD10− CD20− sIg−	CD13+ CD14− CD15+ CD33+	CD41a+ CD61+	HLA-DR+ TdT−		86,100
SKNO-1	CD4+	CD19+	CD13+ CD33+		CD34+		87
SR-91	CD1− CD2− CD3− CD4− CD5− CD7− CD8− CD56+	CD10− CD19− CD20−	CD13− CD14− CD33+	CD41−	CD34+ HLA-DR−	CD54+	88
TI-1	CD1− CD2− CD3− CD4− CD5− CD7− CD8−	CD19− CD20−	CD13+ CD14− CD33+	CD41a−	CD34+		89
UCSD/AML1	CD2− CD4− CD5− CD7+ CD8−	CD19− CD20−	CD13+ CD14− CD15+ CD33+	CD36+ CD41a− CD42b+ GlyA−	CD34+ CD38− CD71+ HLA-DR+ TdT+	CD49b+	90,102
UF-1	CD3− CD4− CD5− CD7+ CD8−	CD10− CD19− CD20−	CD13+ CD14+ CD33+	CD41−	CD34− CD38+ HLA-DR−	CD11b−	91
YJ		CD19− CD20− CD23−	CD14(+) CD16− CD33+		CD34−		92
YNH-1	CD1− CD2+ CD3− CD4− CD5− CD7− CD8−	CD10+ CD19− CD20−	CD13+ CD14− CD33+	CD41a+ CD42b− HLA-DR−	CD34+ HLA-DR−	CD11a+ CD11b+ CD11c+	93
YOS-M	CD2− CD3− CD4+ CD5− CD7− CD8−	CD9− CD10− CD19− CD20− CD21−	CD13(+) CD14+ CD33+		CD34+ HLA-DR+ TdT−		94

[a] +, strong, definite protein expression (mostly more than 10–20% cells positive); (+), weak protein expression, qualitatively and quantitatively (commonly <10% cells positive); −, no protein expression.

[b] Extensive immunophenotype in ref. 98.

Table 3. Myelocytic cell lines: cytokine-related characterization

Cell line	Cytokine receptor expression[a]	Cytokine production[a]	Proliferation response to cytokines[b]	Differentiation response to cytokines[b]	Dependency on cytokines[c]	Ref.
AML14	mRNA: GM-CSFRα+, IL-3Rα+, IL-3Rβ+, IL-5Rα+		GM-CSF+, IL-3+, IL-5+	GM-CSF+, IL-3+, IL-5+		35
Ei501	RT-PCR: IL-2Rγ+ protein: IL-2Rα−, IL-2Rβ+, IL-4Rα+	RT-PCR: TGFβ+				40
EoL-1	IL-2Rα+, Kit−			G-CSF+, TNFα+		97
FKH-1			G-CSF+, GM-CSF+, IL-3+, SCF+		G-CSF or GM-CSF	43
GF-D8	mRNA: G-CSFR+, GM-CSFRα+, GM-CSFRβ+, IL-2Rα+, IL-2Rβ+, IL-2Rγ+, IL-3Rα+, IL-4Rα(+), IL-7Rα(+), IL-9Rα(+) protein: IL-2Rα−, IL-2Rγ−	IL-1β+	GM-CSF+, IFNγ+, IL-3+, PIXY-321+, SCF+ inhibition: TGFβ1+		GM-CSF or IL-3	45,103
GM/SO			GM-CSF+, IFNγ+, IL-1α(+), IL-4+, IL-13+, PIXY-321+, SCF+ inhibition: TGFβ1+		GM-CSF	46,103
HL-60	mRNA: IGF-1R+, IGF-2R+, IL-2Rα(+), IL-4Rα+, IL-7Rα(+), IL-9Rα+ protein: IL-2Rα−, IL-2Rβ+, IL-2Rγ+, Kit−					104
HMC-1	GM-CSFRα+, IL-1Rα(+), IL-2Rα(+), IL-2Rβ(+), IL-3Rα−, IL-3Rβ+, IL-6Rα−, IL-7Rα−, Kit+					10,98
HT93	IL-2Rα−, IL-2Rβ−		G-CSF+, GM-CSF+			51
IRTA17	IL-2Rα(+)		G-CSF+, GM-CSF+, IL-3+, SCF+		G-CSF, GM-CSF or SCF	52
Kasumi-1	Kit+		G-CSF+, GM-CSF+, IL-3+, IL-6+			54
Kasumi-3	IL-2Rα+, Kit+		GM-CSF+, IL-2+, IL-3+, IL-4+, SCF+			55
Kasumi-4			GM-CSF+, IL-3+, IL-6+, SCF+			56
KF-19			inhibition: IFNγ+, TNFα+			61
KG-1	mRNA: IL-4Rα−, IL-7Rα−, IL-9Rα− protein: IL-2Rα+, IL-2Rβ+, IL-2Rγ+, Kit−					99,104
KU-812	EPO-R+, GM-CSFRα+, IL-1Rα+, IL-2Rα−, IL-2Rβ−, IL-3Rα+, IL-3Rβ+, IL-6Rα−, IL-7Rα−, Kit+					98
LW/SO	IL-2Rα+, IL-2Rβ−, Kit−, TNFRI+, TNFRII+					70
MDS92			GM-CSF+, IL-3+, SCF+, TPO(+)	G-CSF+, GM-CSF+, IL-3+, SCF+	IL-3	73,105
MUTZ-2	GM-CSFRα+, IL-2Rα+, IL-3Rα+, Kit+, M-CSFR+		bFGF+, FL+, G-CSF+, IFNβ+, IFN-γ+, IGF-I+, IL-6+, M-CSF+, SCF+, TNFα+		SCF	75,103

Continued on next page

Table 3. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[a]	Proliferation response to cytokines[b]	Differentiation response to cytokines[b]	Dependency on cytokines[c]	Ref.
NKM-1	G-CSFR+, M-CSFR+		G-CSF+, M-CSF+			79
OCI/AML-1	mRNA: Kit−	mRNA: GM-CSF+	G-CSF+, GM-CSF(+), IFNβ+, IGF-1(+), IL-3+, IL-4(+), IL-6(+), M-CSF(+), PIXY-321+, SCF(+) inhibition: TGFβ1+, TNFα+, TNFβ+		G-CSF	80,81,103
OCI/AML-4	mRNA: Kit+, M-CSFR+		G-CSF(+), GM-CSF+, IL-3+, SCF+			82
OCI/AML-5	Kit mRNA−		FL+, G-CSF+, GM-CSF+, IFNβ+, IFNγ+, IL-3+, IL-6+, M-CSF+, PIXY-321+, SCF− inhibition: TGFβ1+, TNFα+		GM-CSF, IL-3	83,103
OHN-GM			GM-CSF+		GM-CSF	84
OIH-1			G-CSF+, GM-CSF+, IL-3+, SCF+		G-CSF, GM-CSF	85
SKNO-1			G-CSF+, GM-CSF+, IFNα+, IFNβ+, IFNγ+, IL-3+, IL-5+, IL-6+, IL-13+, M-CSF+, PIXY-321+, SCF+ inhibition: TNFα+		GM-CSF	87,103
SR-91	IL-2Rα−		GM-CSF+, IL-6(+)			88
UCSD/AML1			GM-CSF+, IL-3+, IL-4+, IL-5+, IL-6+, M-CSF+, PIXY-321+, SCF+ inhibition: TGFβ1+	IL-6+	GM-CSF	90,103
UF-1			GM-CSF+, IL-3+, SCF+ inhibition: TGFβ1+			91
YNH-1			G-CSF+, GM-CSF+, IL-3+		G-CSF, GM-CSF or IL-3	93

[a] Receptor expression or cytokine production at the protein level (ELISA, McAb, RIA), unless otherwise indicated, e.g. at the mRNA level (by RT-PCR, Northern); +, strong, definite expression; (+), weak expression; −, no expression.

[b] Effects of cytokine exposure on proliferation or differentiation (−, no effect; +, positive effect).

[c] Upon growth factor withdrawal these cell lines will die by apoptosis.

Table 4. Myelocytic cell lines: genetic characterization

Cell line	Cytogenetic karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements*	Ref.
AML14	45, XY, −5, +8, −9, +13, −14, −18, −21, +der(3), +der(5q), +mar			35
AR230	44, XX, −2, −11, −14, −17, −17, inv(1)(p31–32p36) or add(1)(p31–32), add(2)(p13), del(9)(q22) or der(9)t(9;?)(q11–13;?), der(9)t(9;?)(p13;?)t(9;22)(q34;q11), add(15)(p11), add(18)(q21), der(11;21)(q10;p10), der(22)t(9;22)(q34;q11), +der(22)t(9;22)(q34;q11), +mar	t(9;22)(q34;q11) → *BCR-ABL* (e19/e18-a2) fusion gene		37
CML-C-1	49, XX, +8, +8, +21, del(3)(q21q23), t(9;22)(q34;q11)	t(9;22)(q34;q11) → *BCR-ABL* fusion gene		38
CTS	46, XX, −17, −22, +2mar, t(6;11)(q27;q23)	t(6;11)(q27;q23) → *MLL-AF6* fusion gene	*IGH* R, *IGK* R, *IGL* G, *TCRB* G, *TCRD* R, *TCRG* G	39
Ei501	46, XX, −7, t(7;8)(q32;q13), t(15;17)(q22;q12)	t(15;17)(q22;q21) → *PML-RARA* fusion gene		40
EM-2	74(70–86)<3n>X, −X, −X, +3, +4, +6, +6, +6, +8, −9, +11, −14, −14, +15, +17, −19, +21, +22, +mar, der(5)t(5;?)(q13/15;?), der(9)t(9;22)(q34;q11), i(17q)x2, in some cells up to three copies of der(9)	t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene	*IGH* G; *P53* D/PM	95,96, 106
EoL-1	50(48–51)<2n> XY, +4, +6, +8, +19, del(9)(q22)			42,95
FKH-1	46, XY, −7, t(6;9)(p23;q34)	t(6;9)(p23;q34) → *DEK-CAN* fusion gene		43
GDM-1	48(46–48)<2n>XX, +8, +13, −16, +mar, t(2;11)(q36;q13), del(6)(q21), t(7;?)(q35;?), del(12)(p13)			95
GF-D8	45, XY, −5, 8q+, +8q+, 11q+, 12p−, −15, −17, +mar, del(7)(q32qter), inv(7)(q31.2q36)		*IGH* G, *TCRD* G; *MYC* amplified	45
GM/SO	45, XX, −9, −17, −19, −22, 7p−, 9q+, +3mar, +der(9)t(9;22)(q34;q11), der(13q)	t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		46
HL-60	82(78–88)<4n>XX, −X, −X, −2, −3, −4, −5, −8, −9, −10, −14, −16, −17, −17, +3m, der(6)t(6;?)(q25;?)/dup(6)(q23;qter)x2, del(9)(p22), del(11)(q22/23), der(16)t(16;17)(q22/23;q21–22)x2	no t(15;17)(q22;q11) *PML-RARA* fusion gene	*P15INK4B* GD or GG, *P16INK4A* GD or GG; *P53* D; *MYC, NEU, NRAS* amplified	95, 107
HMC-1	46, XX, dir ins(10;16)(q2?2;q13q22)		*KIT* PM	15
HNT-34	46, XX, t(3;3)(q21;q26), t(9;22)(q34;q11), 20q−	t(9;22)(q34;q11) → *BCR-ABL* (M/m-bcr) fusion genes t(3;3)(q21;q26) → *EVI1* overexpression		50
HT93	46, XY, t(1;12)(q25;p13), 2q+, t(4;6)(q12;q13), t(15;17)(q22;q11)	t(15;17)(q22;q11) → *PML-RARA* bcr3 fusion gene t(1;12)(q25;p13) → *ETV6-ARG* fusion gene		51

Continued on next page

Table 4. (continued)

Cell line	Cytogenetic karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements*	Ref.
IRTA17	46, XX, t(16;21)(p11;q22), add(22)(p11)	t(16;21)(p11;q22) → *TLS/FUS-ERG* fusion gene		52
K051	48, XY, +1, +2, 7q−, 17p−, 21q−		*P53* PM	53
Kasumi-1	45<2n>X, −Y, −9, −13, −16, +3mar, t(8;21)(q22;q22), der(9)t(9;?)(p22;?), der(15)t(?9;15)(?q11;?p11)	t(8;21)(q22;q22) → *AML1-ETO* fusion gene	*IGH* G, *TCRB* G	54,95, 108
Kasumi-3	46, XY, −8, t(2;5)(p13;q33), t(3;7)(q27;q22), del(5)(q15), del(9)(q32), add(12)(p11), add(16)(q13), +mar	t(3;7)(q27;q22) → *EVI1* overexpression	*IGH* G, *TCRB* G, *TCRD* G, *TCRG* D	55
Kasumi-4	46, XX, inv(3)(q21q26), t(9;22;11)(q34;q11;q13)	t(9;22)(q34;q11) → *BCR-ABL* (b2-a2) fusion gene inv(3)(q21q26) → *EVI1* overexpression	*BCL1* G	56
KBM-7	48, X, −Y, +8, +8, −9, +15, −22, +22q−, del(6)t(6;?)(p23;?), t(9;22)(q34;q11), +t(9;22)(q34;q11), del(12p)	t(9;22)(q34;q11) → *BCR-ABL* (b2-a2) fusion gene	*P53* PM	58
KCL-22	52, XX, +1p−, +6, +8, +8, +8, t(9;22)(q+;q−), +22q−	t(9;22)(q34;q11) → *BCR-ABL* (b2-a2) fusion gene	*P53* D+PM	59
KG-1	45(42–47)<2n>X, −Y, −7, −12, +2mar, dup(7)(q12q33), der(8)t(8;?)(p12;?), der(11)t(1;11)(q11;p11), der(17)t(17;?)(p13;?)		*P53* PM, *RB1* R, *NRAS* mutation	95
KOPM-28	44, XX, −5, −17, −19, t(9;22)(q34;q11), +der(19)t(19;?)(p13;?)	t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		64
KT-1	51, XXYY, +X, +Y, −5, −6, +8, +8, +19, +19, +mar, t(9;22)(q34;q11), t(9;22)(q34;q11)	t(9;22)(q34;q11) → *BCR-ABL* (b2-a2) fusion gene	*IGH* G, *TCR* G	65
KU-812	50–60(58), XY, +1, +4, +6, +6, +8, −9, +14, +15, +19, +21, +21, t(9;22)(q34;q11), +der(9)t(9;22)(q34;q11)x2, +der(11)(q11), i(17q)	t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene	*P53* PM	66,95, 107
KY821	86, X, X, −Y, +1, +1, +3, +3, +4, +4, +5, +5, +6, +6, +7, +7, +8, +8, +10, +10, +11, +11, +12, +13, +13, +13, +13, +14, +14, +15, +15, +16, +16, +18, +19, +19, +20, +20, +21, +22, +22, +der(2)t(2;?)(p25;?), +der(2)t(2;?)(p25;?), del(9)(p22p24), del(9)(p22p24), +mar		*P53* PM	68
KYO-1	47, XY, +15, −16, −18, 6q−, 8q+, 12p+, 15p+, 17p+, 22p+, 22q−(Ph), +22q−(Ph)	t(9;22)(q34;q11) → *BCR-ABL* (b2-a2) fusion gene	*P53* PM	69, 109
LW/SO	45, X, −X, der(9)inv(9)(p12q13), del(9)(p22?)			70
Marimo	44, X, −X, −5, +18, +18, ins(1;?)(q21;?), del(8)(q22), t(10;14;11)(q22;q32;q13), der(14)t(10;14;11)(q22;q32;q13), add(17)(p11), psu dic(18;9)(q23;p21)x2		*MYC* amplified	72
MDS92	44, XY, −7, −12, −13, del(5)(q13q35), add(14)(p11), add(22)(q13), +mar		*NRAS* PM	73
MUTZ-2	48(46–50)<2n>XY, +8, +10		*P53* PM	75,95

Continued on next page

Table 4. (continued)

Cell line	Cytogenetic karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements*	Ref.
NB4	78(71–81)<3n>XX, −X, +2, +6, +7, +7, +11, +12, +13, +14, +17, −19, +20, +4mar, der(8)t(8;?)(q24;?), der(11)t(11;?)(?::11p15↑11q22.1::11q13−>22.1:), der(12)t(12;?)(p11;?), 14p+, t(15;17)(q22;q11–12.1), der(19)t(10;19)(q21.1;p13.3)x2	t(15;17)(q22;q21) → *PML-RARA* (bcr1-2) fusion gene	*P15INK4B* DD, *P16INK4A* DD	76, 95, 110
NKM-1	47, XY, −6, +8, der(2)t(2;?)(q37;?), +der(6)t(6;?)(p23;?)			79
OCI/AML-1	46, XX, −6, +der(6)t(6;8)(p25;q22)			81
OCI/AML-5	48(44–48)<2n>XY, +1, +8, der(1)t(1;19)(p13;p13)			95
OHN-GM	48, XY, −7, +8, +22, del(5)(q11.2q31), t(10;13)(q24;q14), +der(13)t(10;13)(q24;q14)		*P53* PM, RB1 D	84
OIH-1	49, X, −Y, −5, −11, +18, +20, +21, add(7)(q11), add(7)(p21), +del(7)(q22), add(8)(q22), +add(8)(q22), inv(9), add(15)(p11)		*DCC* alteration	85
PL-21	46, XY, −11, +der(11)t(11;?)(11pter–11q23::?)	no t(15,17)(q22;q11) *PML-RARA* fusion gene		86
SKNO-1	44, X, −Y, −16, +der(16q17q), −17.2q, 6q+, t(8;21)(q22;q22), 11p+, 19p+	t(8;21)(q22;q22) → *AML1-ETO* fusion gene	*P53* PM	87
SR-91	50, X, −Y, +3, +6, +7, +13, −14, +18, dic(8;14)(p11;p11), i(17q)		*IGH* G, *TCRB* G; *BCL2* G	88
TI-1	69 (65–71), XXY, +1, +4, +7, +8, +11, +12, +19, 2q+, 6p+, 9p+, 9p−, 13p+			89
UCSD/AML1	45, XX, −7, t(3;3)(q21;q26), t(12;22)(p13;q12)	t(3;3)(q21;q26) → *EVI1* overexpression t(12;22)(p13;q12) → *ETV6/TEL-MN1* fusion gene	*IGH* G, *TCRB* G	75, 90, 108, 111
UF-1	46, XX, add(1)(q44), add(6)(q12), add(7)(q36), t(15;17)(q21;q21)	t(15;17)(q22;q21) → *PML-RARA* fusion gene		91
YJ	45, X, −Y, −2, −5, −9, −14, −14, −15, −16, −17, +8mar, add(3)(q27), del(3)(p13), add(4)(q34), add(6)(q25), del(10)(p12), add(11)(p15), add(13)(q32), add(16)(q23), del(18)(q22)			92
YNH-1	46, XY, der(16)t(16;21)(p11;q22)t(1;16)(q12,q13), der(21)t(16;21)(p11;q22), der(6)t(6;12)(q13;q13), der(12)t(6;12)(q21;q13)	t(16;21)(p11;q22) → *TLS/FUS-ERG* fusion gene	*P53* PM	93
YOS-M	46, XY, −20, del(7)(q22q32), t(9;22)(q34;q11), +mar	t(9;22)(q34;q11) → *BCR-ABL* (b2-a2) fusion gene	*IGH* G	94

* Receptor gene arrangements: D – deleted; G – germline; PM – point mutation; R – rearranged; wt – wild type.

Table 5. Myelocytic cell lines: functional characterization

Cell line	Doubling time	EBV status	Cytochemistry	Inducibility of differentiation	Heterotransplantability into mice	Special functional features	Ref.
AML14	24–36 h			TPA, vit. D3 → mono/macro differentiation; GM-CSF+IL-3+IL-5 → eosino differentiation; retinoic acid → neutro differentiation		spontaneous/induced expression of specific eosino-granular proteins: Charcot-Leyden crystal protein, eosino-cationic protein, eosino-derived neurotoxin, eosino-lysophospholipase, eosino-peroxidase, major basic protein	35, 112
AR230						produces p230 BCR-ABL fusion protein	37
CML-C-1			ACP+, Alcian Blue−, ANBE−, CAE−, MPO−, PAS+, SBB−, Toluidine Blue−		into nude mice		38
CTS	72 h		ACP−, ALP−, ANAE−, ANBE−, CAE-, MPO-, PAS−	(DMSO, hemin, retinoic acid, TPA no effect)			39
Ei501	3–4 d		ANAE−, MPO+, PAS−			PO: BCL2 mRNA+, WT1 mRNA+, target for cytotoxicity assay	40
EM-2	24–48 h	EBV− HTLV-I− HIV−	MSE−	DMSO, retinoic acid → neutro differentiation; TPA → mono/ macro diffferentiation	into SCID mice	colony formation	95, 96, 113
EoL-1	48–60 h	EBV− HTLV-I− HIV−	ACP+, ANAE+, ANBE(+), CAE−, Luxol(+), MPO+, MSE+, PAS(+), SBB+, Toluidine Blue−	cAMP, DMSO, G-CSF, TNFα → eosino differentiation (retinoic acid no effect)		colony formation in agar; no phagocytosis	42, 95, 97
FKH-1	54 h	EBV−	ANBE+, CAE−, MPO−, Toluidine Blue−			granules positive for sulphate glycoproteins	43
GDM-1	96–120 h	EBV− HTLV-I− HIV−	ACP+, ALP+, ANBE+, CAE−, GLC+, Lysozyme+, MPO(+), MSE−, PAS−, SBB+	TPA → macro differentiation		no colony formation in agar; phagocytosis+	44, 95
GF-D8	48–72 h	EBV−	ACP+, ALP−, ANAE+ (NaF inhibitable), ANBE−, MPO(+), MSE−, SBB+	TPA → mono/macro differentiation		clonable/colony formation in agar	45
GM/SO			ANAE−, MPO−, PAS−	TPA → mono/macro differentiation			46
HL-60	25–40 h	EBV− HTLV-I− HIV−	ACP(+), Alcian Blue−, ALP−, ANAE−, ANBE(+), CAE+, Methyl Green−, MPO+, MSE−, NBT−, Oil Red O+, PAS+, Pyronin+, SBB+	various reagents → neutro, mono, macro, eosino, baso differentiation[a]	into nude or SCID mice	colony formation in agar/methylcellulose; lysozyme production+; no phagocytosis; no chemotaxis; no adherence; mRNA: (α-1-antitrypsin+, azurocidin+, cathepsin G+, Charcot Leyden crystals+, defensin+, lactoferrin−, major basic protein+, myeloblastin+, N-elastase+, tryptase−; TF: mRNA: GATA1+, GATA2+, SCL−	48, 95, 115, 116

Continued on next page

Table 5. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Inducibility of differentiation	Heterotransplantability into mice	Special functional features	Ref.
HMC-1	6–8 d	EBNA−	Alcian Blue+, CAE+, Luxol+, Lysozyme−, MPO−, MSE−, Toluidine Blue+	spontaneous ery differentiation	into nude mice	clonable in methylcellulose; no histamine production; no IgE binding; protein: aminocaproate esterase+, cathepsin G(+), chymotrypsin−, CLC protein−, Elastase−, eosino major basic protein+, eosino peroxidase+, eosino- derived neurotoxin−, eosino cationic protein−, tryptase+; mRNA: α-tryptase+, carboxypeptidase A(+), Charcot Leyden crystal protein+, defensin−, granzyme A−, IgE-Rα−/β−/γ+, lactoferrin−, lysozyme(+), mast cell chymase−, mast cell tryptase+, N-elastase−	10, 15, 98
HNT-34	26–27 h		ALP−, ANBE(+), CAE−, MPO−, PAS−	(DMSO, TPA no effect)			50
HT93	48 h		ALP−, ANAE−, MPO(+)	ATRA → neutro/eosino differentiation; ATRA + G-CSF → neutro differentiation; ATRA + GM-CSF, IL-3, IL-5 → eosino/baso differentiation		colony formation in methylcellulose	51
IRTA17	79 h		ANBE+, CAE−, Lysozyme−, MPO−				52
K051	48 h	EBNA−	ANAE−, MPO+, MSE−, PAS−, SBB+	retinoic acid → ery differentiation		MDR-1 mRNA+	53
Kasumi-1	40–72 h	EBV− HTLV-I− HIV−	ALP−, ANBE−, CAE+, MPO+, MSE−	TPA → macro differentiation (DMSO no effect)		TF: mRNA: GATA1−, GATA2+, SCL−	54, 95, 116
Kasumi-3	55–60 h		ACP+, ALP−, ANBE−, CAE−, MPO−, PAS−	TPA → mono differentiation (DMSO, retinoic acid no effect)			55
Kasumi-4	55 h		ACP+, ALP−, ANBE−, CAE−, MPO−, PAS−	(cytokines, DMSO, retinoic acid, TPA no effect)		no α-granules, no demarcation membranes	56
KBM-7	22–24 h	EBNA−	ACP+, ANAE+, ANBE−, CAE+, MPO−, MSE−, PAS+		into nude mice	clonable/colony formation in agar	57, 58
KCL-22	24 h	EBNA−	ACP+, ALP−, ANBE−, Benzidine−, CAE−, MPO−, MSE−, PAS+, SBB−	(DMSO no effect)	into newborn hamsters	no phagocytosis	60
KF-19	27 h		ANAE+, ANBE−, CAE+, MPO+, PAS+	TPA → macro differentiation; DMSO, retinoic acid → neutro differentiation		no phagocytosis; resistant to AraC, adriamycin, vincristine	61
KG-1	40–50 h	EBV− HTLV-I− HIV−	ACP+, CAE+, MPO+, MSE−, PAS+	TPA → mono/macro differentiation (DMSO, Na butyrate no effect)	into nude or SCID mice	no ADCC; no phagocytosis; no chemotaxis; PHA-LCM → colony formation in agar; TF: mRNA: GATA1+, GATA2+, SCL−	63, 95, 113, 116, 117

Continued on next page

Table 5. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Inducibility of differentiation	Heterotransplantability into mice	Special functional features	Ref.
KOPM-28	22–24 h		ACP+, ALP−, ANBE+, CAE+, MPO−, MSE−, PAS−	TPA → meg differentiation (DMSO, retinoic acid no effect)		clonable/colony formation in agar; no phagocytosis	64, 118
KT-1	18–24 h	EBNA−	ANBE−, CAE−, MPO+, PAS(+)			colony formation in methylcellulose	65
KU-812	80–100 h	EBV− HTLV-I− HIV−	ACP+, Alcian Blue(+), ALP−, ANAE+, ANBE(+), Astra Blue+, CAE−, Lysozyme−, MPO−, MSE−, PAS(+), SBB−, Toluidine Blue+	spontaneous ery differentiation; serum-free culture → baso differentiation; TPA → macro differentiation		cloning/colony formation in agar; histamine production; no IgE binding; protein: cathepsin G−, chymase−, elastase−, tryptase−; mRNA: α-tryptase−, carboxypeptidase A+, Charcot Leyden crystal+, chymase−, defensin−, eosino peroxidase−, eosino-derived neurotoxin−, eosino cationic protein−, glycophorin+, granzyme A−, heparin core protein+, IgE-Rα+/β+/γ+, lactoferrin−, lysozyme+, mast cell chymase−, mast cell tryptase+, N-elastase−; α-, β-, γ-, δ-globin mRNA+; δ-aminolevulin synthase mRNA+; HbA+, HbF+, HB Bart's inducible; TF: mRNA: GATA1+, GATA2+, PU1+, SCL+	66, 95, 98, 116, 119
KY821			ANBE(+), CAE+, MPO+, MSE−			colony formation in methylcellulose; *resistant to methotrexate*	68
KYO-1	22 h	EBNA−	ANBE−, CAE−, MPO−, MSE−			colony formation in agar	69
LW/SO			ACP(+), ANAE−, PAS(+), SBB−			not clonable	70
M20		EBNA−	ACP+, ANAE(+), CAE+, MPO+, SBB+	TPA → macro differentiation (DMSO, retinoic acid no effect)		colony formation in methylcellulose; no phagocytosis; secretes lysozyme, prostaglandin E2	71
Marimo	25–28 h		ANAE−, MPO−	DMSO, retinoic acid → neutro differentiation			72
MDS92	80–90 h		MPO+, MSE−	G-CSF, GM-CSF, IL-3, SCF → neutro differentiation		colony formation in agar	73
MOLM-6	24–60 h		MPO+, MSE−				74
MUTZ-2	48 h	EBV− HTLV-I− HIV−	MSE−, TRAP−	TPA → mono differentiation (retinoic acid no effect)		clonable/colony formation in methylcellulose; PO: BCL2 mRNA+	75, 95
NB4	36–48 h	EBV− HTLV-I− HIV−	ALP−, ANBE−, CAE−, MPO+, MSE+	retinoic acid → neutro differentiation			76, 95
NKM-1	36–48 h	EBV−	ANBE−, CAE+, MPO+, NBT−	(DMSO, Na butyrate, retinoic acid, TPA no effect)		colony formation in agar; no phagocytosis	79

Continued on next page

Table 5. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Inducibility of differentiation	Heterotrans-plantability into mice	Special functional features	Ref.
OCI/AML-1	32 h		ANBE(+), CAE+, MPO+, MSE− NBT−	responsive to retinoic acid (but no differentiation)		colony formation in methylcellulose; RARA mRNA+	80
OCI/AML-4	7 d (3 d)[b]		ANBE(+), MPO(+), SBB(+)	sensitive to retinoic acid		colony formation in methylcellulose	82
OCI/AML-5	30–50 h	EBV− HTLV-I− HIV−	MSE−			colony formation in methylcellulose	83, 95
OHN-GM	60 h	EBV−	ANAE(+), MPO+				84
OIH-1	48 h	EBV−	ANBE+, CAE−, MPO−, PAS(+)				85
PL-21	48–64 h	EBNA−	ACP+, ALP−, ANBE(+), CAE+, Lysozyme+, MPO+, MSE+, PAS(+), SBB+	DMSO → macro differentiation		phagocytosis+	86
SKNO-1	48–72 h	EBNA−	ANBE−, CAE+, MPO+	TPA → mono/macro differentiation		PO: p53 overexpressed	87, 120
SR-91	28–32 h	EBV−	ACP−, ANAE(+), CAE−, MSE−, PAS−, SBB−			colony formation in methylcellulose; resistant to NK activity	88
TI-1	14 h		ANBE−, CAE−, MPO−, MSE−	TPA → mono differentiation; hemin → ery differentiation		HbF+ upon induction	89
UCSD/AML1	48–72 h		ACP+, ANAE+, CAE−, MSE−	TPA → macro differentiation; IL-6 → meg differentiation			90
UF-1	72 h		MPO+, NBT−	resistant to retinoic acids TPA			91, 121
YJ	24 h		Fast Green+, neutral+	→ mono differentiation; retinoic acid → neutro differentiation		mRNA: Charcot-Leyden crystal protein+, eosino cationic protein+, eosino-derived neurotoxin+, eosino peroxidase+, major basic protein+; TF: mRNA: C/EBPα+, EGR1(+), GATA1+, GATA2+, MZF1+, PU1+	92
YNH-1	82 h	EBV−	ANBE−, CAE−, MPO+, PAS−				93
YOS-M	5–6 d	EBNA−	ANBE−, CAE(+), MPO+, MSE−				94

[a] Reviewed in [31,114].

[b] Doubling time is shortened by incubation with GM-CSF or IL-3 from 7 d to 3 d.

PHA-LCM – phytohemagglutinin-stimulated peripheral leukocytes conditioned medium; PO – (proto)-oncogenes; TF – transcription factors.

dominant. Thus, KU-812 is currently the prototype cell line for studies on basophil biology. The cell line HMC-1 has been developed from a patient with mast cell leukemia. These cells express a number of characteristic "mast cell-related antigens" and other typical features [10]. The HMC-1 line is widely used as a model for investigating the biology of human mast cells.

3. IMMUNOPHENOTYPE

Myelocytic cell lines generally express the pan-myelomonocytic surface antigens CD13 (41/47,87%), CD15 (18/23,78%) and CD33 (42/47, 82%), while they only rarely display the monocyte-associated marker CD 14 (9/40, 22%) (Table 2). Other antigens physiologically expressed by myelocytic cells, such as CD16, CD32, CD64, CD65, CD68 [144], are mostly positive, but have not been tested on many cell lines. Apart from the relatively frequent expression of T-cell antigens CD4 and CD7 which are also seen on normal cells committed to myelocytic differentiation [145], the majority of these cell lines are negative for surface markers associated with T-cells, B-cells and erythroid-megakaryocytic cells (Table 2). Concerning the progenitor and activation markers, the cell lines were predominantly positive for CD34 (27/45, 60%), CD38 (11/15, 73%) and HLA-DR (26/46, 56%), but negative for the lymphoid nuclear enzyme marker TdT (3/20, 15%). The following profile of myelocytic cell lines emerges: CD4± CD7± CD13+ CD14– CD15+ CD33+ CD34+ CD38+ HLA-DR+, T-antigen negative, B-antigen negative, ery-meg-antigen negative.

4. CYTOKINE-RELATED CHARACTERIZATION

Normal myelocytic cells respond to signals provided by the cytokines G-CSF, GM-CSF and IL-3 regulating cellular proliferation and differentiation [146,147]. Cytokine receptor expression, cytokine production, and proliferative and differentiative responses to cytokines of the myelocytic cell lines are summarized in Table 3. Several cell lines were reported to be constitutively dependent on the addition of specific cytokines to the culture medium (see Appendix 3): FKH-1, GF-D8, GM/SO, IRTA17, MDS92, MUTZ-2, OC1/AML1, OC1/AML5, OHN-GM, OIH-1, SKNO-1, UCSD/AML1, YNH-1 (Table 3). These cells die within a few days when deprived of growth factors [103,148]. The necessary cytokines are usually G-CSF, GM-CSF, IL-3, or SCF. These growth factor-dependent cell lines also respond proliferatively to a variety of other cytokines (reviewed in ref. [103]) (Table 3).

Table 6. Myelocytic cell lines: unconfirmed cell lines (not immortalized, not characterized, not verified, other)

Cell	Patient	Features	Ref.	Remarks*
2L1	from BM of 6 M with t-AML M5 (post-nasopharyngeal embryonal rhabdomyosarcoma and ganglioneuroma)	CD2+ CD10+ CD13+ CD15+ CD33+ CD38+ CD56+; *P53* PM; *MLL* amplified	122	insufficiently characterized
8261	from PB of patient with AML	ALP− CAE− NBT− PAS− SBB−; no phagocytosis; differentiation to monos/macros	123	insufficiently characterized
AML-CL	from PB of 27 F with AML M1 (at diagnosis)	CD13+ CD19− CD33+ CD45+; secretion of IL-1β; 46, XX; established, maintained and serially passaged in SCID mice, but no long-term *in vitro* growth	124	insufficiently characterized; no long-term *in vitro* growth
AML-PS	from BM of 61 M with AML M1 (3rd relapse) (post-sideroblastic anemia MDS)	CD13+ CD19− CD33+ CD45+; secretion of IL-1β; 46, XY/49, XY, −8, −9, +11, +12, +21, +21, +21; established, maintained and serially passaged in SCID mice, but no long-term *in vitro* growth	124	insufficiently characterized; no long-term *in vitro* growth
AML5q−	from patient with AML M2	mRNA: GATA1−, GATA2+, GATA3−; EPO-R+	125,126	insufficiently characterized
AP-1060	from BM of 45 M with AML M3 (at relapse)	46, XY, t(3;14)(p21;q11.2), t(15;17)(q22;q11.2); *PML-RARA* fusion gene; ATRA → terminal differentiation; A_3O_2 → apoptosis	127	insufficiently characterized
CC-AML	from PB of patient with AML M1 (refractory)	CD2− CD3− CD5− CD7− CD10− CD11+ CD13+ CD14+ CD15+ CD16+ CD19− CD20− CD21− CD33+ CD34+ CD45+ CD56− HLA-DR+; PIXY-321, SCF → proliferation; TPA → macro differentiation; DMSO → neutro differentiation	128	insufficiently characterized
EU-4	from child with AML (relapse)	CD2− CD7− CD10− CD13+ CD15+ CD19− CD33+ HLA-DR−; *P15INK4B* D, *P16INK4A* D; mRNA: BAX+, BCL-XL+, MDM2+	129,130	insufficiently characterized
HSM-911	from PB of patient with acute mixed leukemia	CD7+ CD10− CD13+ CD14− CD19− CD33+ CD34+ CD41− GlyA− HLA-DR+; ANBE−, CAE−, MPO−; dependent on GM-CSF, IL-3 or SCF	131	insufficiently characterized

Continued on next page

Table 6. (continued)

Cell	Patient	Features	Ref.	Remarks*
KH-143	from patient with NK-myeloid leukemia	CD2− CD11b− CD13− CD18− CD33+ CD34− CD41+ CD54− CD56+; IL-3Rα+, Kit+; 46−49, XY, Yp+, 1p−, 2p+, +3q−, −8, 9q−, 11q−, 17p+, −18; *IGH* G, *IGK* G, *IGL* G, *TCRB* G, *TCRG* G; responsive to IL-3(+), SCF(+); inhibition by TGFβ+	132	insufficiently characterized
KM-MDS	from BM of patient with MDS	CD2− CD11b+ CD13+ CD18+ CD33+ CD34+ CD41− CD54+ CD56−; G-CSFR+, GM-CSFRα+,IL-3Rα+, Kit+, c-mpl+; 46, XX, −4, 5q−, −7, 13q+, 20q−; responsive to G-CSF(+), IL-3(+), SCF(+), TPO(+); *IGH* G, *IGK* G, *IGL* G, *TCRB* G, *TCRG* G; phagocytosis+; H_2O_2 production+	132	insufficiently characterized
KOPM 30	from peripheral blood of patient with AML	EBV−; *P53* PM; t(9;22)(q34;q11) → *BCR-ABL* (e1-a2) fusion gene	133	insufficiently characterized
KOPM 55	from patient with AML	CD2− CD3− CD10− CD13+ CD19− CD22− CD33+ CD41+ HLA-DR+; ANBE− MPO+; t(9;22)(q34;q11)	101	insufficiently characterized
M24	from PB of patient with AML	HLA-DR− sIg−; EBNA−; ACP+ ANAE+ CAE+ MPO+ SBB+; TPA → macro differentiation; no phagocytosis	134	insufficiently characterized
M26	from PB of patient with AML	HLA-DR− sIg−; EBNA−; ACP+ ANAE− CAE+ MPO+ SBB+; TPA → macro differentiation; no phagocytosis	134	insufficiently characterized
MDS-KZ	from patient with MDS (RAEBT)	dt 2 weeks; complex chromosome abnormalities, −4, 5q−, −7, 13q+, 20q−; responsive to G-CSF; vit. K2 → apoptosis	135	insufficiently characterized
MHH-203	from PB of adult patient with AML	responsive to FL, G-CSF, IGF-I, IL-3, TPO	103	insufficiently characterized
MML-1	from BM of patient with AML M1	biphenotypic: CD3− CD4− CD5− CD8− CD13+ CD14+ CD15+ CD19+ CD20− CD33+ CD37− CD68+ HLA-DR+ cy/sIg−; 46<2n>X, del(2)(p22p23), +8, del(9)(p21p24)	136	insufficiently characterized
MO-91	from patient with AML M0	mRNA: GATA1+, GATA2+, GATA3+; EPO-R+	125,126	insufficiently characterized
MPD	from patient with non-CML MPD	dt 72 h; CD11b+ CD11c+ CD14+ CD15+ CD16+ CD33+ CD54+; r(18); spontaneous neutro, mono, eosino, baso differentiation; mRNA Charcot-Leyden crystal protein+, lactoferrin+, major basic protein+, transcobalamin-1+	137	insufficiently characterized

Continued on next page

Table 6. (continued)

Cell	Patient	Features	Ref.	Remarks*
MTO-94	from BM of patient with MDS	CD7+ CD13+ CD33+ CD34− HLA-DR+; 46, XY, i(17q); responsive to G-CSF, GM-CSF, IL-3, SCF	138	insufficiently characterized
MZ93	from patient with CML-BC	CD1− CD2− CD3− CD4(+) CD7+ CD8(+) CD10− CD13+ CD14− CD15− CD19− CD20− CD21− CD22− CD25+ CD33+ CD34+ CD36− CD38(+) CD41− CD56+ CD122− GlyA− HLA-DR−	99	insufficiently characterized
OCI/AML-6	from PB of 68 F with MDS → AML M4 (at diagnosis) in 1991	responsive to G-CSF, GM-CSF, IFNγ, IL-3, IL-4, IL-13, M-CSF, PIXY-321, SCF	103,139	cross-contaminated with OCI/AML-2
OU-AML-1	from PB of 26 F with AML M4 (at diagnosis)	CD3− CD7− CD13+ CD14− CD19− CD33+ CD34− CD61− CD117− GlyA− MPO−; [hyperdiploid]; *P53* wild-type	140	cross-contaminated with OCI/AML-2
OU-AML-2	from PB of 52 F with AML M2 (at diagnosis)	CD3− CD7− CD13+ CD14− CD19− CD33+ CD34− CD61− CD117− GlyA− MPO−; [diploid]; *P53* wild-type	140	cross-contaminated with OCI/AML-2
OU-AML-3	from PB of 48 M with AML M4 (at diagnosis)	CD3− CD7+ CD13+ CD14− CD19− CD33+ CD34− CD61− CD117− GlyA− MPO−; [diploid]; *P53* wild-type; responsive to ATRA	140	cross-contaminated with OCI/AML-2
OU-AML-4	from PB of 39 M with AML M2 (at 1st relapse)	CD3− CD7+ CD13+ CD14− CD19− CD33+ CD34− CD61− CD117− GlyA− MPO−; [hyperdiploid]; *P53* wild-type	140	cross-contaminated with OCI/AML-2
OU-AML-5	from PB of 70 M with AML M5 (at 2nd relapse)	CD3− CD7+ CD13+ CD14− CD19− CD33+ CD34− CD61− CD117− GlyA− MPO−; [hyperdiploid]; *P53* wild-type	140	cross-contaminated with OCI/AML-2
OU-AML-6	from PB of 47 F with AML M1 (at 1st relapse)	CD3− CD7+ CD13+ CD14− CD19− CD33+ CD34− CD61− CD117− GlyA− MPO−; [hyperdiploid]; *P53* wild-type	140	cross-contaminated with OCI/AML-2
OU-AML-7	from PB of 63 F with AML M4 (at 1st relapse)	CD3− CD7+ CD13+ CD14− CD19− CD33+ CD34− CD61− CD117− GlyA− MPO−; [hyperdiploid]; *P53* wild-type	140	cross-contaminated with OCI/AML-2

Table 6. (continued)

Cell	Patient	Features	Ref.	Remarks*
OU-AML-8	from PB of 63 F with AML M4 (at diagnosis)	CD3− CD7+ CD13+ CD14− CD19− CD33+ CD34− CD61− CD117− GlyA− MPO−; [hyperdiploid]; *P53* wild-type	140	cross-contaminated with OCI/AML-2
RDFD-2	from PB of 56 M with AML M1	ANBE− MPO+; dt 56 h; DMSO, retinoic acid → macro differentiation	141	insufficiently characterized
RED-3	from PB of 24 M with T-ALL → AML (2nd relapse)	CD2+ CD4+ CD13+ CD15+ TdT−; ACP+ ANAE+ MPO+ PAS+ SBB+; DMSO, retinoic acid → neutro differentiation; TPA → macro differentiation; *NRAS* point mutation; c-*MYC* amplification	142	insufficiently characterized; needs to be excluded, cross-contaminated with HL-60
TMM	from PB of 62 M with CML-BC (post-MDS) in 1985	CD3− CD10− CD13− CD14− CD15− CD19+ CD20+ CD33− CD37+ cy/sIg+ HLA-DR+; EBNA+; 46(42−46)<2n>XY	95, 143	not leukemia cell line, but normal EBV+ B-LCL
YS-1	from patient with CML-BC	CD1− CD2− CD3− CD4− CD7− CD8− CD10+ CD13+ CD14− CD15(+) CD19+ CD20+ CD21(+) CD22+ CD25− CD33(+) CD34− CD36− CD38+ CD41− CD56− CD122− GlyA− HLA-DR+	99	insufficiently characterized

* For most cell lines, the insufficient characterization concerns the description of clinical data, authentication, immunoprofile and/or cytogenetics. Present status unknown: no data on this cell line have been published since its original description.

5. GENETIC CHARACTERIZATION

In common with other types of leukemia-lymphoma cell lines, the myelocytic cell lines are characterized by highly complex numerical and structural chromosomal aberrations (Table 4). Of particular note are the various translocations leading to the occurrence of specific fusion genes (Appendix 4, p. 288). The most common balanced translocation among these cell lines is the t(9;22) with the hybrid gene BCR-ABL which is associated in vivo with CML [28]. Fourteen such myelocytic Philadelphia+ cell lines have been developed [96,149,150]. Other unique translocations/fusion genes seen in myelocytic cell lines are t(6;9) *DEK-CAN,* t(8;21) *AML1-ETO*, t(12;22) *ETV6/TEL-MN1*, t(15;17) *PML-RARA*, t(16;21) *TLS/FUS-ERG* and alterations involving bands 11q23 (gene *MLL*) and 3q26 (gene *EV11*). The cell lines provide models for these translocations [19,22,28,108,151–156].

Some cell lines have deletions, rearrangements, point mutations or other alterations of the tumor suppressor genes *P53* (EM-2, HL-60, K051, KBM-7, KCL-22, KG-1, KU-812, KY821, KYO-1, MUTZ-2, OHN-GM, SKNO-1, YNH-1), *RB1* (KG-1, OHN-GM) and *P15INK4B/P16INK4A* (HL-60, NB4); and the oncogenes *NRAS* (HL-60, KG-1, MDS92) and *MYC* (GF-D8, HL-60, Marimo).

6. FUNCTIONAL CHARACTERIZATION

The doubling times of myelocytic cell lines range from 20 hours to 6–8 days (Table 5). All 27 cell lines tested were negative for EBV infection. The following results on the cytochemical staining of myelocytic cell lines were seen: 89% ACP+, 0% ALP, 46% ANAE, 40% ANBE, 48% CAE, 50% lysozyme, 61% MPO, 33% MSE, 48% PAS, 64% SBB (Table 5). A typical cytochemical profile is: ACP+, ALP–, ANAE/ANBE±, CAE±, MPO+, MSE–, PAS±, SBB+. Extensive cytochemical investigations have been reported for cell lines CML-C-1, EoL-1, HL-60, HMC-1 and KU-812. The eosinophilic EoL-1 (Luxol fast blue+, toluidine blue negative), the mast cell line HMC-1 (toluidine blue+) and the basophilic KU-812 (toluidine blue+) showed the staining patterns expected for their cell types (Table 5).

Cellular differentiation induced by various agents has been reported for over 20 cell lines (Table 5). The principal inducers used were retinoic acid and DMSO, leading to neutrophil differentiation; and TPA, causing monocytic/macrophage maturation. The HL-60 cell line is the most widely and best studied target cell line in this regard [31,34,114]. HL-60 cells can be induced to differentiate into neutrophils, monocytes/macrophages, eosinophils or basophils by a multitude of specific inducers [157–159]. Of special

interest are the various retinoic acid-resistant and acid-sensitive parental lines and their subclones (see also Tables 1a and 1b). Heterotransplantation into nude or SCID mice or hamster was reported for only seven cell lines (Table 5, Appendix 5, p. 289). Most cell lines can be cloned and form colonies in methylcellulose. Phagocytosis, adherence and chemotaxis are not properties of myelocytic cell lines. Expression of unique proteins specifically associated with certain subtypes of granulocytic cells (neutrophils, basophils, mast cells) was studied extensively in HL-60, HMC-1 and KU-812.

7. UNCONFIRMED CELL LINES

There is also a panel of myelocytic cell lines which, due to insufficient clinical, immunophenotypic, functional or other characterization were assigned to the category "Unconfirmed Cell Lines" (Table 6). These cell lines were established from patients with AML (Ml, M2, M3, M4), acute mixed leukemia, NK-myeloid leukemia, MDS (RAEBT), MPD, and CML in blast crisis. The present status of some older cell lines (e.g. 8261, RDFD-2) is unknown as no further data have been published. The cells might be lost or a continuous cell line was not established. Of interest are two cell lines that can only be propagated by continuous serial passage in SCID mice (AML-CL, AML-PS). Cell lines RED-3 and TMM might be a cross-contaminated culture and a normal EBV+ B-LCL, respectively.

References

1. Bessis M (ed.) *Blood Smears Reinterpreted.* Springer, Berlin, 1977.
2. Roitt I, Brostoff J, Male D (eds.) *Immunology*, 3rd edn., Mosby, London, 1993.
3. Hoffbrand AV, Pettit JE (eds.) *Sandoz Atlas Clinical Haematology*, Gower, London, 1988.
4. Hayhoe FGJ, Flemans RJ (eds.) *A Colour Atlas of Haematological Cytology*, 3rd edn., Wolfe, London, 1992.
5. Borregaard N, Cowland JB. *Blood* 89: 3503–3521, 1997.
6. Marmont AM, Damasio E, Zucker-Franklin D. In: Zucker-Franklin D, Greaves MF, Grossi CE, Marmont AM (eds.), *Atlas of Blood Cells. Function and Pathology*, 2nd edn., Fischer, Stuttgart, pp 157–253, 1988.
7. Gleich GJ, Adolphson CR. In: Dixon FJ (ed.), *Advances in Immunology 39*, Academic Press, pp. 177–253, 1986.
8. Spry CJF (ed.) *Eosinophils: A Comprehensive Review and Guide to the Scientific and Medical Literature*, Oxford, Oxford University Press, 1988.
9. Zucker-Franklin D. In: Zucker-Franklin D, Greaves MF, Grossi CE, Marmont AM (eds.), *Atlas of Blood Cells. Function and Pathology*, 2nd edn., Fischer, Stuttgart, pp 255–284, 1988.
10. Agis H, Beil WJ, Bankl HC et al. *Leukemia Lymphoma* 22: 187–204, 1996.

11. Weber S, Krüger-Krasagakes S, Grabbe J et al. *Int J Dermatol* 34: 1–10, 1995.
12. Denburg JA. *Blood* 79: 846–860, 1992.
13. Valent P, Ashman LK, Hinterberger W et al. *Blood* 73: 1778–1785, 1989.
14. Zucker-Franklin D. In: Zucker-Franklin D, Greaves MF, Grossi CE, Marmont AM (eds.), *Atlas of Blood Cells. Function and Pathology,* 2nd edn., Fischer, Stuttgart, pp 285–320, 1988.
15. Butterfield JH, Weiler D, Dewald G et al. *Leukemia Res* 12: 345–355, 1988.
16. Bennett JM, Catovsky D, Daniel MT et al. *Br J Haematol* 33: 451–458, 1976.
17. Bennett JM, Catovsky D, Daniel MT et al. *Br J Haematol* 78: 325–329, 1991.
18. Grignani F, Fagioli M, Ferrucci PF et al. *Blood Rev* 7 *:* 87–93, 1993.
19. Grignani F, Fagioli M, Alcalay M et al. *Blood* 83: 10–25, 1994.
20. Warrell RP, de Thé H, Wang ZY et al. *N Engl J Med* 329: 177–189, 1993.
21. Kita K, Shirakawa S, Kamada N et al. *Leukemia Lymphoma* 13: 229–234, 1994.
22. Nucifora G, Rowley JD. *Leukemia Lymphoma* 14: 353–362, 1994.
23. Chen Z, Chen SJ. *Leukemia Lymphoma* 8: 253–260, 1992.
24. Bennett JM, Catovsky D, Daniel MT et al. Br *J Haematol* 51: 189–192, 1982.
25. Beris P. *Semin Hematol* 26: 216–233, 1989.
26. Koeffler HP. *Semin Hematol* 23: 223–236, 1986.
27. Bennett JM, Catovsky D, Daniel MT et al. *Br J Haematol* 87: 746–754, 1994.
28. Melo JV. *Blood* 88: 2375–2384, 1996.
29. Kantarjian HM, Deisseroth A, Kurzrock R et al. *Blood* 82: 691–703, 1993.
30. Koeffler HP, Golde DW. *Blood* 56: 344–350, 1980.
31. Koeffler HP. *Blood* 62: 709–721, 1983.
32. Lübbert M, Koeffler HP. *Blood Rev* 2: 121–133, 1988.
33. Lubbert M, Koeffler HP. *Cancer Rev* 10: 33–62, 1990.
34. Lübbert M, Herrmann F, Koeffler HP. *Blood* 77: 909–924, 1991.
35. Paul CC, Tolbert M, Mahrer S et al. *Blood* 81: 1193–1199, 1993.
36. Paul CC, Mahrer S, Tolbert M et al. *Blood* 86: 3737–3744, 1995.
37. Wada H, Mizutani S, Nishimura J et al. Cancer Res 55: 3192–3196, 1995.
38. Kubonishi I, Takeuchi S, Uemura Y et al. *Jpn J Cancer Res* 86: 451–459, 1995.
39. Kakuda H, Sato T, Hayashi Y et al. *Br J Haematol* 95: 306–318, 1996.
40. Weidmann E, Brieger J, Karakas T et al. *Leukemia* 11: 709–713, 1997.
41. Keating A, Martin PJ, Bernstein ID et al. In: Golde DW, Marks PA (eds.), *Normal and Neoplastic Hematopoiesis*, Liss, New York, pp 513–520, 1983.
42. Saito H, Bourinbaiar A, Ginsburg M et al. *Blood* 66: 1233–1240, 1985.
43. Hamaguchi H, Nagata K, Yamamoto K et al. *Br J Haematol* 102: 1249–1256, 1998.
44. Ben-Bassat H, Korkesh A, Voss R et al. *Int J Cancer* 6: 743–752, 1982.
45. Rambaldi A, Bettoni S, Tosi S et al. *Blood* 81: 1376–1383, 1993.
46. Oez S, Tittelbach H, Fahsold R et al. *Blood* 76: 578–582, 1990.
47. Collins SJ, Gallo RC, Gallagher RE. *Nature* 270: 347–349, 1977.
48. Gallagher R, Collins S, Trujillo J et al. *Blood* 54: 713–733, 1979.
49. Dalton WT Jr, Ahearn MJ, McCredie KB et al. *Blood* 71: 242–247, 1988.
50. Hamaguchi H, Suzukawa K, Nagata K et al. *Br J Haematol* 98: 399–407, 1997.
51. Kishi K, Toba K, Azegami T et al. *Exp Hematol* 26: 135–142, 1998.
52. Hiyoshi M, Yamane T, Hirai M et al. *Br J Haematol* 90: 417–424, 1995.
53. Abo J, Inokuchi K, Dan K et al. *Blood* 82: 2829–2836, 1993.
54. Asou H, Tashiro S, Hamamoto K et al. *Blood* 77: 2031–2036, 1991.
55. Asou H, Suzukawa K, Kita K et al. *Jpn J Cancer Res* 87: 269–274, 1996.
56. Asou H, Eguchi M, Suzukawa K et al. *Br J Haematol* 93: 68–74, 1996.

57. Andersson B, Beran M, Pathak S et al. *Cancer Genet Cytogenet* 24: 335–343, 1987.
58. Andersson B, Collins VP, Kurzrock R et al. *Leukemia* 9: 2100–2108, 1995.
59. Kubonishi I, Machida KI, Sonobe H et al. *Gann* 74: 319–322, 1983.
60. Kubonishi I, Miyoshi I. *Int J Cell Cloning* 1: 105–117, 1983.
61. Fukuda T, Kamishima T, Kakihara T et al. *Leukemia Res* 20: 931–939, 1996.
62. Koeffler HP, Golde DW. *Science* 200: 1153–1154, 1978.
63. Koeffler HP, Billing R, Lusis AJ et al. *Blood* 56: 265–273, 1980.
64. Mori T, Nakazawa S, Nishino K et al. *Leukemia Res* 11: 241–249, 1987.
65. Yanagisawa K, Yamauchi H, Kaneko M et al. *Blood* 91: 641–648, 1998.
66. Kishi K. *Leukemia Res* 9: 381–390, 1985.
67. Fukuda T, Kishi K, Ohnishi Y et al. *Blood* 70: 612–619, 1987.
68. Saito H, Kishi K, Narita M et al. *Leukemia Res* 16: 217–226, 1992.
69. Ohkubo T, Kamamoto T, Kita K et al. *Leukemia Res* 9: 921–926, 1985.
70. Oez S, Trautmann U, Smetak M et al. *Ann Hematol* 72: 307–316, 1996.
71. Treves AJ, Halperin M, Barak V et al. *Exp Hematol* 13: 281–288, 1985.
72. Yoshida H, Kondo M, Ichihashi T et al. *Cancer Genet Cytogenet* 100: 21–24, 1998.
73. Tohyama K, Tsutani H, Ueda T et al. *Br J Haematol* 87: 235–242, 1994.
74. Tsuji-Takayama K, Kamiya T, Nakamura S et al. *Human Cell* 7: 167–172, 1994.
75. Hu ZB, Ma W, Zaborski M et al. *Leukemia* 10: 1025–1040, 1996.
76. Lanotte M, Martin-Thouvenin V, Najman S et al. *Blood* 77: 1080–1086, 1991.
77. Dermime S, Grignani F, Clerici M et al. *Blood* 82: 1573–1577, 1993.
78. Duprez E, Ruchaud S, Houge G et al. *Leukemia* 6: 1281–1287, 1992.
79. Kataoka T, Morishita Y, Ogura M et al. *Cancer Res* 50: 7703–7709, 1990.
80. Wang C, Curtis JE, Minden MD et al. *Leukemia* 3: 264–269, 1989.
81. Nara N, Suzuki T, Nagata K et al. *Jpn J Cancer Res* 81: 625–631, 1990.
82. Koistinen P, Wang C, Yang GS et al. *Leukemia* 5: 704–711, 1991.
83. Wang C, Koistinen P, Yang GS et al. *Leukemia* 5: 493–499, 1991.
84. Nagai M, Fujita M, Ikeda T et al. *Br J Haematol* 98: 392–398, 1997.
85. Hamaguchi H, Inokuchi K, Nara N et al. *Int J Hematol* 67: 153–164, 1998.
86. Kubonishi I, Machida KI, Niiya K et al. *Blood* 63: 254–259, 1984.
87. Matozaki S, Nakagawa T, Kawaguchi R et al. *Br J Haematol* 89: 805–811, 1995.
88. Klingemann HG, Gong HJ, Maki G et al. *Leukemia Lymphoma* 12: 463–470, 1994.
89. Taoka T, Tasaka T, Tanaka T et al. *Blood* 80: 46–52, 1992.
90. Oval J, Jones OW, Montoya M et al. *Blood* 76: 1369–1374, 1990.
91. Kizaki M, Matsushita H, Takayama N et al. *Blood* 88: 1824–1833, 1996.
92. Yamaguchi Y, Nishio H, Kasahara T et al. *Leukemia* 12: 1430–1439, 1998.
93. Yamamoto K, Hamaguchi H, Nagata K et al. *Leukemia* 11: 599–608, 1997.
94. Yasukawa M, Yanagisawa K, Kohno H et al. *Br J Haematol* 82: 515–521, 1992.
95. Drexler HG, Dirks W, MacLeod RAF et al. (eds.) *DSMZ Catalogue of Human and Animal Cell Cultures*, 7th edn., DSMZ, Braunschweig, 1999.
96. Keating A. *Clin Haematol* 2: 1021–1029, 1987.
97. Mayumi M. *Leukemia Lymphoma* 7: 243–250, 1992.
98. Nilsson G, Blom T, Kusche-Gullberg M et al. *Scand J Immunol* 39: 489–498, 1994.
99. Toba K, Kishi K, Koike T et al. *Exp Hematol* 24: 894–901, 1996.
100. Drexler HG, Quentmeier H, MacLeod RAF et al. *Leukemia Res* 19: 681–691, 1995.
101. Kojika S, Sugita K, Inukai T et al. *Leukemia* 10: 994–999, 1996.
102. Taetle R, Payne C, Dos Santos B et al. *Cancer Res* 53: 3386–3393, 1993.
103. Drexler HG, Zaborski M, Quentmeier H. *Leukemia* 11: 701–708, 1997.
104. Schumann RR, Nakarai T, Grass HJ et al. *Blood* 87: 2419–2427, 1996.

105. Tohyama K, Tohyama Y, Nakayama T et al. *Br J Haematol* 91: 795–799, 1995.
106. Feinstein E, Cimino G, Gale RP et al. *Proc Natl Acad Sci USA* 88: 6293–6297, 1991.
107. Drexler HG. *Leukemia* 12: 845–859, 1998.
108. Drexler HG, MacLeod RAF, Borkhardt A et al. *Leukemia* 9: 480–500, 1995.
109. Bi S, Lanza F, Goldman JM. *Leukemia* 7: 1840–1845, 1993.
110. de Thé H, Chomienne C, Lanotte M et al. *Nature* 347: 558–561, 1990.
111. Oval J, Smedsrud M, Taetle R. *Leukemia* 6: 446–451, 1992.
112. Paul CC Ackerman SJ, Mahrer S et al. *J Leuk Biol* 56: 74–79, 1994.
113. Uckun FM. *Blood* 88: 1135–1146, 1996.
114. Collins SJ. *Blood* 70: 1233–1244, 1987.
115. Abrink M, Gobl AE, Huang R et al. *Leukemia* 8: 1579–1584, 1994.
116. Shimamoto T, Ohyashiki K, Ohyashiki JH et al. *Blood* 86: 3173–3180, 1995.
117. Machado EA, Gerard DA, Lozzio CB et al. *Blood* 63: 1015–1022, 1984.
118. Tsuda H, Sakaguchi M, Kawakita M et al. *Int J Cell Cloning* 6: 209–220, 1988.
119. Blom T, Huang R, Aveskogh M et al. *Eur J Immunol* 22: 2025–2032, 1992.
120. Kawaguchi R, Yoshioka JI, Hikiji K et al. *Cancer Genet Cytogenet* 67: 157, 1993.
121. Kizaki M, Ueno H, Matsushita H et al. *Leukemia Lymphoma* 25: 427–434, 1997.
122. Felix CA, Megonigal MD, Chervinsky DS et al. *Blood* 91: 4451–4456, 1998.
123. Barak Y, Shore NA, Higgins GR et al. *Br J Haematol* 27: 543–549, 1974.
124. Giavazzi R, Di Berardino C, Garofalo A et al. *Int J Cancer* 61: 280–285, 1995.
125. Okabe M, Kunieda Y, Shoji M et al. *Leukemia Res* 19: 933–943, 1995.
126. Okabe M, Kunieda Y, Nakane S et al. *Leukemia Lymphoma* 16: 493–503, 1995.
127. Kim SH, Ding W, Cannizzaro L et al. *Blood* 92: 609a, 1998.
128. Srikanth S, Sokol L, Canfield M et al. *Blood* 82: 23a, 1993.
129. Zhou M, Gu L, James CD et al. *Leukemia* 9: 1159–1161, 1995.
130. Zhou M, Yeager AM, Smith SD et al. *Blood* 85: 1608–1614, 1995.
131. Takahashi M, Shigeno N, Takahashi H et al. *Leukemia Res* 21: 1115–1123, 1997.
132. Takiguchi T, Sakuma M, Kim C et al. *Br J Haematol* 102: 24, 1998.
133. Wada H, Asada M, Nakazawa S et al. *Leukemia* 8: 53–59, 1994.
134. Treves AJ, Barak V, Halperin M et al. *Immunol Lett* 12: 225–230, 1986.
135. Miyazawa K, Kawanishi Y, Yaguchi M et al. *Blood* 92: 253b, 1998.
136. Komada Y, Zhou YW, Zhang XL et al. *Blood* 86: 3848–3860, 1995.
137. Paul CC, Aly E, Baumann MA. *Blood* 92: 147b, 1998.
138. Mizobuchi N, Takahashi I, Horimi T et al. *Acta Medica Okayama* 51: 227–232, 1997.
139. Tohda S, Yang GS, Ashman LK et al. *J Cell Physiol* 154: 410–418, 1993.
140. Zheng A, Castren K, Saily M et al. *Br J Cancer* 79: 407–415, 1999.
141. Lucas DL, Knight RD, Khan EC et al. *Blood* 62: 152a, 1983.
142. Mallet M, Mane SM, Meltzer SJ et al. *Leukemia* 3: 511–515, 1989.
143. McCarty TM, Rajamaran S, Elder FFB et al. *Blood* 70: 1665–1672, 1987.
144. Drexler HG, Minowada J. *Leukemia Res* 10: 279–290, 1986.
145. Drexler HG, Thiel E, Ludwig WG. *Leukemia* 7: 489–498, 1993.
146. Callard R, Gearing A (eds.) *The Cytokine FactsBook*, Academic Press, London, 1994.
147. Nicola NA (ed.) *Guidebook to Cytokines and their Receptors*, Sambrook, Oxford, 1994.
148. Oval J, Taetle R. *Blood Rev* 4: 270–279, 1990.
149. Drexler HG. *Leukemia Res* 18: 919–927, 1994.
150. Drexler HG, MacLeod RAF, Uphoff CC. *Leukemia Res* 23: 207–215, 1999.
151. Nucifora G, Rowley JD. *Blood* 86: 1–14, 1995.
152. Rubnitz JE, Behm FG, Downing JR. *Leukemia* 10: 74–82, 1996.

153. Ohyashiki K, Ohyashiki JH, Shimamoto T et al. *Leukemia Lymphoma* 23: 431–436, 1996.
154. Golub TR, McLean T, Stegmaier K et al. *Biochim Biophys Acta* 1288: M7–M10, 1996.
155. Rabbitts TH. *Nature* 372: 143–149, 1994.
156. Drexler HG, Borkhardt A, Janssen JWG. *Leukemia Lymphoma* 19: 359–380, 1995.
157. Fischkoff SA, Pollak A, Gleich GJ et al. *J Exp Med* 160: 179–196, 1984.
158. Hutt-Taylor SR, Harnish D, Richardson M et al. *Blood* 71: 209–215, 1988.
159. Tomonaga M, Gasson JC, Quan SG et al. *Blood* 67: 1433–1441, 1986.

Chapter 7

Monocytic Cell Lines

Hans G. Drexler
DSMZ-German Collection of Microorganisms and Cell Cultures, Department of Human and Animal Cell Cultures, Braunschweig, Germany. Tel: +49-531-2616-160; Fax: +49-531-2616-150; E-mail: hdr@dsmz.de

1. INTRODUCTION

The cells comprising the "mononuclear phagocyte system" include promonocytes and their precursors in the bone marrow, monocytes in the circulation and macrophages in tissues. The notion that these cells are parts of a system is derived from their common origin, functions and similar morphology. An older, but now revised concept was the so-called "reticuloendothelial system". The ideas and definitions of the monocytic series (including its precursors and progeny) have undergone drastic changes in recent decades [1]. Matters are further complicated by different denominations for the same type of cell, an example being "histiocyte" (used mainly by pathologists) and "macrophage" (applied predominantly by immunologists and hematologists). The classical theory according to which monocytes are derived from histiocytes (called reticulum cells by early investigators – hence the term "reticuloendothelial system") was found to be incorrect and was replaced by the reverse hypothesis.

The mononuclear phagocyte system forms a network which is found in many organs. It includes circulating cells, monocytes in the peripheral blood, and cells resident in tissues or fixed to the endothelial layer of blood capillaries, such as Kupffer cells in the liver, intraglomerular mesangial cells in the kidney, alveolar and serosal macrophages in the lung and pleura/peritoneum, microglia cells in the brain, and the sinus macrophages in the spleen and lymph node [2].

The monocytic cell lineage originates from the stem cell in the bone marrow as a common committed progenitor cell for the granulocyte (myelocytic) and monocyte-macrophage pathways; the so-called colony-forming unit granulocyte-macrophage (CFU-GM). This cell typically expresses cytokine receptors for GM-CSF and M-CSF, which stimulate further differentiation to

J.R.W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol. III, 237–257.

the promonocyte, the earliest morphologically identifiable cell of the series [3]. The promonocyte is capable of endocytosis and adherence to glass, plastic or other substrates. The subsequent stage, the monocyte, is phagocytic and microbiocidal. Some monocytes migrate into the organs and tissue systems to become macrophages. Once in the tissues, monocytes do not re-enter the circulation. Rather, they undergo transformation into tissue-specific macrophages with morphological and functional properties that are characteristic of the tissue in which they reside.

The mononuclear phagocyte system has two functions, performed by two different types of cells. First, the removal of particulate antigens by the "professional" phagocytic monocyte-macrophage; and second, the presentation of antigens to lymphocytes by the "antigen-presenting cell" [2].

There are various forms of acute and chronic leukemia in which a cell committed to monocytic differentiation appears to be the target of leukemogenesis. Morphologists discern the myelomonocytic subgroup AML FAB M4 (with its variant M4eo in which an increased percentage of eosinophils is seen) and the monocytic subgroup AML FAB M5 (with further distinction of the immature subtype M5a and the mature subtype M5b) [4]. Despite its high percentage of monocytic blasts, chronic myelomonocytic leukemia (CMML) has been assigned to the myelodysplastic syndromes (MDS) [5]. Finally, CML can occasionally lead to monoblastic blast crisis (instead of the more common myelocytic blast crisis).

More than 30 leukemia cell lines have been established since 1974 (Tables 1 and 6). Thirty-one monocytic cell lines have been described adequately (Table 1), while the remaining cell lines require further characterization, have been lost or their current status is not known (Table 6). The oldest monocytic cell line is U-937 [38]. Despite the original diagnosis of "generalized diffuse histiocytic lymphoma" (this nomenclature is no longer used), the U-937 cells do have clear-cut monocyte-associated features. U-937 and the next cell line to be established, THP-1 [35] are the prototype monocytic leukemia cell lines. Both lines are widely used and are available through several cell line banks (Appendix 1, p. 284). The lines listed in Table 1 are useful models for cells representing the different steps of monocytic maturation [84,85].

2. CLINICAL CHARACTERIZATION

Thirty-one cell lines with monocytic characteristics are listed in Table 1. These cell lines were derived mainly from patients with either AML M4 (n = 10, 32%) or AML M5 (n = 16, 51%); two cell lines were established from patients with CML in blast crisis. The age of the patients ranged from 1 to 76 years (among them 8 children). Specimens were obtained predominantly

Table 1. Monocytic cell lines: clinical characterization

Cell line[a]	Patient age/sex[b]	Diagnosis[c]	Treatment status[d]	Specimen site[e]	Authentication[f]	Year est.	Culture medium[g]	Availability[h]	Primary ref.
AML-1	12 M	AML M4	R		yes		IMDM + FBS		6
AML-193	13 F	AML M5	R		yes		IMDM + GM-CSF or IL-3	ATCC	6
CTV-1	40 F	AML M5	R	PB	no	1982	RPMI 1640 + FBS	DSMZ	7
DOP-M1	1 F	AML M5a	T	cerebro-spinal fluid	yes	1989	RPMI 1640 + FBS		8
FLG29.1	38 F	AML M5a	D	BM	no	1987	RPMI 1640 + FBS		9
IMS-M1	33 M	AML M5a		BM	no	1988	IMDM + FBS		10,11
KBM-3	32 F	AML M4	resistant	PB	yes		IMDM + FBS	Author	12
KBM-5	67 F	CML-mono BC	BC	PB	yes		IMDM + FBS	Author	13
KP-1	2 F	AML M5	D	PB	no	1986	RPMI 1640 + FBS		14
KP-MO-TS	12 M	AML M5b	D	PB	yes	1987	RPMI 1640 + FBS	Author	15
ME-1[i]	40 M	AML M4eo	2nd R	PB	yes	1988	RPMI 1640 + FBS	Author	16,17
ML-2[j]	26 M	T-NHL → T-ALL → AML M4	D	PB	yes	1978	RPMI 1640 + FBS	DSMZ	18
MOLM-13[k]	20 M	MDS (RAEB) → AML M5a	R	PB	yes	1995	RPMI 1640 + FBS	Author	19
Mono Mac 6[l]	64 M	myeloid metaplasia → AML M5	R	PB	no	1985	RPMI 1640 + FBS	DSMZ	20
MUTZ-3	29 M	AML M4	D	PB	yes	1993	α-MEM + FBS + 5637 CM or GM-CSF, IL-3, PIXY-321	DSMZ	21
MV4-11	10 M	AML M5	D		yes		IMDM or RPMI 1640 + FBS or IMDM + GM-CSF	ATCC, DSMZ	6

Continued on next page

Table 1. (continued)

Cell line[a]	Patient age/sex[b]	Diagnosis[c]	Treatment status[d]	Specimen site[e]	Authentication[f]	Year est.	Culture medium[g]	Availability[h]	Primary ref.
NOMO-1	31 F	AML M5a	2nd R	BM	no	1985	RPMI 1640 + FBS or serum-free	Author	22
OCI/AML-2	65 M	AML M4	D	PB	no	1986	α-MEM + FBS	DSMZ	23,24
OMA-AML-1	? M	AML M4	5th R	PB	no	1989	RPMI 1640 + FBS + HS	Author	25,26
P31/Fujioka	7 M	AML M5	R	PB	no	1980	RPMI 1640 + FBS	JCRB	27
P39/Tsugane	69 M	MDS (CMML) → AML M2	D	PB	no	1983	RPMI 1640 + FBS	JCRB	28
PLB-985	38 F	AML M4	R	PB	yes	1985	RPMI 1640 + FBS or serum-free	DSMZ	29
RC-2A[m]	adult M	AML M4	refractory	PB	no		RPMI 1640 + FBS		30
RWLeu-4		CML-BC	BC	PB	no		RPMI 1640 + FBS	Author	31,32
SCC-3	20 F	NHL (diffuse large cell – stage IV-A)	R	PE	no	1985	RPMI 1640 + FBS	JCRB	33
SKM-1	76 M	MDS (RAEBT) → AML M5	refractory	PB	yes	1989	RPMI 1640 + FBS	JCRB	34
THP-1	1 M	AML M5	R	PB	no	1978	RPMI 1640 + FBS	ATCC, DSMZ, JCRB, RIKEN	35
TK-1[n]	22 M	T-lymphoblastic lymphoma → biclonal leukemia (T-ALL L2 + AML M4)	D	PB	no	1984	RPMI 1640 + FBS	Author	36,37
U-937	37 M	generalized, diffuse histiocytic lymphoma	refractory	PE	no	1974	RPMI 1640 + FBS (originally Ham's F10 + NCS)	ATCC, DSMZ, IFO, JCRB, RIKEN	38

Continued on next page

Table 1. (continued)

Cell line[a]	Patient age/sex[b]	Diagnosis[c]	Treatment status[d]	Specimen site[e]	Authentication[f]	Year est.	Culture medium[g]	Availability[h]	Primary ref.
UG3	26 F	AML M5	T	PB	no	1997	IMDM + FBS + GM-CSF or IL-3	Author	39
YK-M2	31 M	AML M5	T	PB	yes	1985	RPMI 1640 + FBS	Author	40

[a] Names of cell lines are as given in the original literature; subclones (variant cell lines derived from a parental cell line) and sister cell lines (derived independently from the same patient from different specimens or at different time points) are indicated for each cell line.
[b] Age at the time of establishment of cell line.
[c] Diagnoses are indicated as given in the original literature; →: disease progressed from a pre-malignancy/first malignancy into the final malignancy.
[d] BC – at blast crisis; CP – in the chronic phase; D – at diagnosis (prior to therapy); R – at relapse; T – during therapy.
[e] BM – bone marrow; PB – peripheral blood; PE – pleural effusion; Tu – tumor.
[f] Evidence (e.g. cytogenetic marker chromosomes, immunoprofile, others) that this cell line was derived from the patient indicated.
[g] Culture media as indicated in the original literature; cell line might also grow with other medium and/or supplements.
[h] Availability from cell banks (ATCC; DSMZ; IFO; JCRB; RIKEN) or from the original investigator (author).
[i] Five subclones (ME-2, ME-3, ME-F_1, ME-F_2, ME-F_3) with some different morphological, cytochemical and cytogenetic features were established.
[j] Sister cell lines ML-1 and ML-3 with different cytogenetic aberrations were established from the same specimen of the same patient.
[k] Sister cell line MOLM-14 with some different immunophenotypic and minor cytogenetic features established from the same specimen.
[l] Sister cell line Mono Mac 1 was established from the same specimen and the same patient.
[m] Subclone of original cell line RC-2 which was growth factor-dependent; sister cell line CESS-B is an EBV+ LCL and was established from the same specimen.
[n] Subclones TK-1B (pseudodiploid with complex chromosomal aberrations) and TK-1D (diploid normal karyotype) were established by cloning from the parental line TK-1.

Table 2. Monocytic cell lines: immunophenotypic characterization

Cell line	T-/NK-cell marker*	B-cell marker	Myelomonocytic marker	Erythroid-megakaryocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
AML-1	CD2– CD3– CD5– CD7+	CD10–	CD13+ CD14+ CD15+ CD33+		HLA-DR+		6
AML-193	CD2– CD3– CD4– CD5– CD7– CD8–	CD10– CD19–	CD13+ CD14– CD15+ CD16– CD33+	CD41a– GlyA–	CD34– CD38– CD71+ HLA-DR+		6,41, 42
CTV-1	CD2– CD3– CD4– CD5– CD6+ CD7+ CD8– CD28– CD57+ TCR$\alpha\beta$– TCRδ–	CD10– CD19– CD20– CD21– cy/sIg–	CD13– CD14(+) CD15+ CD33– CD68–	CD41a– CD61–	CD34– HLA-DR– TdT–	CD11b–	7
DOP-M1	CD2– CD3– CD4– CD7– CD8–	CD10– CD19–	CD13– CD14– CD15+ CD33+ CD65+	CD41–	HLA-DR–	CD11b–	8
FLG29.1	CD1a– CD2– CD3– CD4– CD5– CD7– CD8–	CD19– CD20– CD21– CD22– CD23– CD24–	CD13+ CD14(+) CD15(+) CD32+ CD33(+) CD35(+) CD68+	CD9+ CD31– CD36– CD41a– CD42b(+) CD61+	CD34+ CD71+ HLA-DR–	CD11a– CD11b– CD18– CD44+ CD51(+) CD54+ CD55+	9
IMS-M1	CD4+		CD14+ CD33+		CD34+ CD38+ HLA-DR+		11
KBM-3	CD2+ CD3– CD4+ CD5– CD8–	CD10– CD19–	CD14+ CD33+		CD34– HLA-DR+ TdT–		12
KBM-5	CD2– CD4+	sIg–	CD13+ CD14– CD33+		CD34– HLA-DR– TdT–	CD11b–	13
KP-1	CD2– CD3– CD4+ CD8–	CD19– CD20–	CD13+ CD14+ CD33+		HLA-DR+	CD11a+ CD11c+ CD18+	14
KP-MO-TS	CD1a– CD2– CD3– CD4+ CD5– CD8–	CD10– CD19– CD20– sIg–	CD13– CD14– CD15+ CD33+ CD35–		HLA-DR+ TdT+	CD11b+	15
ME-1	CD2– CD7–	CD10– CD20–	CD13+ CD14+ CD33+		CD34+ HLA-DR+	CD11b(+)	16
ML-2	CD1– CD2– CD3– CD4+ CD5– CD7+ CD8– CD28– CD57– TCR$\alpha\beta$– TCRδ–	CD10(+) CD19– CD20– CD21–	CD13+ CD14(+) CD15+ CD33+	CD9– CD41a– CD61(+)	CD34– CD38+ CD71+ HLA-DR+ TdT–	CD11b+	24
MOLM-13	CD1– CD2– CD3– CD4(+) CD5– CD7– CD8– CD57–	CD10– CD19– CD20– CD21– CD22– CD23– CD40–	CD13– CD14– CD15+ CD32+ CD33+ CD64– CD65– CD68– CD87(+) CD91– CD92(+) CD93+ CD155–	CD9– CD41a– CD42b– CD61– CD62P–	CD34– HLA-DR– TdT–	CD11a+	19
Mono Mac 6	CD2– CD3– CD4(+)	CD19– CD20–	CD13+ CD14(+) CD15+ CD33+ CD68+	GlyA–	CD34– HLA-DR+ TdT–	CD11b+	20,24

Continued on next page

Table 2. (continued)

MUTZ-3	CD1– CD3– CD4+ CD5– CD7– CD8– CD56–	CD10– CD19– CD20– CD23–	CD13+ CD14+ CD15+ CD16– CD32+ CD33+ CD64(+) CD65+ CD68+	CD41a– CD42b– CD61– GlyA–	CD30+ CD34(+) CD38+ CD71+ HLA-DR+ TdT–	CD11b+	21
MV4-11	CD2– CD3– CD4+ CD5– CD7– CD8–	CD10– CD19– CD21– CD37– CD138+ cy/sIg–	CD13+ CD14(+) CD15+ CD16– CD33+		CD34– CD38– HLA-DR+		6,24, 41
NOMO-1	CD2–				HLA-DR+		22
OCI/AML-2	CD3–	CD19–	CD13+ CD14– CD15+ CD33+				24
OMA-AML-1			CD13+ CD14(+) CD15+ CD33+		CD34+ HLA-DR–	CD11c+	25
P31/Fujioka	CD1– CD2– CD3– CD4– CD5– CD8–	CD10– CD20– CD80– CD86+ cy/sIg–	CD14+ CD15+		HLA- DR+ TdT–	CD11b+ CD54+	27
P39/Tsugane	CD1– CD2– CD3– CD4+ CD5– CD8–	CD10– CD20– CD80– CD86– cy/sIg–	CD13+ CD14+ CD15+		HLA-DR– TdT–	CD11b– CD54+	28
PLB-985	CD3–	CD19–	CD13+ CD14– CD15+ CD33+		HLA-DR–	CD11b+	24,29
RC-2A			CD14–		HLA-DR+	CD11b+	43
RWLeu-4	CD8–	CD21+ sIg–	CD14– CD15+		HLA-DR(+)	CD11b+	31
SCC-3	CD1– CD2– CD3– CD4– CD5– CD8– CD57–	CD10+ CD19– CD20– CD21– CD24– PCA-1+	CD13+ CD14+ CD15– CD16– CD33–	CD36–	CD38+ CD71+ HLA-DR–	CD11b–	33
SKM-1	CD2– CD3– CD4+ CD5– CD7– CD8–		CD13+ CD14– CD33+		HLA-DR+	CD11b+	34
THP-1	CD1– CD2– CD3– CD4+ CD7+ CD28– CD57– TCR$\alpha\beta$–	CD19– CD20– CD23(+) CD24+ CD80– CD86+ cy/sIg–	CD13+ CD14(+) CD15+ CD33+ CD68–	CD9– CD41a+ CD61+	CD34– CD38+ CD71+ HLA-DR+	CD11b+ CD54+	24,35
TK-1	CD1– CD2– CD3– CD4– CD5– CD7– CD8– TCR$\alpha\beta$–	CD10– CD20– cy/sIg–	CD13– CD14– CD15+		HLA-DR+ TdT–	CD11b–	36
U-937	CD1– CD2– CD3– cyCD3– CD4+ CD7+ CD8– CD28– CD57– TCR$\alpha\beta$– TCRδ–	CD10– CD19– CD20– CD21– CD23+ CD24(+) CD75– CD80– CD86– cy/sIg–	CD13+ CD14(+) CD15+ CD17– CD33+ CD35+ CD68+ CD88(+)	CD9– CD41a– CD42b– vWF–	CD34– CD38(+) CD71+ HLA-DR– TdT–	CD11b+ CD44+ CD54+	24
UG3			CD13+ CD14(+) CD16– CD33+ CD64+ CD68+	CD36+	CD71+ HLA-DR+	CD11b+ CD11c– CD54+	39
YK-M2	CD1a– CD2– CD3– CD4– CD5– CD8– TCR$\alpha\beta$–	CD10– CD20– CD21– sIg–	CD13+ CD14+ CD15+		HLA- DR+ TdT–	CD11b–	40

* +, strong, definite protein expression (mostly more than 10–20% cells positive); (+), weak protein expression, qualitatively and quantitatively (commonly <10% cells positive); –, no protein expression.

Table 3. Monocytic cell lines: cytokine-related characterization

Cell line	Cytokine receptor expression[a]	Cytokine production[a]	Proliferation response to cytokines[b]	Differentiation response to cytokines[b]	Dependency on cytokines[c]	Ref.
AML-193	IL-2Rα–		G-CSF+, GM-CSF+, IGF-I+, IL-3+, PIXY-321+ inhibitory: TGF-β1+		GM-CSF or IL-3	6,41,44
FLG29.1	G-CSFRα–, GM-CSFRα–, gp80+, IFN-γR+, IL-2Rα–, IL-3Rα+, IL-4Rα–, IL-6Rα–, Kit+, TNF-RI/II+					9
IMS-M1	Kit–		G-CSF+, GM-CSF+, M-CSF+			11
KBM-3	Northern: M-CSFR+	Northern: GM-CSF+				12
KBM-5		RT-PCR: TNF-α+	inhibitory: TNF-α+			13
ME-1			GM-CSF+, IL-3+	GM-CSF+, IL-3+, IL-4+		16
MOLM-13	GM-CSFRα+, IFN-γR+, M-CSFR–, TNFRI/II–			IFN-γ+, IFN-γ+, TNF-α+		19
Mono Mac 6	mRNA: IL-2Rα+, IL-2Rβ+, IL-2Rγ(+), IL-4Rα(+), IL-7Rα(+), IL-9Rα+ protein: IL-2Rγ–	IL-1+		IFN-γ+		45
MUTZ-3[d]	GM-CSFRα+, IL-2Rα–, IL-3Rα+, Kit+, M-CSFR+		G-CSF+, GM-CSF+, IFN-β+, IFN-γ+, IGF-I+, IL-3+, IL-4+, IL-6+, IL-7+, insulin+, M-CSF+, MIP-1α+, PIXY-321+, SCF+		GM-CSF, IL-3, PIXY-321	21,44
MV4-11	RT-PCR: IGF-1R+, IGF-2R+, IL-2Rα–	RT-PCR: IGF-1+	G-CSF+, GM-CSF+, IL-3+			6,41
OCI/AML-2			G-CSF+, GM-CSF+, M-CSF+			23
OMA-AML-1			G-CSF+, GM-CSF+, IL-3+, IL-6+, LIF+	GM-CSF+, IL-3+, LIF+		25,26
SKM-1			GM-CSF+			34
U-937	RT-PCR: IL-2Rα+, IL-4Rα+, IL-7Rα+, IL-9Rα+ protein: GM-CSFRα+, IL-1Rα (+), IL-2Rα–, IL-2Rβ+, IL-2Rγ+, IL-3Rα(+), IL-3Rβ+, IL-6Rα+, IL-7Rα–, Kit–	PDGF–		IFN-γ+		45
UG3	G-CSFRα+					39

[a] Receptor expression or cytokine production at the protein level (ELISA, McAb, RIA), unless otherwise indicated, e.g. at the mRNA level (by RT-PCR, Northern); +, strong, definite expression; (+), weak expression; —, no expression. [b] Effects of cytokine exposure on proliferation or differentiation (–, no effect; +, positive effect). [c] Upon growth factor withdrawal these cell lines will die by apoptosis. [d] Detailed report on proliferative response to cytokines in ref. [21].

Table 4. Monocytic cell lines: genetic characterization

Cell line	Cytogenetic karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements[a]	Ref.
AML-1	51, XY, +1p–, +6, +8, +8, +19, 2p–, 12p+		*IGH* G, *IGK* G, *IGL* G, *TCRB* G	6
AML-193	49, X, +3, +6, +8, –17, +der(17)t(17;17)(p13.1;q21.3)		*IGH* G, *IGK* G, *IGL* G, *TCRB* G	6
CTV-1	92(89–94)<4n> XX/XXY/XXYY, –1, +6, +6, –17, t(1;7)(p34.2;q34)x2, del(3)(p21), i(6q)x2, t(12;16)(q24.32;q11)x2		*P53* PM	7, 24
DOP-M1	47, X, –X, –13, +19, +20, +mar			8
FLG29.1	45–69, 3p+		*IGH* G, *TCRB* G, *TCRG* G	9
IMS-M1	46, XY, –7, +8, –10, +12, 1p–, t(9;11)(p22;q23)	t(9;11)(p22;q23) → *MLL-AF9* fusion gene	*P53* PM	10, 11
KBM-3	48(41–51)<2n>, –4, –12, +13, del(12)ins(12q+?), del(5)(p12 → ter), del(9)(p11(ter), del(16)t(16q+?), del(7)ins(7p+HSR)		*P53* PM	12
KBM-5	69–87, XX, +6, +7, +8, +8, +8, –9, –9, 9q+, –11, –11, –13, 14q–, +15, 17p+, +20, +22q–, +22q, – multiple Ph, +frag, +DMs	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene	*BCR*, *ABL* amplified; *P53* PM	13, 46
KP-1	47(46–48)XX, 1p–, 10p+, 16q+, 19p+, +mar, t(11q+;19p–)			14
KP-MO-TS	46, XY, –17, t(10;11)(p13;q21), +mar		*IGH* G, *IGK* R, *TCRB* G, *TCRG* G	15
ME-1	47, XY, +8, inv(16)(p13q22), del(17)(p12p13)	inv(16)(p13q22) → *CBFB-MYH11* fusion gene		16, 47
ML-2	92(84–94)<4n>XX, –Y, –Y, –7, –9, –10, –10, +11, +12, +12, +13, +13, –15, –16, –17, –17, +18, +18, –20, –20, +4mar, der(1)t(1;?)(p21;?)x2, del(6)(q23)x2, der(6)t(6;11)(q27;?q23)x2, ?der(11)t(6;11)(q27;?q23)/del(11)(q23)x2, der(11)t(11;?)(? → 11p11 → 11q23:)x2, der(11)t(11;?)(q11-13;?), dup(13)(q32 → qter)x2, der(18)t(15;?;18)(q21;?;q11)x2	t(6;11)(q27;q23) → *MLL-AF6* fusion gene	*P16INK4A* D; *P53* PM; *RB1* R[b]; *IGH* GR, *TCRA* GG, *TCRB* GR, *TCRG* GR[c]	24, 48–51
MOLM-13	49<2n>XY, +6, +8, +13, ins(11;9)(q23;p22p23), del(14)(q23.3q31.3)	ins(11;9)(q23;p22p23) → *MLL-AF9* fusion gene	*P15INK4B* D, *P16INK4A* D	19
Mono Mac 6[d]	84–90<4n>XX/XXX, –Y, +6, +7, –12, –13, –13, –16, – 16, +2mar, t(9;11)(p22;q23)x2, add(10)(p11)x2, add(12)(q21), del(13)(q13q14) der(13)t(13;14)(p11;q12)x2, der(17)t(13;17)(q21;p11)x2	t(9;11)(p21;q23) → *MLL-AF9* fusion gene		24, 52
MUTZ-3	46(44–48)<2n>XY, t(1;3)(q43;q13)inv(3)(q21q26), t(2;7)(q36;q36)inv(7)(p15q36), t(12;22)(p13;q12)	t(12;22)(p13;q12) → *ETV6/TEL-MN1* fusion gene?		21, 24, 53
MV4-11	48(46–48)<2n>XY, +8, +18, +19, –21, t(4;11)(q21;q23)	t(4;11)(q21;q23) → *MLL-AF4* fusion gene	*IGH* G, *IGK* RG, *IGL* G, *TCRB* G	6, 24
NOMO-1	47, XX, 1p+, 7q+, 13p+			22
OCI/AML-2	48(43–49)<2n>XY, +6, +8, der(1)inv(1)(p36q31)t(1;6)(q13;p12), der(2)t(2;17)(p23;q24.1)del(2)(q14.2q36), der(3)t(1;3)(p36;p25), ins(3;2)(q21;q14.2q36), t(5;8)(q11.2;q24), der(6)t(1;6)(q31;p12)t(3;6)(q26;q24), inv(12)(p13.3q13.2), t(13;14)(q32/33;q24.2), der(17)t(2;17)(p23;q24.1)			24

Continued on next page

Table 4. (continued)

Cell line	Cytogenetic karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements[a]	Ref
OMA-AML-1	46, XY, t(2;7;11)(p21;p12;p15), +mar			25
P31/Fujioka	47, XY, −10, +15, +19, t(7;11)(7qter → 7p13::11q22 → 11qter;11pter → 11q22:), inv(9)(pter → p11::q13 → q11:q13 → qter), del(17)(qter → p11:)		*NRAS* mutation	27
P39/Tsugane	45(39–46)XY, −7, −8, −16, −17, +22, +del(6)(pter → q15:), t(9;?)(q34;?), t(14;16)(14pter → 14q24::16q21 → 16qter; 16pter → 16q21::14q24 → 14qter)		*NRAS* mutation	28
PLB-985	46(44–50)<2n>X, −X, +6, dm, dup(1)(q21q41), del(3)(p21), del(6)(q21)x2			24
RC-2A	47, XY, +11, t(11;12)			30
RWLeu-4	56, 7p+, 12p+, 17p+, Ph	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b2-a2) fusion gene		31
SCC-3	50, XX, −2, −4, −7, −15, +16, −20, +1p−, 1q−, 3q−, 6q−, 7p+, 8q−, 10q+, +11q+, +12q+, 12p−, 13p+, 17p+, +5 mar			33
SKM-1	46, XY, del(9)(q13q22), der(17)t(17;?)(p13;?)		*P53* PM	34
THP-1	94(88–96)<4n>XY/XXY, −Y, +1, +3, +6, +6, −8, −13, −19, −22, −22, +2mar, add(1)(p11), del(1)(q42.2), i(2q), del(6)(p21)x2-4, i(7p), der(9)t(9;11)(p22;q23)i(9)(p10)x2, der(11)t(9;11)(p22;q23)x2, add(12)(q24)x1-2, der(13)t(8;13)(p11;p12), add(?18)(q21)	t(9;11)(p21;q23) → *MLL-AF9* fusion gene	*P15INK4B* DD, *P16INK4A* DD; *P53* D; *RB1* R	24, 49, 54
TK-1	46, XY, −14, −17, +der(14)t(14;17)(14pter(14q22::17q23(17qter), +der(17)t(11;14;17)(17pter(17q23::14q22(14qter::11q13(11qter)		*IGH* G, *TCRB* G, *TCRG* R	37, 40
U-937	63(58–69)<3n>XXY, −2, −4, −6, +7, −9, −20, −21, +3mar, t(1;12)(q21;p13), der(5)t(1;5)(p22;q35), add(9)(p22), t(10;11)(p14;q23), i(11q), i(12p), add(16)(q22), add(19)(q13)	t(10;11)(p14;q23) → *MLL-CALM* fusion gene	*P53* PM	24, 55
UG3	46, XX, −7, +8, t(9;11)(p22;q23)	t(9;11)(p22;q23) → *MLL-AF9* fusion gene		39
YK-M2	68<3n>X, −Y, +3, −4, −4, −5, +6, +7, −10, −11, −16, del(17)(p11), +4mar			40

[a] Receptor gene arrangements: D – deleted; G – germline; PM – point mutation; R – rearranged.

[b] Data from sister cell line ML-1.

[c] ML-2 is negative for the *IGH*, *TCRA*, *TCRB*, *TCRG* mRNAs.

[d] Mono Mac 1 is diploid sister cell line with fewer *in vitro* rearrangements [24].

Table 5. Monocytic cell lines: functional characterization

Cell line	Doubling time	EBV status	Cytochemistry	Inducibility of differentiation	Heterotransplantability into mice	Special functional features	Ref.
AML-1		EBNA– HTLV-II–	ANAE+, CAE+, MPO–, PAS–				6
AML-193		EBNA– HTLV-II–	ANAE(+), CAE–, MPO–, MSE–, PAS(+)				6
CTV-1	30–40 h	EBV– HTLV-I– HIV–	ACP–, ANAE+ (NaF inhibitable), ANBE+, MPO–, MSE–, PAS+, SBB–, TRAP–	(AraC, DMSO, retinoic acid, TPA → no effect)			7,24
DOP-M1	48 h		ANBE+ (NaF inhibitable), CAE+, MPO+	(IFN-γ no effect)		no phagocytosis	8
FLG29.1		EBV–	ACP+, ANAE+ (NaF inhibitable), CAE–, MSE+, NBT+, PAS–, SBB–, TRAP+	TPA → osteoclast differentiation (vit. D3 no effect)		responsive to calcitonin; expression of calcitonin and estrogen receptors	9
IMS-M1	36–48 h		ANBE+, CAE–, MPO–	vit. D3 → mono/macro differentiation		retinoic acids induce apoptosis	11
KBM-3	23 h		ANBE+, CAE+, MPO+, MSE+, PAS–		into nude mice or SCID mice	colony formation in agar	12
KBM-5	24–60 h	EBNA–	ACP+, ANBE+, CAE+, MPO(+), MSE+, PAS–	(DMSO, hemin, retinoic acid, TPA no effect)	into SCID mice	colony formation in agar/ methylcellulose; resistant to NK activity, IFN-α, IFN-γ; no phagocytosis	13
KP-1	96 h		ANBE+ (NaF inhibitable), CAE–, MPO–, PAS–	TPA → macro differentiation		produces lysozyme; phagocytosis+; scavenger receptor+	14
KP-MO-TS	48 h	EBNA–	ACP+, ANBE+, CAE(+), MPO+, PAS–	retinoic acid, TPA → mono/macro differentiation (DMSO, IFN-γ no effect)		α1-antitrypsin+; phagocytosis(+); lysozyme production	15
ME-1	4–5 d	EBNA–	ANBE(+), CAE+, Luxol(+), MPO+, MSE+, Toluidine Blue(+)	GM-CSF, IL-3, IL-4, PHA-LCM → macro differentiation; serum-free culture → neutro differentiation; PHA-LCM → eosino differentiation; (DMSO, retinoic acid, vit. D3 no effect)		produces lysozyme; phagocytosis+; weak colony formation in methylcellulose	16,17,56
ML-2	60 h	EBV– HTLV-I– HIV–	MSE+	AraC, DMSO, TPA → mono/macro differentiation		TF: GATA-1 mRNA–, GATA-2 mRNA+, SCL mRNA–	24,57,58
MOLM-13	3–4 d	MPO–, MSE+		IFN-γ (+ TNF-α) → macro differentiation (GM-CSF, M-CSF no effect)			19
Mono Mac 6	50–60 h	EBV– HTLV-I– HIV–	ACP–, ANAE+ (NaF inhibitable), CAE–, MPO–, MSE+, PAS–	IFN-γ → mono differentiation		phagocytosis+; lysozyme+; mRNA: α1-antitrypsin–, azurocidin+, C3 complement+, cathepsin G+, defensin–, N-elastase+, lactoferrin–, myeloblastin+, tryptase+	20,24,59

Continued on next page

Table 5. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Inducibility of differentiation	Heterotransplantability into mice	Special functional features	Ref.
MUTZ-3	90–110 h	EBV– HTLV-I– HIV–	MSE+, MPO+, TRAP+	retinoic acid, TPA → mono/macro differentiation		colony formation in methylcellulose	21,24
MV4-11	50 h	EBV– HTLV-I– HIV–	ANAE+, CAE(+), MPO–, MSE+, PAS–			colony formation in agar	6,24
NOMO-1	24 h	EBNA–	ACP+, ANBE+, CAE–, MPO+, MSE+, NBT+	TPA → macro differentiation		phagocytosis+; lysozyme production+	22,60
OCI/AML-2		EBV– HTLV-I– HIV–	MSE+, NBT–			colony formation in methylcellulose; retinoic acid receptor+	23,24
OMA-AML-1			ANAE+ (NaF inhibitable), NBT–	GM-CSF, IL-3 → eosino differentiation	into nude or SCID mice	phagocytosis+; clonable in suspension; forming cobblestone areas	25,26
P31/Fujioka	80 h	EBNA–	ACP+, ALP–, ANBE+ (NaF inhibitable), CAE+, MPO–, PAS+			phagocytosis+	27
P39/Tsugane			ACP+, ANBE+ (NaF inhibitable), CAE+, MPO–, PAS+		into nude mice	phagocytosis+	28
PLB-985	24–48 h	EBV– HTLV-I– HIV–	ALP–, ANAE+, MPO+, MSE+, PAS(+), SBB+	Bt-cAMP, DMSO, retinoic acid → neutro differentiation; TPA → mono/macro differentiation	into nude mice	colony formation in agar; phagocytosis inducible	24,29
RC-2A	48 h	EBNA–	ACP(+), ANAE+, MSE–	*PHA-LCM* → mono/macro differentiation		cloning/colony formation in agar; weak antigen presentation	30,43
RWLeu-4	25–26 h	EBNA–	CAE+, MPO+, MSE+, NBT–, PAS+	DMSO, TPA, Vit. D3 → macro differentiation	into nude mice	PO: c-MYC+	31,32
SCC-3	48 h	EBNA HTLV-I–	ANBE+ (NaF inhibitable), CAE–, MPO–, MSE–, PAS+	TPA → macro differentiation		phagocytosis(+)	33
SKM-1	48 h	EBNA–	ANBE+, MPO+, MSE+			cloning/colony formation in agar; secretion of MPO; p53 overexpression	34,61
THP-1	35–40 h	EBV– HTLV-I– HIV–	ALP–, ANBE+ (NaF inhibitable), CAE–, MPO–, MSE+, PAS+, SBB–	TPA → macro differentiation		antigen presentation+; ADCC–; phagocytosis+; lysozyme production+; mRNA: α1-antitrypsin–, azurocidin+, C3 complement+, cathepsin G–, defensin–, N-elastase+, lactoferrin–, myeloblastin+, tryptase–; TF: GATA-1 mRNA–, GATA-2 mRNA–, SCL mRNA–	24,35,58, 59,62
TK-1	36–48 h	EBNA–	ANAE+ (NaF inhibitable), CAE+, MPO+, MSE+, NBT–	TPA, Vit. D3 → mono/macro differentiation		clonable; no phagocytosis	36

Continued on next page

Table 5. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Inducibility of differentiation	Heterotransplantability into mice	Special functional features	Ref.
U-937	30–40 h	EBV– HTLV-I– HIV–	ACP+, Alcian Blue(+), ALP–, ANAE+ (NaF inhibitable), ANBE+, CAE+, GLC+, MPO–, MSE(+), Oil Red O–, PAS+, SBB+, Toluidine Blue–, TRAP–	IFN-γ, retinoic acid, TPA, Vit. D3 → mono/macro differentiation		ADCC+, phagocytosis+; no colony formation in agar; mRNA: α1-antitrypsin+, azurocidin–, C3 complement+, cathepsin G–, defensin–, N-elastase–, lactoferrin–, myeloblastin–, tryptase(+); lysozyme *production*	24,38,59
UG3	60–70 h		ALP–, ANBE+, CAE+, MPO–	G-CSF → neutro differentiation; GM-CSF, M-CSF → macro differentiation; M-CSF + IL-4/IL-13 → osteoclast differentiation		colony formation in methylcellulose (with cytokines)	39,63
YK-M2	60 h	EBNA–	ANBE+ (NaF inhibitable), CAE(+), MPO+, NBT–	Vit. D3 → mono/macro differentiation		phagocytosis+	40

PHA-LCM – phytohemagglutinin-stimulated peripheral leukocytes conditioned medium; PO – (proto)-oncogenes; TF – transcription factors.

Table 6. Monocytic cell lines: unconfirmed cell lines (not immortalized, not characterized, not verified, other)

Cell line	Patient	Features	Ref.	Remarks[a]
230	from PB of 35 M with AML M4 in 1976	CD2– HLA-DR+ sIg– TdT–; ACP+ ANBE(+) ALP– CAE– EBNA– MPO– PAS+ SBB–	64	insufficiently characterized; present status unknown
2MAC[b]	normal donor	well-characterized normal macrophage cell line (immunoprofile, cytokines)	65	not leukemia cell line
AMoL I	from BM of child with AML M5 (at diagnosis)	CD3– CD11b– cy/sIg– HLA-DR+; ANAE+ (NaF inhibitable); no antigen presentation; no phagocytosis; produces IL-1	66	insufficiently characterized; present status unknown
AMoL II	from BM of child with AML M5 (at diagnosis)	CD3– CD11b– cy/sIg– HLA-DR+; ANAE+ (NaF inhibitable); no antigen presentation; no phagocytosis; produces IL-1	66	insufficiently characterized; present status unknown
DD	from PB of patient with malignant histiocytosis in leukemic phase	CD2– CD11a(+) CD14(+) CD16– CD19– CD32+ CD64– HLA-DR–; ACP– ANAE(+); LPS, TPA → mono/macro differentiation	67	insufficiently characterized
HBM-MI-1 and -2[b]	from BM of 1 M with diffuse cutaneous mastocytosis	established by SV40 transformation of parental, not immortal line HBM-M; CD11b(+) CD11c– CD13(+) HLA-DR–; ACP+ Alcian blue(+) ALP+ ANAE+ CAE+ MPO+ PAS+ Toluidine blue(+)	68	not leukemia cell lines
HL-92	from PB of patient with AML M4	CD2– CD11b+ HLA-DR+ sIg– TdT–; ACP(+) ANAE+ CAE+ EBNA– MPO– NBT+ SBB+; phagocytosis+; DMSO, retinoic acid → neutro differentiation; TPA → mono/macro differentiation	69	cell line apparently lost
J6-1	from patient with AML M5 in 1976	CD15+; M-CSFR mRNA+, TGF-β mRNA+; produces M-CSF; GM-CSF, IL-3, M-CSF stimulate proliferation	70	insufficiently characterized
J-111	from PB of 25 F with AML M5		71	cross-contaminated: in reality cell line HELA (ref. 72,73)
JOSK-I	from PB of 72 F with AML M4 in 1983	immunophenotypically, cytogenetically, etc. well characterized	74	cross- contaminated: in reality cell line U-937 (ref. 24)
JOSK-K	from PB of 54 M with AML M5 in 1984	immunophenotypically, cytogenetically, etc. well characterized	74	cross- contaminated: in reality cell line U-937 (ref. 24)
JOSK-M	from PB of 37 M with CML-mono BC in 1984	immunophenotypically, cytogenetically, etc. well characterized	74	cross- contaminated: in reality cell line U-937 (ref. 24)
JOSK-S	from PB of 66 F with AML M5 in 1983	immunophenotypically, cytogenetically, etc. well characterized	74	cross- contaminated: in reality cell line U-937 (ref. 24)
K1m[b]	from PB	well-characterized normal macrophage cell line (immunoprofile); phagocytosis+	75	not leukemia cell line
KMT-2[b]	from normal umbilical cord blood	CD13+ CD15+ CD33+ CD34+ GlyA– HLA-DR+; ACP+ ANAE+ (NaF inhibitable) CAE– MPO– PAS+ SBB–; GM-CSF, IL-3, M-CSF stimulate proliferation	76	not leukemia cell line
KOCL-48	from 0.5 F with first ALL L2, then AML M4	CD2+ CD3– CD5+ CD7+ CD10+ CD13+ CD14+ CD15+ CD19+ CD22– CD33+ CD41b– HLA-DR+ cyIgM+ sIg–; ANBE+, MPO–; 50, X, t(4;11)(q21;q23) → *MLL-AF4* fusion gene; *IGH* RD, *IGK* G, *TCRB* G, *TCRG* RG	77,78	insufficiently characterized
MOBS-1		MSE+		cross-contaminated: in reality cell line U-937
Na	from BM of 69 F with AML M2 → AML M4	CD11c+ CD13+ CD14+; ANBE+ lysozyme+; t(8;17); *MYC* rearranged, amplified	79	insufficiently characterized

Continued on next page

Table 6. (continued)

Cell line	*Patient*	*Features*	*Ref.*	*Remarks*[a]
OCI-M3	from PB of patient with AML M4 in 1986		80	insufficiently characterized; present status unknown
OCI/AML-3	from PB of 57 M with AML M4 in 1987	CD7+ CD8+ CD13+ CD14+ CD19+ CD33+ CD34(+) TdT+; retinoic acid receptor mRNA+; MSE+	81	insufficiently characterized
OTC-4	from PB of patient with AML M4	G-CSF, GM-CSF inhibit proliferation, induce macro differentiation; TPA, vit. D3 → macro differentiation	82	insufficiently characterized
YAP[b]	from PB of 42 M with psoriasis vulgaris in 1993	CD2– CD3– CD4+ CD8– CD11b+ CD14– CD16– CD19– CD25+ CD33+ CD56+ CD68–; ANBE+ (NaF inhibitable) MPO– PAS+; complex, highly rearranged karyotype; EBNA– SV40–; responsive to TPA	83	not leukemia cell line

[a] For most cell lines, the insufficient characterization concerns the description of essential features, including clinical data, authentication, immunoprofile and/or cytogenetics. Present status unknown: no data on this cell line have been published since its original description.

[b] These cell lines were described to have monocytic/macrophage features, but were not derived from patients with leukemias or other hematological neoplasms.

at diagnosis (n = 9) or at relapse/refractory stage (n = 16). Patient material was obtained from the peripheral blood (n = 22), bone marrow (n = 3) or extra-hematological sites (n = 3). Cell lines were established (or when this was not indicated, at least described) in the 1970s (n = 3), 1980s (n = 23) and 1990s (n = 5). Authentication was provided for 14/31 (45%) cell lines. Three types of media are used to culture these cell lines, namely RPMI 1640 (n = 22), IMDM (n = 7) and α-MEM (n = 2); most lines require addition of 10–20% FBS. Four cell lines could be cultured under serum-free conditions (AML-193, MV4-11, NOMO-1, PLB-985) (Appendix 2, p. 286). Cell lines AML-193, MUTZ-3 and UG3 are absolutely growth factor-dependent and will die in the absence of growth factors (Appendix 3, p. 287). Fourteen cell lines can be obtained from major cell banks (Appendix 1, p. 284). Subclones with variant immunological or cytogenetic features have been derived from the parental lines ME-1 and TK-1. Sister cell lines of ML-2, MOLM-13, and Mono Mac 6 were established from the same patient, but either at different time points, or the original material was split prior to or very early in the cell culture.

3. IMMUNOPHENOTYPE

Monocytic cell lines usually express the pan-myelomonocytic cell surface markers CD13 (20/25, 80%), CD15 (21/22, 95%), CD33 (22/24, 92%), and CD68 (5/7, 62%) (Table 2). A typical monocyte-associated cell surface antigen is CD14, found on 19/30 (63%) cell lines. It appears that expression of this marker is lost during *in vitro* culture of the originally CD14-positive primary AML cells [59]. The cell lines are commonly negative for typical B-cell, T-cell and erythroid-megakaryocytic-associated antigens, except for CD4 and CD7 which are also known to be expressed by normal monocytic precursor cells [86]. With regard to the progenitor cell markers CD34 and HLA-DR, 33% (5/15) and 69% (20/29) of lines, respectively, were described as positive. Finally, most cell lines display adhesion molecules CD11b and CD54. A typical immunoprofile of a monocytic leukemia cell line is: CD4± CD7± CD11b+ CD13+ CD14± CD15+ CD33+ CD34± CD54+ HLA-DR+, T-antigen negative, B-antigen negative, ery-meg-antigen negative.

4. CYTOKINE-RELATED CHARACTERIZATION

The cytokine which is uniquely effective on cells committed to monocyte differentiation is M-CSF. Several cell lines were described as responding proliferatively to this growth factor (IMS-M1, MUTZ-3, OCI/AML-2, UG3).

The related cytokines GM-CSF and IL-3 are effective on normal monocytic precursor cells and also induce the growth of various monocytic leukemia cell lines (AML-193, IMS-MI, ME-1, MUTZ-3, MV4-11, OCI/AML-2, OMA-AML-1, SKM-1, UG3) [44]. IFN-γ, known as the typical *in vitro* inducer of monocytic activation and differentiation, was also effective on monocytic cell lines. Three cell lines (AML-193, MUTZ-3, UG3) are absolutely growth factor-dependent on GM-CSF, IL-3 or PIXY-321 and will die within about one week in the absence of growth factor (Appendix 3, p. 287).

5. GENETIC CHARACTERIZATION

The monocytic cell lines display rather complex karyotypes (Table 4). Non-random unique translocations were found in several cell lines. Two cell lines (KBM-5, RWLeu-4) have the Philadelphia chromosome t(9;22)(q34;q11) which is typical for CML, the breakpoints lying in the major breakpoint cluster region (M-bcr) (Appendix 4, p. 288). Inversion (16)(p13q22), which is specific for the AML subtype M4eo, occurs in the cell line ME-1. Apart from these latter two alterations and the rare t(12;22) seen in cell line MUTZ-3, all other cell lines with specific translocations have a breakpoint at chromosome 11q23 involving the gene *MLL*: IMS-M1, ML-2, MOLM-13, Mono Mac 6, MV4-11, THP-1, U-937, UG3 (Appendix 4, p. 288). While 11q23-involving translocations were detected in various types of ALL and AML, monocytic AML commonly has this cytogenetic aberration [54]. Of note also are the deletions of the *P15INK4B*, *P16INK4A* and *P53* genes, the *NRAS* mutations, and the rearrangement of the *RB1* gene in various cell lines [87].

6. FUNCTIONAL CHARACTERIZATION

The monocytic cell lines have somewhat longer doubling times than other cell types, ranging from 1 to 4–5 days, mostly in the 24–48 h range (15/25, 60%) (Table 5). All cell lines tested were negative for EBV, HTLV-I/II and HIV (Table 5). With regard to the cytochemical profile of monocytic leukemia cell lines, the following results were reported: 8/10 (80%) ACP+, 0/5 ALP+, 26/26 ANAE/ANBE+, 13/21 (62%) CAE+, 11/25 (44%) MPO+, 17/21 (81%) MSE+, 2/7 (29%) NBT+, 9/17 (53%) PAS+, 2/5 (40%) SBB+, 2/4 (50%) TRAP+. The most prevalent monocytic enzyme marker is α-naphthyl acetate or butyrate esterase [88], which is positive in all cell lines. The majority of this enzymatic activity stems from one unique isoenzyme, the so-called monocyte-specific esterase (MSE), detected in 80% of the cell

lines tested. Importantly, this monocytic-specific activity can be inhibited by sodium fluoride [89–91].

Various agents can induce differentiation in human monocytic leukemia cells: including DMSO, IFN-γ, retinoic acid, TPA and vitamin D3 [92]. These biomodulators induce monocytic-macrophage differentiation in most cell lines examined (Table 5). U-937 and THP-1 cell lines provide two useful models for investigating the mechanisms of action of these agents [92–94]. Several cell lines can be heterotransplanted into nude or SCID mice (Table 5).

Functional features specific for monocytic cell lines, in concordance with their normal physiological counterparts [2], are phagocytosis (seen in 12 out of 15 cell lines tested), lysozyme production (in all six cell lines tested), and antigen presentation and antigen-dependent cell-mediated cytotoxicity. Unfortunately, the last two features, unique to monocytic cells, have rarely been examined.

7. UNCONFIRMED CELL LINES

The panel of cell lines requiring further characterization is large and diverse (Table 6). There are cell lines which are cross-contaminated with U-937 or the cervix carcinoma cell line HELA (the JOSK-series, J-111, MOBS-1). Several cell lines have interesting features and might be useful additions to the spectrum of available, well-characterized monocytic cell lines (e.g. OCI-M3, OCI/AML-3, OTC-4). The cell lines remain insufficiently characterized and in many cases their availability is not known and they might have been lost, including 230, AMOL I, AMOL II, DD, HL-92, J6-1, KOCL-48, Na, OCI-M3, OCI/AML-3. There are some cell lines with specific monocytic or macrophage properties which are not derived from patients with monocytic leukemias or hematological neoplasms, but were reported to be established from normal monocytic cells or macrophages (see references in Table 6). They seem to be representative *in vitro* models for this type of hematopoietic cell: 2MAC, HBM-MI-1/-2, K1m, KMT-2, YAP.

The osteoclast originates from an early precursor common to the monocytic and granulocytic lineages. However, tissue macrophages and monocytes isolated from the circulation significantly differ from osteoclasts in morphology, antigenic properties and other biological features. The cell line FLG29.1 (listed in Table 1 under monocytic leukemia cell lines) is quite unique in presenting monocyte-macrophage and osteoclast features [9]. Following induction of differentiation with TPA, these cells lose monocytic characteristics and acquire more osteoclast-associated markers. Similarly, UG3

cells were described as acquiring osteoclast-like features upon treatment with cytokines [39,63].

References

1. Bessis M (ed.) *Blood Smears Reinterpreted*, Springer, Berlin, 1977.
2. Roitt I, Brostoff J, Male D (eds.) *Immunology*, 3rd edn., Mosby, London, 1993.
3. Johnston RB Jr, Zucker-Franklin D. In: Zucker-Franklin D, Greaves MF, Grossi CE, Marmont AM (eds.), *Atlas of Blood Cells. Function and Pathology*, 2nd edn., Fischer, Stuttgart, pp 321–357, 1988.
4. Bennett JM, Catovsky D, Daniel MT et al. *Br J Haematol* 33: 451–458, 1976.
5. Bennett JM, Catovsky D, Daniel MT et al. *Br J Haematol* 51: 189–192, 1982.
6. Lange B, Valtieri M, Santoli D et al. *Blood* 70: 192–199, 1987.
7. Chen PM, Chiu CF, Chiou TJ et al. *Gann* 75: 660–664, 1984.
8. Sugita K, Sakakibara H, Eguchi M et al. *Int J Hematol* 55: 45–51, 1992.
9. Gattei V, Bernabei PA, Pinto A et al. *J Cell Biol* 116: 437–447, 1992.
10. Yamamoto K, Seto M, Iida S et al. *Blood* 83: 2912–2921, 1994.
11. Nagamura F, Tojo A, Tani K et al. *Blood* 86: 150a, 1995.
12. Andersson BS, Bergerheim USR, Collins VP et al. *Exp Hematol* 20: 361–367, 1992.
13. Beran M, Pisa P, O'Brien S et al. *Cancer Res* 53: 3603–3610, 1993.
14. Adachi N, Takaki K, Nakamura R et al. *Pediatr Res* 34: 258–264, 1993.
15. Ikushima S, Yoshihara T, Fujiwara F et al. *Acta Haematol Jpn* 53: 678–687, 1990.
16. Yanagisawa K, Horiuchi T, Fujita S. *Blood* 78: 451–457, 1991.
17. Yanagisawa K, Sato M, Horiuchi T et al. *Blood* 84: 84–93, 1994.
18. Herrmann R, Han T, Barcos MP et al. *Cancer* 46: 1383–1388, 1980.
19. Matsuo Y, MacLeod RAF, Uphoff CC et al. *Leukemia* 11: 1469–1477, 1997.
20. Ziegler-Heitbrock HWL, Thiel E, Fütterer A et al. *Int J Cancer* 41: 456, 461, 1988.
21. Hu ZB, Ma W, Zaborski M et al. *Leukemia* 10: 1025–1040, 1996.
22. Kato Y, Ogura M, Okumura M et al. *Acta Haematol Jpn* 49: 277, 1986.
23. Wang C, Curtis JE, Minden MD et al. *Leukemia* 3: 264–269, 1989.
24. Drexler HG, Dirks W, MacLeod RAF et al. (eds.) *DSMZ Catalogue of Human and Animal Cell Cultures*, 7th edn., DSMZ, Braunschweig, 1999.
25. Pirruccello SJ, Jackson JD, Lang MS et al. *Blood* 80: 1026–1032, 1992.
26. Pirruccello SJ, Jackson JD, Sharp G. *Leukemia Lymphoma* 13: 169–178, 1994.
27. Hirose M, Minato K, Tobinai K et al. *Gann* 73: 735–741, 1982.
28. Nagai M, Seki S, Kitahara T et al. *Gann* 75: 1100–1107, 1984.
29. Tucker KA, Lilly MB, Heck L Jr et al. *Blood* 70: 372–378, 1987.
30. Bradley TR, Pilkington G, Garson M et al. *Br J Haematol* 51: 595–604, 1982.
31. Lasky SR, Bell W, Huhn RD et al. *Cancer Res* 50: 3087–3094, 1990.
32. Wiemann M, Hollmann A, Posner M et al. *Clin Res* 33: 460A, 1985.
33. Kimura Y, Toki H, Okabe K et al. *Jpn J Cancer Res* 77: 862–865, 1986.
34. Nakagawa T, Matozaki S, Murayama T et al. *Br J Haematol* 85: 469–476, 1993.
35. Tsuchiya S, Yamabe M, Yamaguchi et al. *Int J Cancer* 26: 171–176, 1980.
36. Ohno H, Doi S, Fukuhara S et al. *Int J Cancer* 37: 761–767, 1986.
37. Nosaka T, Ohno H, Doi S et al. *J Clin Invest* 81: 1824–1828, 1988.
38. Sundström C, Nilsson K. *Int J Cancer* 17: 565–577, 1976.
39. Ikeda T, Saski K, Ikeda K et al. *Blood* 91: 4543–4553, 1998.
40. Ohno H, Fukuhara S, Doi S et al. *Cancer Res* 46: 6400–6405, 1986.

41. Santoli D, Yang YC, Clark SC et al. *J Immunol* 139: 3348–3354, 1987.
42. Taetle R, Payne C, Dos Santos B et al. *Cancer Res* 53: 3386–3393, 1993.
43. Lyons AB, Ashman LK. *Leukemia Res* 11: 797–805, 1987.
44. Drexler HG, Zaborksi M, Quentmeier H. *Leukemia* 11: 701–708, 1997.
45. Schumann RR, Nakarai T, Gruss HJ et al. *Blood* 87: 2419–2427, 1996.
46. Blick MB, Andersson BS, Gutterman JU et al. *Leukemia Res* 10: 1401–1409, 1986.
47. Liu P, Claxton DF, Marlton P et al. *Blood* 82: 716–721, 1993.
48. Tanabe S, Zeleznik-Le NJ, Kobayashi H et al. *Genes Chromosomes Cancer* 15: 206–216, 1996.
49. Nakamaki T, Kawamata N, Schwaller J et al. *Br J Haematol* 91: 139–149, 1995.
50. Ohyashiki K, Ohyashiki JH, Sandberg AA. *Cancer Res* 46: 3642–3647, 1986.
51. Ohyashiki JH, Ohyashiki K, Toyama K et al. *Cancer Genet Cytogenet* 37: 193–200, 1989.
52. MacLeod RAF, Voges M, Drexler HG. *Blood* 82: 3221–3222, 1993.
53. MacLeod RAF, Hu ZB, Kaufmann M et al. *Genes Chromosomes Cancer* 16: 144–148, 1996.
54. Drexler HG, MacLeod RAF, Borkhardt A et al. *Leukemia* 9: 480–500, 1995.
55. Dreyling MH, Martinez-Climent JA, Zheng M et al. *Proc Natl Acad Sci USA* 93: 4804–4809, 1996.
56. Yanagisawa K, Hasegawa H, Fujita S. *Br J Haematol* 80: 293–297, 1992.
57. Takeda K, Minowada J, Bloch A. *Cancer Res* 42: 5152–5253, 1982.
58. Shimamoto T, Ohyashiki K, Ohyashiki JH et al. *Blood* 86: 3173–3180, 1995.
59. Abrink M, Gobl AE, Huang R et al. *Leukemia* 8: 1579–1584, 1994.
60. Hamaguchi M, Morishita Y, Takahashi I et al. *Blood* 77: 94–100, 1991.
61. Kawaguchi R, Hosokawa Y, Komine A et al. *Leukemia* 6: 1296–1301, 1992.
62. Tsuchiya S, Kobayashi Y, Goto Y et al. *Cancer Res* 42: 1530–1536, 1982.
63. Ikeda T, Ikeda K, Sasaki K et al. *Biochem Biophys Res Commun* 253: 265–272, 1998.
64. Karpas A, Khalid G, Burns GF et al. *Br J Cancer* 37: 308–315, 1978.
65. Dialynas DP, Tan PC, Huhn GD et al. *Cell Immunol* 177: 182–193, 1997.
66. Giller RH, Mori M, Hayward AR. *J Clin Immunol* 4: 429–438, 1984.
67. Kávai M, Ádány R, Pásti G et al. *Cell Immunol* 139: 531–540, 1992.
68. Townsend M, MacPherson J, Krilis S et al. *Br J Haematol* 85: 452–461, 1993.
69. Salahuddin SZ, Markham PD, McCredie KB et al. *Leukemia Res* 6: 729–741, 1982.
70. Wu KF, Rao Q, Zheng GG et al. *Leukemia Res* 18: 843–849, 1994.
71. Osgood EE, Brooke JH. *Blood* 10: 1010–1022, 1955.
72. Drexler HG, Häne B, Hu ZB et al. *Leukemia* 7: 2077–2078, 1993.
73. Nelson-Rees WA, Flandermeyer RR. *Science* 191: 96–97, 1976.
74. Ohta M, Furukawa Y, Ide C et al. *Cancer Res* 46: 3067–3074, 1986.
75. Dialynas DP, Lee MJ, Shao LE et al. *Blood* 88: 158a, 1996.
76. Tamura S, Sugawara M, Tanaka H et al. *Blood* 76: 501–507, 1990.
77. Iida S, Saito M, Okazaki T et al. *Leukemia Res* 16: 1155–1163, 1992.
78. Kojika S, Sugita K, Inukai T et al. *Leukemia* 10: 994–999, 1996.
79. Kimura Y, Miura I, Hashimoto K et al. *Blood* 76: 289a, 1990.
80. Tweeddale M, Jamal N, Nguyen A et al. *Blood* 74: 572–578, 1989.
81. Wang C, Koistinen P, Yang GS et al. *Leukemia* 5: 493–499, 1991.
82. Ono Y, Iwasaki H, Otsuka T et al. *Blood* 84: 574a, 1994.
83. Hamamoto Y, Nagai K, Muto M et al. *Arch Dermatol Res* 288: 225–229, 1996.
84. Lübbert M, Koeffler HP. *Blood Rev* 2: 121–133, 1988.
85. Lübbert M, Herrmann F, Koeffler HP. *Blood* 77: 909–924, 1991.

86. Drexler HG, Thiel E, Ludwig WD. *Leukemia* 7: 489–498, 1993.
87. Drexler HG. *Leukemia* 12: 845–859, 1998.
88. Hayhoe FGJ, Flemans RJ (eds.) *A Colour Atlas of Haematological Cytology*, 3rd edn., Wolfe, London, 1992.
89. Uphoff CC, Gignac SM, Metge K et al. *Leukemia* 7: 58–62, 1993.
90. Uphoff CC, Hu ZB, Gignac SM et al. *Leukemia* 8: 1510–1526, 1994.
91. Gignac SM, Hu ZB, Denkmann SA et al. *Leukemia Lymphoma* 22: 143–151, 1996.
92. Koeffler HP. *Blood* 62: 709–721, 1983.
93. Koeffler HP. *Semin Hematol* 23: 223–236, 1986.
94. Harris P, Ralph P. *J Leuk Biol* 37: 407–422, 1985.

Chapter 8

Erythroid – Megakaryocytic Cell Lines

Hans G. Drexler
DSMZ-German Collection of Microorganisms and Cell Cultures, Department of Human and Animal Cell Cultures, Braunschweig, Germany; Tel: +49-531-2616-160; Fax: +49-531-2616-150; E-mail: hdr@dsmz.de

1. INTRODUCTION

For reasons outlined below, leukemia cell lines derived from erythroid or megakaryocytic cells are combined in this chapter. These cell lines represent *in vitro* models of immature erythroid and megakaryocytic cells and their distinct or common precursors.

Morphologically, the erythrocytic series consists of a succession of cells which begins with a pronormoblast and ends with the erythrocyte (or red blood cell) [1]. These circulating red cells and their precursors may be considered as a functional unit which has been designated as the erythron. The cells of this unit are not restricted to those recognizable morphologically in the bone marrow and in the peripheral blood, but also include elements which are not morphologically identifiable, namely the committed precursors of the erythroid line, the existence of which has been demonstrated by functional assays [2].

The thrombocytic series is a succession of cells which starts with the basophilic megakaryoblast in the bone marrow and ends with the circulating thrombocyte or platelet [1]. In normal human bone marrow, the megakaryocyte is the largest cell, measuring 20–150 μm in diameter. The cells are polyploid, but not multinucleated, in contrast to the other giant bone marrow-derived cells, the osteoclasts. Normal megakaryopoiesis is maintained by morphologically unidentifiable precursors which are capable of differentiating into morphologically recognizable megakaryoblasts or of reproducing themselves [3].

As both erythrocytes and thrombocytes are terminally differentiated, anucleated end-stage cells, immature progenitor and precursor cells are considered to be the targets in the leukemogenic process. The acute myeloid leukemias originating in these cell lineages have been termed AML M6

J.R. W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol. III, 259–294.

(erythroid) and AML M7 (megakaryocytic) [4,5]. Erythroid and/or megakaryocytic cells can also represent a subpopulation of the main leukemic population in the myeloid blast crisis of CML [6].

Over the last two decades, a large panel of erythroid-megakaryocytic leukemia cell lines has been established from patients with AML, CML in blast crisis or rare hematological disorders [7–9]. The features displayed by a cell line assigned to this category are not single lineage-specific, but in most instances extend to both lineages. Indeed, data from both normal and malignant cells support this notion. Multiple lines of evidence underscore the concept of a close relationship between erythroid and megakaryocytic lineages [10]. The two share a number of transcription factors (including NF-E2, GATA-1, GATA-2, SCL) [11–13]. Furthermore, erythroid and megakaryocytic cell surface markers (including GlyA, CD41, CD42, CD61) are found on both types of leukemia cell and this dual expression is found on the same cell [14–17]. The cytokines EPO and TPO, originally considered to be cell lineage-specific, also have stimulatory effects on the megakaryocytic cell system and the erythron, respectively [18–20]. The receptor for EPO, which is the principal growth factor regulating the production of erythrocytes, has also been detected on megakaryocytes [21]. A bipotent normal erythroid-megakaryocytic progenitor could be isolated from human bone marrow [22]. Finally, most of the "erythroid" cell lines available display, or can be induced to display, features of megakaryocytic differentiation. The converse is true for cell lines initially thought to be exclusively megakaryocytic.

While a given cell line may show a preponderance of erythroid or megakaryocytic features, the notion of a close lineage relationship, extended here to the assignment of such cell lines to a common category, is borne out by the extensive published data, summarized here in Tables 1–6. Most erythroid-megakaryocytic cell lines were established in the 1980s (49%) and 1990s (47%), with K-562 being the oldest cell line [45].

2. CLINICAL CHARACTERIZATION

Forty-nine cell lines with erythroid and megakaryocytic characteristics are listed in Table 1. These cell lines were derived mainly from patients with CML in blast crisis (41%), *de novo* or secondary AML M6 (14%) or AML M7 (33%). It is of note that 4/7 AML M6 and 8/16 AML M7 cases were either secondary to or accompanied by other hematological disorders or (pre)malignancies. The ages of the patients ranged from 0.5 to 73 years with 3 infants (<1 year) and 10 other children. Specimens were obtained at diagnosis (n = 12), at relapse (n = 11) or in blast crisis (n = 20). Taking CML blast crisis as an indicator of relapse, then 63% of these cell lines were

Table 1. Erythroid-megakaryocytic cell lines: clinical characterization

Cell line[a]	Patient[b] age/sex	Diagnosis[c]	Treatment status[d]	Specimen site[e]	Authentication[f]	Year est.	Culture medium[g]	Availability[h]	Primary Ref.
AP-217	42 M	CML-BC	BC	PB	yes	1992	RPMI 1640 + FBS	Author	23
AS-E2	62 M	AML M6	R	BM	yes	1993	IMDM + FBS + EPO	Author	24
B1647	14 M	AML M2		BM	no		IMDM + FBS		25
CHRF-288-11[i]	2 M	AML M7 (+ myelofibrosis)	T	Tu	yes	1988	Fischer + HS	Author	26,27
CMK[j]	1 M	AML M7 (+ Down's syndrome)	R	PB	yes	1985	RPMI 1640 + FBS	DSMZ, IFO	28–30
CMS	2 F	granulosarcoma → AML M7	R	PB	yes	1991	RPMI 1640 + FBS		31
CMY	2 M	AML M7 (+ Down's syndrome)	T	BM	no	1991	RPMI 1640 + FBS		32
ELF-153	48 M	Myelofibrosis → AML M7	R	BM	no	1988	RPMI 1640 + FBS + GM-CSF	Author	33,34
F-36P[k]	68 M	MDS (RAEB) → AML M6	D	PE	no	1989	RPMI 1640 + FBS + GM-CSF or IL-3	RIKEN	35
GRW	4 F	AML M7 (+ Down's syndrome)	R	PB	yes	1992	α-MEM + FBS + SCF	Author	36
HEL	30 M	Hodgkin → AML M6 (post-BMT)	R	PB	yes	1980	RPMI 1640 + FBS	ATCC, DSMZ, JCRB	37
HIMeg-1[l]	25 F	CML	CP	PB	no		IMDM/RPMI 1640 + FBS	Author	38
HML	2 M	AML M7 (+ Down's syndrome)	D	PB	no		α-MEM + FBS + GM-CSF		39
HU-3[m]	69 F	AML M7	D	BM	no	1991	RPMI 1640 + human serum + GM-CSF	Author	40–42
JK-1	62 M	CML-ery BC	BC	Tu	no	1987	RPMI 1640 + FBS or human serum	DSMZ	43
JURL-MK1[n]	73 M	CML-BC	BC	PB	no	1993	DMEM + FBS	Author	44
K-562	53 F	CML-BC	BC	PE	yes	1970	RPMI 1640 + FBS	ATCC, DSMZ, JCRB, RIKEN	45
KH184	25 M	Megakaryocytic sarcoma	R	PB	no	1984	RPMI 1640 + FBS	Author	46
KH88[o]	70 M	CML-ery BC	BC	PB	yes	1988	IMDM + FBS	Author	47
KMOE-2[p]	2 F	Acute erythremia (erythroblastosis)	D	PB	no	1978	Ham's F12 + FBS	DSMZ	48
LAMA-84[q]	29 F	CML-my/meg BC	BC	PB	yes	1984	RPMI 1640 + FBS	DSMZ	49,50

Continued on next page

Table 1. (continued)

Cell line[a]	Patient[b] age/sex	Diagnosis[c]	Treatment status[d]	Specimen site[e]	Authentication[f]	Year est.	Culture medium[g]	Availability[h]	Primary Ref.
M-07e[r]	0.5 F	AML M7	D	PB	no	1987	IMDM + FBS + IL-3	DSMZ	42,51,52
M-MOK[s]	1 F	AML M7	R	BM	no	1989	RPMI 1640 + FBS + GM-CSF	Author	42,53
MB-02	70 M	Myelofibrosis + myeloid metaplasia → AML M7	D	PB	yes	1988	RPMI 1640 + human serum + GM-CSF	Author	54
MC3	51 F	CML-meg BC	BC	PB	yes	1986	RPMI 1640 + FBS	Author	55
MEG-01[t]	55 M	CML-meg BC	BC	BM	no	1983	RPMI 1640 + FBS	ATCC, DSMZ, IFO	56,57
MEG-A2	24 M	CML-meg BC	BC	PB	yes	1991	IMDM + FBS	Author	58
MG-S	3 M	AML M0 (+ Down's syndrome)	R	PB	no	1991	RPMI 1640 + FBS		59
MHH 225	60 M	AML M7	D	BM	yes	1993	RPMI 1640 + FBS or serum-free	Author	60,61
MKPL-1	66 M	AML M7	D	BM	yes	1989	RPMI 1640 + FBS	Author	62
MOLM-1	41 M	CML-BC	BC	BM	no	1988	RPMI 1640 + FBS	Author	63
MOLM-7[u]	44 M	CML-my BC	BC	PB	no	1992	RPMI 1640 + FBS	Author	64
M-TAT	3 M	MDS (RAEBT)	R	PB	no	1992	RPMI 1640 + FBS + GM-CSF		65
NS-Meg	44 F	CML-meg BC	BC	PB	yes	1992	RPMI 1640 + FBS	Author	66
OCIM1	62 M	CLL → AML M6			no	1984	IMDM + FBS	Author	67
OCIM2	56 M	MDS → AML M6			no	1984	IMDM + FBS	Author	67
RM10	44 F	CML-BC	BC	BM	no	1982	IMDM or RPMI 1640 + FBS		68
RS-1	2 F	AML M7	R	PB	no		RPMI 1640 + FBS	Author	69
SAM-1	22 M	CML-BC	BC	PB	yes	1987	RPMI 1640 + FBS	Author	70
SET-2	71 F	essential thrombocythemia (leukemic conversion)	D	PB	yes	1995	DMEM + FBS	Author	71
SKH1		CML-meg BC	BC		no		RPMI 1640 + FBS	Author	72
T-33	62 F	CML-meg BC	BC	PB	no	1985	RPMI 1640 + FBS or serum-free	Author	73
TF-1[v]	35 M	AML M6	D	BM	no	1987	RPMI 1640 + FBS + GM-CSF	ATCC, DSMZ	42,74,75
TS9;22	56 M	CML-BC	BC	PB	yes	1992	RPMI 1640 + FBS	Author	76

Continued on next page

Table 1. (continued)

Cell line[a]	Patient[b] age/sex	Diagnosis[c]	Treatment status[d]	Specimen site[e]	Authentication[f]	Year est.	Culture medium[g]	Availability[h]	Primary Ref.
UoC-M1	68 M	AML M1	D	BM	yes	1992	McCoy's 5A + FBS		77
UT-7[w]	64 M	AML M7	D	BM	no	1988	IMDM + FBS + GM-CSF or IL-3 or EPO	DSMZ	78–81
Y-1K	57 F	CML-ery BC	BC	PB	no	1990	IMDM + FBS		82
YN-1	35 M	CML-ery BC	BC	PB	no	1980	IMDM + FBS		82
YS9;22	23 F	CML-BC	BC	PB	yes	1992	RPMI 1640 + FBS	Author	76

[a] Names of cell lines are indicated as given in the original literature; subclones (variant cell lines derived from a parental cell line) and sister cell lines (derived independently from the same patient from different specimens or at different time points) are indicated for each cell line. [b] Age at the time of establishment of cell line. [c] Diagnoses are indicated as given in the original literature; → : disease progressed from a pre-malignancy/first malignancy into the final malignancy. [d] BC – at blast crisis; CP – in the chronic phase; D – at diagnosis (prior to therapy); R – at relapse; T – during therapy. [e] BM – bone marrow; PB – peripheral blood; PE – pleural effusion; Tu – tumor. [f] Evidence (e.g. cytogenetic marker chromosomes, immunoprofile, others) that this cell line was derived from the patient indicated. [g] Culture media as indicated in the original literature; cell line might also grow with other medium and/or supplements. [h] Availability from cell banks (ATCC; DSMZ; IFO; JCRB; RIKEN) or from the original investigator (author) [i] Cell line was established in 1985 from a solid tumor line (CHRF-288) that was passaged by heterotransplantation into nude mice. [j] CMK6 (poorly differentiated) and CMK11-5 (well differentiated) are subclones established from CMK. [k] A subclone termed F-36E is EPO-dependent/responsive and is grown with 20 U/ml EPO or 1 ng/ml GM-CSF or 1 ng/ml IL-3. [l] This subclone termed HIMeg-1 was established from the parental line HIMeg. [m] A TPO-dependent subclone termed HU-3/TPO was established. [n] A subclone termed JURL-MK2 was established. [o] Two subclones KH88 B4D6 and KH88 C2F8 with slightly different immunophenotypical and cytogenetic features were established. [p] Sister cell lines KMOE-1 and KMOE-3N from the same patient were established from the bone marrow in suspension culture and by heterotransplantation of KMOE-1 cells into an athymic nude mouse, respectively. [q] Subclones LAMA-87 (with an erythroid-eosinophilic phenotype) and LAMA-88 (with an eosinophilic-monocytic phenotype) were established by heterotransplantation of LAMA-84 into athymic mice. [r] This subclone termed M-07e was established from the parental growth factor-independent line M-07 at passage 15; TPO-dependent subclone M-07e/TPO was established. [s] A TPO-dependent subclone M-MOK/TPO was established. [t] A slightly different subclone named MEG-01s was established. [u] Seven cell lines from a single sample from the same patient were established: MOLM-7 and MOLM-11 represent the megakaryocytic lineage; MOLM-6, –8, –9, –10, –12 represent the myelocytic lineage. [v] Originally termed MFD-1; a TPO-dependent subclone TF-1/TPO was established. [w] EPO- and TPO-dependent subclones UT-7/Epo and UT-7/TPO were established.

Table 2. Erythroid-megakaryocytic cell lines: immunophenotypic characterization

Cell line	T-/NK-cell marker[a]	B-cell marker	Myelomonocytic marker	Erythroid-megakaryocytic marker	Progenitor/activation marker	Adhesion marker	Ref
AP-217	CD2− CD3−	CD19− CD22−	CD15+ CD33+	CD36+ CD41a+ GlyA(+)	CD34− HLA-DR+	CD11a+ CD11b+	23
AS-E2	CD2− CD3−	CD10− CD19−	CD13− CD14− CD33−	CD36+ CD41− GlyA+	CD34− CD38+ CD71+ HLA-DR−	CD11b(+)	24
B1647	CD3− CD4− CD8− CD56− CD57−	CD19− CD20− CD23−	CD14− CD16− CD33+	CD41+ CD42b− GlyA+ vWF+	CD34− CD38+ HLA-DR+	CD11c− CD54+	25
CHRF-288-11	CD1− CD2− CD3− CD4− CD5− CD7− CD8− CD56−	CD10− CD19− CD20−	CD13+ CD14− CD33+	CD36+ CD41a+ GlyA− PPO+ vWF+	HLA-DR+		27
CMK[b]	CD1− CD2− CD3− CD4(+) CD7(+) CD8− CD56−	CD10− CD19− CD20− CD21− CD22−	CD13+ CD14(+) CD15− CD33+	CD36+ CD41a+ CD42b(+) GlyA+ PPO+ vWF+	CD34+ CD38− CD71+ HLA-DR+		28,30
CMS	CD1− CD2− CD3− CD4− CD5− CD7− CD8−	CD19−	CD13− CD33+	CD41+ CD42b(+) GlyA− PPO+	CD34+ CD38− HLA-DR+		31
CMY	CD3− CD4+ CD7+ CD8−	CD10− CD19− CD20−	CD13+ CD14− CD33+	CD41a+ GlyA− PPO+	CD34+		32
ELF-153	CD2− CD3− CD4+	CD10− CD19− CD20−	CD13+ CD14− CD15− CD33+	CD9+ CD31+ CD36− CD41+ CD42a− CD42b− CD61+ CD62− CD63+ CD107a+ CD107b+ GlyA− PPO+ vWF(+)	CD34+ CD38− HLA-DR+	CD11b− CD11c− CD51+	33
F-36P	CD4− CD5− CD8−	CD10− CD19−	CD13+ CD14− CD33+	CD41a+ CD42b− GlyA+ PPO(+)	CD34+		35
GRW	CD3− CD4− CD7+	CD10− CD19− sIg−	CD14− CD33+	CD41a+ CD42b(+) CD61+ CD62P− GlyA−	CD34+ CD38+ CD71+ HLA-DR+		36
HEL	CD1− CD2− CD3− CD4+ CD5− CD7− CD8− CD28− CD56− TCR$\alpha\beta$−	CD10− CD19− CD20(+) CD21(+) CD22− Ig−	CD13+ CD14− CD15+ CD33+	CD9+ CD36+ CD41a+ CD42a− CD42b(+) CD61+ CD62− GlyA+ PPO− vWF(+)	CD34− CD38− CD71+ HLA-DR(+)	CD11b+ CD44+	83,84
HIMeg-1			CD14− CD16+ CD33+	CD41a(+) CD41b− GlyA+ PPO+	CD34−	CD11c+	38
HML	CD2− CD3− CD4+ CD8−	CD19−	CD13+ CD14+ CD15− CD33+	CD36+ CD41b+ CD42b− GlyA+ vWF− PPO(+)	CD34− CD38− CD71+ HLA-DR+	CD11b(+)	39
HU-3	CD1a− CD2− CD3− CD4+ CD7− CD8−	CD10− CD19− CD21+ CD23−	CD13+ CD14+ CD15+ CD16− CD32+ CD33+ CD35+	CD31+ CD36+ CD41a+ CD41b− CD42a− CD42b− CD61+ GlyA+	CD34+ CD38+ CD71+ HLA-DR+	CD11a− CD11b+ CD11c− CD18− CD44+ CD49b− CD49d+ CD49e+ CD49f+ CD51− CD54+ CD58+	40,41
JK-1	CD2− CD3− CD4+ CD8−	CD10− CD19− CD20− CD21−	CD13+ CD14−	CD41+ CD42+ GlyA+	HLA-DR−	CD11b−	43,83
JURL-MK1	CD2− CD7− CD56−	CD19− CD24− CD40−	CD13+ CD33+	CD9− CD36+ CD41a+ CD42b− CD61+ CD62P− CD63+ GlyA+	CD30− CD34− CD69+ HLA-DR+	CD11a− CD11b− CD11c− CD43+ CD44− CD54− CD62L−	44

Continued on next page

Table 2. (continued)

Cell line	T-/NK-cell marker[a]	B-cell marker	Myelomonocytic marker	Erythroid-megakaryocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
K-562	CD1− CD2− CD3− CD4− CD5− CD7− CD8− CD28− CD56− CD57− TCR$\alpha\beta/\delta$−	CD10− CD19− CD20− CD21− CD22− CD80+ CD86−	CD13+ CD14− CD15+ CD33−	CD9+ CD36− CD41(+) CD42b− CD61(+) GlyA+ PPO− vWF−	CD34− CD38− CD71+ HLA-DR− TdT−	CD11b(+) CD44+ CD54+	28,83
KH184	CD2− CD4− CD5− CD7−	CD10− CD19− CD20−	CD13(+) CD14− CD33+	CD36(+) CD41a+ CD42b+ GlyA− PPO−	CD34− HLA-DR(+)		46
KH88	CD1− CD2− CD3− CD4− CD8− CD56−	CD10− CD19− CD20− CD21− CD22−	CD13− CD14− CD15− CD33−	CD36+ CD41b− CD42− CD61− GlyA− PPO+	CD34− CD38− CD71+ HLA-DR−	CD11b+	28,47
KMOE-2	CD3−	CD19− cy/sIg−	CD13+	GlyA+	TdT−		48,83
LAMA-84[c]	CD2− CD3− CD4− CD5− CD7+ CD8− CD56−	CD10− CD19− CD20+ CD21− CD22− CD23− CD24+ CD37+ CD40+	CD13+ CD14− CD15− CD16− CD17+ CD32+ CD33+ CD35+ CD65− CD66a− CD66b− CD68+	CD9− CD31+ CD41a+ CD41b+ CD42b− CD61+ CD63+ GlyA− PPO+ vWF−	CD34− CD69+ CD71+ HLA-DR+ TdT−	CD11a+ CD11b+ CD11c− CD43+ CD44+ CD54−	49,50,83,85
M-07e[d]	CD3−	CD19−	CD13+ CD14− CD15(+) CD33+	CD41a+ CD42b+ GlyA− PPO(+)	CD34(+) CD71+ HLA-DR−	CD44+	83,86
M-MOK	CD1− CD3− CD4− CD7− CD8−	CD10− CD19− CD20−	CD13+ CD14− CD33+	CD41b+ CD42b+ GlyA− PPO−	CD34+ HLA-DR−	CD11a+ CD11b− CD11c− CD18+ CD54+	53
MB-02	CD1a− CD2− CD3− CD4− CD7− CD8−	CD10− CD19− CD20− CD21+ CD23−	CD13+ CD14− CD15− CD16− CD32+ CD33+ CD35+	CD31+ CD36+ CD41a− CD41b− CD42a− CD42b− CD61+ GlyA+	CD34+ CD38+ CD45+ CD71+ HLA-DR+	CD11a+ CD11b+ CD11c− CD18+ CD44+ CD49b− CD49d+ CD49e+ CD49f CD51− CD54+ CD58+	54
MC3	CD3− CD7−	CD10− CD19+ CD20− cyIgM−	CD13+ CD14− CD33+	CD41a+ CD42b+	CD34+ CD38+ HLA-DR−	CD54+	55,87
MEG-01	CD1− CD2− CD3− CD4+ CD5− CD7− CD8− CD28− CD56− CD57(+)	CD10− CD19− CD20− CD21+ CD22−	CD13+ CD14− CD15+ CD16− CD33+ CD68−	CD9+ CD36+ CD41a+ CD42a− CD42b(+) CD61+ CD62P− GlyA− PPO+ vWF+	CD34− CD38− HLA-DR+ TdT−	CD11b+ CD44+	28,57,84
MEG-A2	CD1− CD2− CD3− CD4+ CD5− CD7+ CD8−	CD10− CD19− CD20−	CD13+ CD14− CD33+	CD41a+ CD42b− GlyA− PPO− vWF−	CD34+ HLA-DR+		58
MG-S	CD1a− CD2− CD3− CD4+ CD5− CD7+ CD8− CD56− CD57−	CD10− CD19− CD20− CD21− cyCD22+ CD24+	CD13+ CD14− CD15(+) CD16− CD33+	CD36+ CD41a+ CD42b− GlyA− PPO+	CD34+ CD38+ HLA-DR+ TdT+	CD11b+ CD11c−	59
MHH 225	CD2− CD3− CD4− CD5− CD7− CD8−	CD10− CD19− CD20− CD21−	CD13+ CD15+ CD33+ CD65+	CD41+ CD42b− CD62+ GlyA+ PPO+	CD34+ CD38− HLA-DR+ TdT−	CD11a− CD11b− CD11c− CD54−	60,61
MKPL-1	CD3− CD4− CD5− CD8−	CD10− CD19− CD20−	CD13+ CD33+	CD36+ CD41a+ CD42b− CD61+ GlyA− PPO− vWF−	CD30−		62
MOLM-1	CD1− CD2− CD3− CD4(+) CD5− CD7+ CD8− CD57−	CD10− CD19− CD20− sIg−	CD13+ CD14− CD15− CD33+	CD9+ CD41(+) CD42a− CD42b− CD61+ CD62(+) vWF−	CD34+ CD71+ HLA-DR+ TdT−	CD44+	63

Continued on next page

Table 2. (continued)

Cell line	T-/NK-cell marker[a]	B-cell marker	Myelomonocytic marker	Erythroid-megakaryocytic marker	Progenitor/activation marker	Adhesion marker	Ref.
MOLM-7	CD3− CD4+ CD7+ CD8−	CD10− CD19− CD20− CD24−	CD13+ CD14− CD15− CD33+	CD9+ CD41a+ CD42a− CD42b+ CD61+ CD62− vWF−	CD34+ CD71+ HLA-DR+	CD11a(+) CD44+	64,84
M-TAT				GlyA+			65
NS-Meg	CD3− CD4+ CD7+ CD56−	CD10+ CD19−	CD13+ CD14− CD33+	CD36+ CD41a+ CD42a− CD61+ CD62− GlyA+ PPO+	CD34+ HLA-DR+	CD11b+ CD18+	66
OCIM1	CD1− CD2− CD3− CD4− CD5− CD7− CD8−	CD10− CD19− sIg−	CD13+ CD15− CD33+	CD36+ CD41(+) CD42b− GlyA+ vWF−	CD34+ CD38− HLA-DR+ TdT−		67
OCIM2	CD1− CD2− CD3− CD4− CD5− CD7− CD8−	CD10− CD19− sIg−	CD13+ CD15− CD33+	CD36+ CD41+ CD42b− GlyA+ vWF+	CD34+ CD38− HLA-DR− TdT−		67
RM10	CD1a− CD2− CD3− CD4− CD8− CD57−	CD10+ CD19− sIg−	CD13+ CD14+ CD16− CD33+	CD41− CD42− GlyA+ vWF−	HLA-DR+	CD11b−	68
RS-1	CD2−	CD20−	CD13+ CD33+	CD41+ CD61+	CD34+	CD11b−	69
SAM-1	CD2− CD3− CD4− CD5− CD8− CD57−	CD19− CD20−	CD13− CD33−	CD41+ CD42b+ GlyA+ PPO+	CD34− CD38− HLA-DR−	CD11b−	70
SET-2	CD2− CD3− CD4+ CD5− CD7+	CD10− CD19− CD20−	CD13+ CD14− CD33+	CD9− CD36+ CD41+ CD42b− CD61+ CD62P− GlyA−	CD34+ CD38+ CD71+ CD95− HLA-DR+		71
T-33	CD3− CD4− CD5− CD8− CD57−	CD10− CD19−	CD13+ CD14+ CD15− CD33+	CD41a+ CD42b+ CD61+ GlyA− PPO+ vWF+	HLA-DR+		73
TF-1	CD3 CD4− CD5− CD7− CD8− CD57−	CD10− CD19−	CD13+ CD14− CD15− CD33+	CD41+ CD42a(+) CD42b+ CD61+ CD62− GlyA(+) PPO−	CD34+ CD38+ HLA-DR+	CD11a− CD11b+ CD11c+ CD18+ CD44+ CD54+ CD56− CD62L− CD106−	75,83
TS9;22	CD1a− CD2− CD3− CD4− CD5− CD7− CD8− CD28− CD56− CD57− TCR$\alpha\beta/\delta$−	CD10− CD19− CD20− CD21− CD22− CD24+ cy/sIg−	CD13+ CD14− CD15− CD16− CD33+	CD9+ CD36+ CD41a+ CD42b− CD61(+) GlyA−	CD34+ CD38+ CD71+ HLA-DR+ TdT−	CD11b−	76
UoC-M1	CD1a− CD2− CD3− CD4− CD5− CD7+ CD8− CD56−	CD10− CD19− CD20− CD24+, sIg−	CD13+ CD14− CD15− CD33+	CD41− CD42− CD61+ GlyA−	CD34+ CD38+ CD45+ HLA-DR+		77
UT-7	CD2− CD3−	CD10− CD19− CD20−	CD13+ CD14− CD15+ CD16+ CD33+	CD41a+ CD41b+ CD42b+ CD61+ GlyA+ PPO+	CD34+ HLA-DR+	CD11b+ CD44(+)	78
Y-1K	CD3− CD4+ CD8−	CD19− CD20−	CD13+ CD33+	CD41+ GlyA+	CD34+ HLA-DR+		82
YN-1	CD3− CD4− CD8−	CD19− CD20−	CD13+ CD33+	CD41− GlyA+	CD34− HLA-DR−		82
YS9;22	CD1a− CD2− CD3− CD4− CD5− CD7− CD8− CD28− CD56+ CD57−	CD10− CD19− CD20− CD21− CD22− CD24− sIg−	CD13+ CD14− CD15− CD16− CD33+	CD9+ CD36+ CD41a+ CD42b− CD61+ GlyA−	CD34+ CD38+ CD71+ HLA-DR− TdT−	CD11b−	76

[a] +, strong, definite protein expression (mostly more than 10% cells positive); (+), weak protein expression, qualitatively and quantitatively (<10% cells positive); −, no protein expression. [b] Part of the immunophenotype is that of CMK11-5 subclone. [c] Extensive immunophenotype in ref. [85]. [d] Extensive immunophenotype on parental cell line M-07 in ref. [51].

Table 3. Erythroid-megakaryocytic cell lines: cytokine-related characterization

Cell line	Cytokine receptor expression[a]	Cytokine production[a]	Proliferation response to cytokines[b]	Differentiation response to cytokines[b]	Dependency on cytokines[c]	Ref.
AP-217	RT-PCR: EPO-R+; protein: MPL+					23
AS-E2	EPO-R+, IL-2Rα−		EPO+		EPO	24
B1647	EPO-R+, IL-2Rα−, IL-2Rβ−, KIT(+), MPL+		TPO+			25
CHRF-288-11	RT-PCR: FGF-R1+, FGF-R2+, GM-CSFRα+, IFNαR+, IFNβR+, IL-1R+, IL-6R+, Kit+, M-CSFR+, TNFαR+; protein: IL-2Rα−, MPL+	RT-PCR: bFGF+, GM-CSF+, IFNα+, IL-1β+, IL-3+, IL-7+, IL-8+, IL-11+, SCF+, TGFβ+, TNFα+; ELISA: bFGF+ GM-CSF+, IFNα+, SCF+, TGFβ+, TNFα+				88
CMK	IL-2Rα+, IL-2Rβ−, MPL(+)	GM-CSF+, IL-1α+, IL-1β+, IL-6+, TGFβ+, TNFα+	GM-CSF+, IL-3+, TPO+			30
CMY			GM-CSF(+), IL-3(+), IL-6(+), TPO(+)			32
ELF-153	EPO-R−, GM-CSFRα+, gp130+, IL-6Rα+, Kit+, MPL+		GM-CSF+, IL-3+, IL-4+, IL-6(+), PIXY-321+, SCF+		GM-CSF	33,34
F-36P	GM-CSFRα+, IL-2Rα−		GM-CSF+, IFNγ+, IL-3+, IL-5+, PIXY-321+	EPO+	GM-CSF or IL-3	35,42
GRW	RT-PCR: G-CSFR+, IL-1Rα+, IL-2Rα+, IL-3Rα+, IL-4Rα+, IL-6Rα+, IL-7Rα+, IL-9R+, IL-11R+, Kit+, TNFαR+		SCF+	EPO+, GM-CSF+, IL-3+	SCF	36
HEL	RT-PCR: IL-2Rα(+), IL-2Rβ+, IL-2Rα+, IL-4Rα(+), IL-9Rα+ protein: EPO-R+, IL-2Rα−, IL-2Rβ+, IL-2Rγ+, Kit+, MPL+	PDGF+				16,86
HIMeg-1	MPL+		IL-6+			38
HML	gp130+, IL-6Rα−, Kit+		GM-CSF+, SCF+; inhibition: TGFβ+	SCF + IL-5 or SCF + EPO	GM-CSF	39
HU-3	IL-2Rα−	RT-PCR: GM-CSF+, IL-1β+, IL-6+, IL-7+, IL-10+, IL-13+, SCF+, TGFβ+, TNFα+	EPO+, GM-CSF+, IFNβ+, IFNγ+, IL-1α+, IL-3+, IL-4+, IL-5+, IL-6+, LIF+, NGF+, OSM+, PIXY-321+, SCF+, TNFα+, TNFβ+, TPO+	EPO+	GM-CSF, IL-3 or TPO	41, 42, 89
JK-1	EPO-R+, Kit+		EPO+			43
JURL-MK1	IL-2Rα+, IL-2Rβ−, Kit+		SCF(+)			44

Continued on next page

Table 3. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[a]	Proliferation response to cytokines[b]	Differentiation response to cytokines[b]	Dependency on cytokines[c]	Ref.
K-562	RT-PCR: IL-2Rα(+), IL-2Rβ(+), IL-2Rγ+, IL-4Rα(+), IL-9Rα+, IL-11Rα+ protein: EPO-R+, IL-2Rα−, IL-2Rβ(+), IL-2Rγ+, Kit(+)	PDGF+, TGFβ+				16, 86
M-07e	RT-PCR: IL-2Rα+, IL-2Rβ+, IL-2Rγ+, IL-11Rα+; protein: IL-2Rα−, IL-2Rβ+, IL-2Rγ+, MPL+		GM-CSF+, IFNα+, IFNβ+, IFNγ+, IL-2+, IL-3+, IL-4+, IL-6+, IL-9+, IL-15+, NGF+, PIXY-321+, SCF+, TNFα+, TPO+; inhibitory: TGFβ1+		GM-CSF, IL-3 or TPO	42, 52, 89
M-MOK	Kit+, MPL+		GM-CSF+, IFNβ+, IFNγ+, IL-3+, IL-4+, IL-9+, IL-13+, IL-15+, PIXY-321+, SCF+, TNFα+, TNFβ+, TPO+; inhibitory: TGFβ+		GM-CSF or TPO	42, 53, 89, 90
MB-02	IL-2Rα−	RT-PCR: GM-CSF+, IL-1β+, IL-7+, IL-13+, TGFβ+	EPO+, GM-CSF+, IL-1β+, IL-3+, PIXY-321+, SCF+, TNFα+, TNFβ+; inhibitory: IFNα+, IFNβ+, TGFβ+,	EPO+	GM-CSF	42, 54
MC3	RT-PCR: EPO-R+, MPL+		IL-1β+, IL-3+; inhibitory: TGFβ+			55
MEG-01	IL-2Rα−, IL-2Rβ−	RT-PCR: PDGF+; ELISA: β-TG+, PDGF+, PF-4+, TGFβ+				57
MEG-A2		RT-PCR: G-CSF+, GM-CSF+, IL-1α+, IL-1β+, IL-3+, IL-4+, IL-6+, M-CSF+, TPO+	EPO+, GM-CSF+, IL-3+	EPO(+), GM-CSF(+), IL-3(+), IL-6(+)		58
MG-S	IL-2Rα−, Kit+		GM-CSF+			59
MHH 225	IL-2Rα−		SCF(+); inhibitory: IFNα(+), TNFα(+)	IL-4+		60, 61
M-TAT	EPO-R+		EPO+, GM-CSF+, IL-3+, SCF+		EPO, GM-CSF or SCF	65
NS-Meg	Northern: EPO-R+		EPO+, GM-CSF+, IL-3+	EPO+		66
OCIM1	EPO-R+, Kit+	PDGF+				16,67
OCIM2	Kit+	PDGF+				16,67
RM10	EPO-R+, IL-2Rα−, Kit−					68
RS-1			IL-3+			69
SAM-1	EPO-R−, Kit−		IL-6+			70

Continued on next page

Table 3. (continued)

Cell line	Cytokine receptor expression[a]	Cytokine production[a]	Proliferation response to cytokines[b]	Differentiation response to cytokines[b]	Dependency on cytokines[c]	Ref.
SET-2	gp130+, IL-2Rα–, IL-6Rα+, Kit+, MPL+	IL-6(+)	GM-CSF(+), IL-3(+), IL-6(+)			71
T-33			EPO+			73
TF-1	RT-PCR: GM-CSFRβ+, IL-1R II+, IL-4Rα+, IL-11Rα+, TGFβR II+, EPO-R+, GM-CSFRα+, IL-3Rα+, IL-5Rα+, MPL(+) protein: GM-CSFRα+, IL-3Rα+, IL-5α+, Kit+		CNTF+, EPO+, GM-CSF+, IFNγ+, IL-1+, IL-3+, IL-4+, IL-5+, IL-6+, IL-13+, LIF+, NGF+, OSM+, PIXY-321+, SCF+, TNFα+, TNFβ+, TPO+ inhibitory: IFNα+, IFNβ+, TGFβ+		GM-CSF, IL-3 or TPO	42, 74, 75, 89
UT-7	EPO-R+, GM-CSFRα+	ELISA: β-TG(+), PF-4(+)	EPO+, G-CSF+, GM-CSF+, IFNβ+, IFNγ+, IL-3+, IL-4+, IL-5+, IL-6+, NGF+, PIXY-321+, SCF+; inhibitory: TGFβ+		GM-CSF, IL-3, EPO or TPO	42, 78–81, 89

[a] Receptor expression or cytokine production at the protein level (ELISA, McAb, RIA), unless otherwise indicated, e.g. at the mRNA level (by RT- PCR, Northern); +, strong, definite expression; (+), weak expression; –, no expression.

[b] Effects of cytokine exposure on proliferation or differentiation (–, no effect; +, positive effect).

[c] Following growth factor withdrawal, these cell lines will die by apoptosis.

established from patients with a refractory disease. Apart from tumors and pleural effusions, peripheral blood (n = 28) and bone marrow (n = 14) were the preferred sites from which specimens for cell culture were obtained.

While no authentication was provided for 28 cell lines, evidence for a derivation from the assumed patient was given in 21 instances, mostly in the form of a comparison of the patient's and cell line's cytogenetic profile, but also by comparing immunoprofiles and genotypic profiles (e.g. gene banding patterns in Southern blots). The media used were RPMI 1640 (n = 30), IMDM (n = 12), (α-MEM (n = 2), Dulbecco's MEM (n = 2), Fisher's (n = 1), McCoy's 5A (n = 1), and Ham's F12 (n = 1). Usually, the cultures require addition of 10–20% FBS. Cell lines said to require horse serum or human serum (CHRF-288-11, HU-3, JK-1, MB-02) could also be adapted to grow with FBS alone. Only two cell lines could be cultured under serum-free conditions (MHH 225, T-33) (Appendix 2, p. 286). Several cell lines are constitutively growth factor-dependent, undergoing apoptosis in the absence of the respective cytokines: AS-E2, ELF-153, F-36P, GRW, HU-3, M-07e, M-MOK, MB-02, M-TAT, TF-1, and UT-7 [89] (Table 3; Appendix 3). Most of these cell lines can be obtained from the original investigators or cell banks (CMK, F-36P, HEL, JK-1, K-562, KMOE-2, LAMA-84, M-07e, MEG-01, TF-1, UT-7) (Appendix 1, p. 284).

Subclones with features significantly different from those of their parental lines have been derived from several cell lines. The differences concern mainly immunological and cytogenetic characteristics (parental lines: JURL-MK1, KH88, LAMA-84, MEG-01), stage of differentiation and ability to differentiate along a certain maturation pathway (parental lines: CMK, LAMA-84), and responsiveness/dependence on various growth factors (parental lines: F-36P, HU-3, M-07e, M-MOK, TF-1, UT-7). Of particular interest are cell lines derived from different patient sites or at different stages of the disease (parental lines: KMOE-2, MOLM-7).

3. IMMUNOPHENOTYPE

The erythroid-megakaryocytic cell lines display characteristic immunoprofiles (Table 2). Apart from the occasional positivity for CD4 and CD7, which are also expressed by myeloid precursor cells [110], the cells are usually negative for classical T-/NK-cell markers (including CD1, CD2, CD3, CD4, CD5, CD7, CD8, CD56, CD57, TCR) and B-cell markers (including CD 10, CD 19, CD20, CD21, Ig). Most cell lines express the two pan-myeloid surface antigens CD 13 and CD33 and are generally negative for the granulocytic and monocytic markers CD 15 and CD 14, respectively.

Table 4. Erythroid-megakaryocytic cell lines: genetic characterization

Cell line	Karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene *rearrangements**	Ref.
AP-217	87, XXYY, −7, −9, der(5)t(5;?)(q31 or q32;?), t(9;22)(q34;q11), del(17)(p11), der(19)t(11;19)(q13;q13), der(21)t(21;?)(p13;?)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		23
AS-E2	67–82, XXYY, −2, −2, −4, −5, −10, −10, −13, −13, +14, −15, −15, −16, −16, −17, −18, −19, −19, −22, −22, +mar, del(1)(p32p36), del(3)(p21), del(3)(q21), der(4)t(1;4)(q21;p16)x2, add(8)(p23), add(8)(q24)x2, add(9)(q34), +del(9)(q11q22), add(10)(p14), add(11)(q23), i(11)(q10), add(15)(p12)			24
B1647	53, XY, +2, +5, +8, +13, −14, +19, +21, t(10;11)(p11;q21), +der(14)t(14;?)(p11;?)			25
CHRF-288-11	50, XY, 1p+, 6p−, +6q−, +8, −10, 12p+, −15, +17, +19p+			27
CMK	85-90<4n>XY, −X, −Y, −2, −3, +5, −6, −6, −8, +11, −15, −15, +16, −17, −19, +21, +22, +7-11mar, add(1)(p36), add(1)(q31), add(3)(q11), del(3)(p14)x2–3, add(5)(q11), add(5)(q13), dup(8)(q11q21), add(8)(q13–21), del(8)(q11), del(9)(p21)x2, add(9)(q11)x2, del(10)(q22q24), der(11;17)(q10;q10), der(11)dup(11)(p13p15)t(5;11)(q11;p15)x1–2, del(11)(q23), add(12)(p13)x2, add(17)(p1?), add(18)(q23)x2–3, add(19)(p13), der(20)t(1;20)(q2?5;q1?2)x2, add(22)(q13)		*P53* PM	30, 83
CMS	46, X, del(X)(q23), der(2)t(1;2)(q22–24;q35), add(5)(q15), der(7)t(7;8)(p22;q21), add(17)(p13), add(19)(q13)			31
CMY	del(17p)		*P53* PM	32
ELF-153	43, XY, −7, −14, −17, del(5)(q13q31), t(12;14)(p11.2;q11.2)			33
F-36P	43, Y, Xp+, −5, −7, −13, −16, −17, −19, −21, 2q−, 9p+, 10q+		*P16INK4A* DD; *P53* PM	35, 91

Continued on next page

Table 4. (continued)

Cell line	Karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements*	Ref.
GRW	44, XX, −2, −4, −5, −7, −18, −19, +2mar, add(4)(p14), add(7)(q36), +der(15)t(15;21)(q10;q10)x2			36
HEL	63(60–64)<3n>XXY, −2, −9, −10, −10, −11, −14, −16, −16, −17, −19, +20, +21, +r, der(2)t(2;7)(q14;q32), add(3)(p14/21.3), der(4)t(4;17)(q13.3;q12), add(5)(q12), der(6)t(1;6)(p31.3;p23), der(6)t(3;6)(p21;q21), der(7)t(2;7)(q14;q32)t(7;?18)(p14;q21), der(9)qdp(9)(p11p24)t(9;20)(p24;?q11)t(11;20)(q13;?q13), add(17)(p12), add(20)(q11), r(20)(p11q11), dup(21)(qter;q11), add(22)(p11); masked 5q− and 20q−		*P15INK4B* DD, *P16INK4A* DD	83, 91–94
HML	47, XY, +21, del(3)(q24q26.1)			39
HU-3	73(70-76)XXX, +7, +7, +8, +8, −17, −17, −22			41
JK-1	48(45–48)<2n>XY, +8, +22, t(1;7)(q10;q10), der(9)t(9;22)(q34;q11), der(22)t(9;22)(q34;q11)x2; carries two Ph	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b2-a2) fusion gene	*P16INK4A* DG	43, 83, 92, 94
JURL-MK1	39(33–43)<2n>XY, −4, −5, −9, −11, −12, −18, −19, +mar, der(9)t(9;22)(q34;q11), i(17), der(22)t(9;22)(q34;q11)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		44
K-562	61-68<3n>XX, −X, −3, +7, −13, −18, +3mar, del(9)(p11/13), der(14)t(14;?)(p11;?), der(17)t(17;?)(p11/13;?), der(?18)t(15;?18)(q21;?q12), del(X)(p22), 2 markers appear from FISH to have arisen from Ph	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene	*P15INK4B* DD, *P16INK4A* DD; *ABL* amplified	83, 91–94
KH184	44, X, −Y, −1, −2, −2, −6, −16, −17, −18, +19, −20, −20, −22, −22, +der(?Y)t(?Y;22)(p11;q11), t(1;8)(q11;q11), +der(2)t(2;?)(p23;?), +der(6)t(6;?)(p23;?), del(9)(q13q22), t(10;17)(p12;q11), t(11;19)(q23;q13.3), +der(17)t(17;?), +der(20)t(3;20)(q13;p13), +der(22)t(22;?)(p13;?)			46

Continued on next page

Table 4. (continued)

Cell line	Karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements*	Ref.
KH88	71, XY, +Y, +3, +5, +6, +8, −9, +10, −12, −14, −14, −15, −15, −17, −18, −20, +21, −22, +3mar, del(X)(q22q28), del(2)(p11.1), add(7)(p11.2), del(9)(p13), t(9;22)(q34;q11), i(11)(q10), der(17)t(2;17)(p13;p13)x2, add(18)(p11.2), +der(19)t(7;19)(q10;q10), +add(19)(p13)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		46
KMOE-2	82(80–88)<4n>XX, −X, −X, −10, −13, −14, −16, −18, −20, −22, +3mar, *der(1)t(1;?)(q21;?)x2*, *del(7)(p15)x2*, del(9)(p12), i(17q)			83
LAMA-84	73/74(69–77)<3n>XX-X, +1, −2, +5, +6, +8, +13, −14, +17, +17, −18, +22, +mar, del(7)(p15), der(9)t(9;22)(q34;q11)x2, i(11q), add(13)(q33), del(17)(p12), der(22)t(9;22)(q34;q11)x4; carries Ph (4 copies)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene	*P15INK4B* DD, *P16INK4A* DD; *P53* PM	49, 83, 93
M-07e	46(45–46)<2n>XX, t(11;21)(p11;p13), add(13)(p13), add(22)(p13)		*BCL2* G, *BCR* G, *IGL* G,	51, 83
M-MOK	46, XX, 2q+, 14p+		*IGH* G, *TCRB* G	53
MB-02	43–50, XY, −1, −3, −4, −10, −11, −12, −14, −14, +15, +16, +6mar, +der(1)t(1;2)(q42;q23), +del(3)(q21), +der(3)t(3;?)(p25;?)			54
MC3	55, XX, +6, +8, +11, +15, +19, +2mar, t(1;5)(q25;q15), t(9;22)(q34;q11)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene	*IGH* G, *IGK* G; *P53* R	55
MEG-01	54(53–56)<2n>XY, +6, +19, +19, +21, +3–4mar, t(1;15)(p13;p13), ?inv(3)(p25q26), i(4q), add(5)(p15), der(9)t(9;22)(q34;q11)x2, add(10)(p14), dup(13)(q13q33–34), add(14)(p11), der(22)t(9;22)(q34;q11)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b2-a2) fusion gene	*P15INK4B* DD, *P16INK4A* DD; *P53* PM	56, 83, 92, 94
MEG-A2	55(51–56)XY, +1, +6, +8, +11, +18, −20, +21, +21, t(1;6)(p13;q22), t(9;22)(p34;q11), del(17)(p11), +dic(19)t(8;19)(p22;q13), +dic(20)(8;20)(p22;q13), +der(22)t(9;22)(p34;q11)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		58

Continued on next page

Table 4. (continued)

Cell line	Karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements*	Ref.
MG-S	46, XY, −4, −12, −17, −17, +21, +2mar, +der(17)t(4;17)(4qter → 4q21::17p11 → 17qter), 7p+, 7q+, 13p+			59
MHH 225	del(7)(p13), t(9;21)(q10;q10), 11q+, 9p−, r(21), t(9;11)(p?;q23)	t(9;11)(p?;q23) → *MLL-AF9* fusion gene?		61
MKPL-1	92, +3mar			62
MOLM-1	72, X, +1, +2, +2, +2, +4, +4, +6, +8, +8, +10, +11, +12, +13, +13, +14, +15, +16, +19, +19, +20, +21, +21, +22q−, inv(3)(q21q26), +inv(9)t(9;22)(q34;q10), +der(17)t(17;?)(p1?1;?)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b2-a2) fusion gene; inv(3)(q21q26) → *EVI1* overexpression		63, 91, 95
NS-Meg	53, XY, +6, +7, +8, +9, +11, +14, +19, +21, t(9;22)(q34;q11), der(17)t(1;17)(p11;p13), +der(22)t(9;22)(q34;q11)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		66
RM10	44–212(63) XX, +4, −8, +9p−, −11, +12, +13, +14q+, +15, +16, +18q+, +18q+, +19, +20, +21, +22q−, +3mar, +i(11q), Ph+	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (M-bcr) fusion gene	*ABL* amplified	68
RS-1	46, XX, −16, +mar			69
SAM-1	t(9;22)(q34;11)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		70
SET-2	47–48, X, −5, −9, −14, −21, −22, +1-3r, +5-7mar, add(X)(p22), del(X)(p21), add(1)(p36), add(4)(p1?2), add(7)(q32), del(10)(q24), add(12)(p13), add(13)(q3?2), add(16)(q22), add(17)(q25), add(19)(p13)			71
SKH1	t(3;21)(q26;q22), t(9;22)(q34;q11)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene; t(3;21)(q26;q22) → *EVI1-AML1* fusion gene		72, 91
T-33	51, XX, 1p+, +4, +9, +9, +19, +Ph, t(9;22)(q34;q11)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		73

Table 4. (continued)

Cell line	Karyotype	Unique translocations (→ fusion genes)	Unique gene alterations, receptor gene rearrangements*	Ref.
TF-1	52–57<2n>XY/XXY, +3, +5, +6, +8, +12, +15, +19, +19, +20, +20, +3mar, der(1)?dup(1)(p21p31)t(1;8)(p36;q11), t(2;12)(q32;q14), t(3;12)(p13–14;p12–13), add(3)(q21), add(5)(q11–13), der(8)t(1;8)(p36;q11), der(12)t(3;12)(p13–14;p12–13)t(1;12)(q31–32;q24), add(14)(p12), iso(17)(q10), add(17)(q21), add(19)(q13), trp(19)(q12;q13.3), der(21)t(19;21)(q13.1;q22)dup(19)(q13.1q13.3)t(11;19)(q13;q13.3), der(22)t(19;22)(q11;p11)			75, 83
TS9;22	43, XY, −7, −9, −10, −13, −14, −15, add(1)(p22), add(4)(p15), t(9;22)(q34;q11), add(17)(p12), +3mar	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		76
UoC-M1	42, X, −Y, −7, −9, −16, −19, +21, der(9)t(Y;9)(q1?2;p22)t(9;19)(q1?2;p12 or q12), del(5)(q1?2q3?4), der(5)t(5;9)(p1?5;q1?3), dic(11)t(9;11;19), der(14;21)(q10;q10)del(14)(q1?3), der(17)t(7;17)(p14;p12), del(19)(p1?1q1?2), der(21)t(11;21)(q22;q22)dup(21)(q11q22), +der(21)t(16;21)(p11;p11), der(22)t(19;22)(p12 or q12;p1?1)	*MLL* amplification		77
UT-7	92(92–96)XXYY, −2, −2, −3, +6, −11, −11, −13, −13, −13, −14, −17, −17, −18, −18, −19, −20, −21, −21, +18mar, +der(2)t(2;5)(p11;q11)x2		*P16INK4A* DD; *P53* PM	78, 91
Y-1K	66(64–67)XX, t(9;22)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		82
YN-1	53, XY, +8, +8, +14, +14, +19, +21, +22q−, t(9;22)(q34;q11)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		82
YS9;22	46, X, −X, +19, der(1)t(1;3)(p32;p12), dic(3)(q26;p12), t(9;22)(q34;q11), add(16)(p13)	Ph+ t(9;22)(q34;q11) → *BCR-ABL* (b3-a2) fusion gene		76

* Receptor gene arrangements: D – deleted; G – germline; PM – point mutation; R – rearranged.

Table 5. Erythroid-megakaryocytic cell lines: functional characterization

Cell line	Doubling time	EBV status	Cytochemistry	Inducibility of differentiation	Heterotransplantability into mice	Special functional features	Ref.
AP-217	24–30 h			TPA → meg differentiation; hemin, retinoic acid → ery differentiation		β-, γ-globin mRNA+; cloning/colony formation in methylcellulose; PO: c-myc mRNA+; TF: GATA-1 mRNA+	23, 96
AS-E2	49 h	EBV−	ANBE−, Benzidine+, CAE−, MPO−, PAS(+)			γ-globin mRNA+; colony formation in methylcellulose; TF: GATA-1 mRNA+, GATA-2 mRNA+	24
B1647				hemin, hydroxyurea, TPA → ery differentiation		α-, β-globin mRNA+; γ-globin+; α-tubulin+; colony formation in agar	25
CHRF-288-11			MPO−	TPA → meg differentiation	into nude mice	α-granules+	27
CMK	40–50 h	EBV− HTLV-I− HIV−	ACP+, ALP−, ANAE+, MPO−, PAS+	TPA → meg differentiation		α-granules+; demarcation system+; colony formation in methylcellulose; PO: c-sis mRNA+; TF: GATA-1 mRNA+, GATA-2 mRNA+	28–30,83
CMS	42 h		ACP+, ALP−, ANAE+, MPO−, PAS+	TPA → multinucleated cells		colony formation	31
CMY	46 h		ACP+, ALP−, ANBE+ (NaF resistant), MPO−, PAS+	cytokines → meg differentiation		colony formation in methylcellulose; α-granules+	32
ELF-153	36 h		Benzidine−		into nude mice	ϵ-, γ-globin mRNA−; demarcation system+; cloning/colony formation in methylcellulose; TF: GATA-1 mRNA+, GATA-2 mRNA+	33, 34
F-36P			ANAE−, ANBE−, MPO−, PAS+	EPO → ery differentiation			35
GRW	91 (40–120) h		ACP+, ANAE+, PAS+, SBB−	EPO, GM-CSF, IL-3, SCF → ery, meg or baso differentiation			36
HEL	24–36 h	EBV− HTLV-I− HIV−	ACP+, ANAE+, ANBE+, Benzidine−, CAE−, MPO−, PAS+, SBB−	hemin → ery differentiation; TPA → meg differentiation		α-, β-, γ-, ϵ-, ζ-globin+; δ-aminolevulin synthase mRNA+, Hb Bart's+; clonable in methylcellulose; TF: GATA-1 mRNA+, GATA-2 mRNA+, SCL mRNA+	68, 82, 83, 97
HIMeg-1				TPA → mono differentiation; leukocyte conditioned medium, retinoic acid, vit. D3 → meg differentiation			38
HML			ACP+, ALP−, ANBE+, CAE−, MPO−, PAS+, SBB−	TPA → meg differentiation; SCF + IL-5 + retinoic acid → eosino differentiation; SCF + EPO → ery differentiation		eosino POX−, major basic protein(+), Biebrich scarlet−, Luxol fast blue−, toluidine blue−, PF4−, β-TG−	39

Continued on next page

Table 5. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Inducibility of differentiation	Heterotransplant-ability into mice	Special functional features	Ref.
HU-3				TPA → meg differentiation; EPO → ery differentiation		clonable; TF: NF-E2+	41
JK-1	48 h	EBV− HTLV-I− HIV−	ACP+, ANBE−, Benzidine+, CAE−, MPO−, PAS+	spontaneous ery differentiation; δ-ALA → ery differentiation; (ARA-C, DMSO, hemin, Na butyrate no effect)		β-, δ-globin mRNA+; HbF+; (red) colony formation in methylcellulose	43, 68, 83
JURL-MK1	48 h		ALP−, ANAE(+), MPO−, PAS+	TPA → meg differentiation; (ARA-C, ATRA, DMSO, hemin no effect)			44
K-562	24–30 h	EBV− HTLV-I− HIV−	ALP−, ANBE−, Benzidine+, MPO−, PAS+	TPA → meg differentiation; hemin → ery differentiation	into nude or SCID mice	α-, γ-, ε-, ζ-globin mRNA+; δ-aminolevulin synthase mRNA+; Hb Bart's+, Hb Portland+, Hb Gower1+, Hb Gower II+, HbF+; colony formation in agar; no phagocytosis; target for NK assay; no α-granules, no demarcation system, TF: GATA-1 mRNA+, GATA-2 mRNA+, SCL mRNA+	45, 68, 82, 83, 97
KH184	36–60 h		ACP+, ANAE−, CAE−, MPO−, PAS−	(TPA no effect)		globin mRNA−; no α-granules, no demarcation system	46
KH88	20–26 h		ACP+, ANAE+, Ferritin−, Lactoferrin−, MPO−	TPA → meg differentiation; hemin → ery differentiation; (DMSO, retinoic acid no effect)		HbA+, HbF+	47
KMOE-2	24 h	EBV− HTLV-I− HIV−	ANAE−, Benzidine−, MPO−, PAS+, SBB−	Na butyrate → ery differentiation	into nude mice	α-, β-, γ-, δ-globin mRNA+; HbA+; colony formation in agar	48, 68, 83, 99
LAMA-84	30 h	EBV− HTLV-I− HIV−	ACP+, CAE+, Lactoferrin−, Luxol−, MPO−, PAS+, SBB(+), Vimentin+	hemin → ery differentiation; DMSO, Na butyrate, TPA → meg differentiation; (retinoic acid, vit. D3 no effect)	into estrone-treated nude mice	α-, β-, γ-globin mRNA+; colony formation in agar; no α-granules, no demarcation system; no NBT reduction, no phagocytosis, no lysozyme	49, 50, 83, 85
M-07e	32–50 h	EBV− HTLV-I− HIV−	ACP+, ANAE+, ANBE−, CAE−, MPO−, PAS−		into SCID mice	cloning/colony formation in methylcellulose; spontaneous production of platelet-like particles; demarcation system+	51, 52, 83, 98
M-MOK		EBV−	ANBE−, CAE−, MPO−, PAS−, SBB−	TPA → meg differentiation; (ATRA not effective)		TF: GATA-1 mRNA−, GATA-2 mRNA+, GATA-3 mRNA+	53

Continued on next page

Table 5. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Inducibility of differentiation	Heterotransplantability into mice	Special functional features	Ref.
MB-02		EBNA−	ACP+, ANAE−, Benzidine−, CAE−, MPO−, PAS+	TPA → meg differentiation; DMSO, EPO → ery differentiation		β-, γ-, ε-globin mRNA+, ζ-globin mRNA-; HbF+; not clonable; colony formation in methylcellulose or agar; no phagocytosis; TF: NF-E2+	54
MC3			ACP+, ANBE−, CAE−, MPO−, PAS−, SBB−	TPA → meg differentiation		TF: GATA-2 mRNA+	87
MEG-01	36–48 h	EBV− HTLV-I− HIV−	ACP+, ALP−, ANAE+, ANBE−, CAE−, MPO−, PAS(+)	TPA → meg differentiation		no α-granules, no demarcation system; no lysozyme, no phagocytosis	28, 56, 57, 83, 84
MEG-A2	26–30 h	EBV−	ACP+, ALP−, ANAE−, ANBE+, CAE−, MPO−, PAS+	cytokines, TPA → meg differentiation; (DMSO, hemin, retinoic acid no effect)		no α-granules, demarcation system+	58
MG-S	36 h		ACP+, ANBE−, CAE−, MPO−, PAS+	TPA → meg differentiation		no α-granules, no demarcation system	59
MHH 225	38–48 h		ACP(+), ANAE−, ANBE−, PAS+, SBB−	IL-4 → myeloid differentiation		SCF protects against apoptosis	60, 61
MKPL-1	30 h	EBNA−	ACP+, ALP−, ANAE+, ANBE+, CAE−, MPO−, PAS+		into nude mice	α-granules+, demarcation system+	62
MOLM-1	43 h		MPO−	TPA → meg differentiation		no NBT reduction, no phagocytosis	63
MOLM-7	24–60 h		MPO−	TPA → meg differentiation			64, 84
M-TAT			Benzidine+	EPO → ery differentiation		γ-globin mRNA+, δ-aminolevulin synthase mRNA+; TF: GATA-1+, GATA-2+	65
NS-Meg			ACP+, ANAE+, ANBE−, Benzidine−, MPO−, PAS+, SBB−	cytokines, TPA → meg differentiation; EPO → ery differentiation; (δ-ALA, DMSO, hemin, Na butyrate no effect)		α-, γ-globin mRNA+; induction of HbF; α-granules+, demarcation system+; TF: GATA-1 mRNA+; spontaneous production of platelet-like particles	66
OCIM1			ANAE+, Benzidine+, CAE−, MPO−, PAS+	δ-ALA → ery differentiation; TPA → macro/meg differentiation		α-, γ-, ε-globin+; induction of Hb Bart's, HbF; cloning/(red) colony formation in methylcellulose	67, 68
OCIM2			ANAE+, Benzidine+, CAE−, MPO−, PAS+	δ-ALA → ery differentiation; TPA → macro/meg differentiation	into SCID mice	α-, γ-, δ-, ε-, ζ-globin+; induction of Hb Bart's, HbF, Hb Portland; cloning/(red) colony formation in methylcellulose	67, 68, 98

Continued on next page

Table 5. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Inducibility of differentiation	Heterotransplantability into mice	Special functional features	Ref.
RM10	18–20 h	EBNA− HTLV-I−	ACP−, ANBE−, Benzidine+, CAE−, MPO−, PAS+, SBB−	AraC, hemin → ery differentiation; TPA → mono differentiation; (δ-ALA, DMSO, Na butyrate, retinoic acid, vitamin D3 no effect)		α-, γ-, ε-, ζ-globin mRNA+; Hb Bart's+, Hb Portland+, Hb Gower1+; no phagocytosis	68
RS-1	72 h		ANAE−, CAE−, MPO−	retinoic acid, TPA inhibit proliferation		colony formation in methylcellulose (with IL-3); emperipolesis+	69
SAM-1				TPA → differentiation		γ-globin mRNA+; TF: GATA-1 mRNA+, GATA-2 mRNA+, GATA-3 mRNA−	70
SET-2	36 h	EBV−	ANBE+, CAE−, MPO−, PAS(+)	TPA → meg differentiation		spontaneous production of platelet-like particles; α-granules+; β-TG+, PF-4+	71
T-33	24–36 h		ACP+, ALP+, ANAE+, ANBE(+), CAE−, GLC+, MPO−, PAS+, SBB+	TPA → meg differentiation		α-granules+, demarcation system+; HbA−	73
TF-1	70 h	EBV− HTLV-I− HIV−	ACP−, ANAE−, CAE−, Fe−, MPO−, PAS+	δ-ALA, hemin → ery differentiation; TPA → macro/meg differentiation; (DMSO, retinoic acid, vit. D3 no effect)	into SCID mice	α-, β-, γ-globin mRNA+; induction of HbF, HbA; cloning/colony formation in agar/methylcellulose	74, 75, 83
TS9;22						PO: EVI1 mRNA−; TF: GATA-1 mRNA+, GATA-2 mRNA+, SCL mRNA+	76, 97
UoC-M1			ACP+, ANAE+ (NaF inhibitable), ANBE−, MPO−			no demarcation system	77
UT-7	36–48 h	EBV−	ACP+, ALP−, ANAE+, ANBE+, CAE−, MPO−, PAS(+), SBB−	TPA → meg differentiation		α-granules+, no demarcation system; clonable; PO: c-myb mRNA+; TF: GATA-1 mRNA+, GATA-2 mRNA+	78
Y-1K	62 h		ALP+, ANBE+, Benzidine−, CAE−, MPO−, PAS+	(hemin, EPO no effect)		γ-globin mRNA+; δ-aminolevulin synthase mRNA+; TF: GATA-1 mRNA+	82
YN-1	20–24 h		ALP−, ANBE−, Benzidine+, CAE−, MPO−, PAS+	hemin, Ara C → ery differentiation (EPO no effect)		γ-globin mRNA+; δ-aminolevulin synthase mRNA+; Hb Bart's+, Hb Portland+, HbF+, HbA(+); TF: GATA-1 mRNA+	82
YS9;22						PO: EVI1 mRNA+; TF: GATA-1 mRNA+, GATA-2 mRNA+, SCL mRNA+	76, 97

PO – (proto)-oncogenes; TF – transcription factors.

Table 6. Erythroid-megakaryocytic cell lines: unconfirmed cell lines (not immortalized, not characterized, not verified, other)

Cell line	Patient	Features	Ref.	Remarks*
AML-HJ	from BM of AML M7	CD9(+) CD33+ CD34+ CD36(+) CD41(+) CD42(+) CD61(+) GlyA(+) HLA-DR+	100	insufficiently characterized
B-403	from PB of AML	CD41a+ CD42+ CD71+ HLA-DR+; ANAE+ EBNA− MPO− PAS+; produces PDGF; polyploid	101	probably EBV+ B-LCL
DAMI	from PB of 57 M with AML M7 in 1986	extensive immunological and cytogenetic characterization (e.g. CD41a+ CD42b+ GlyA+)	102	cross-contaminated: in reality cell line HEL [103]
EST-IU	from BM of secondary AML		104, 105	not immortalized cell line
KG-91	from PB of AML	CD34+ CD71+ GlyA+; HbF+, Benzidine+; γ-, α-, ζ-globin+; AraC, hemin, Na butyrate $\rightarrow$ ery differentiation	106	insufficiently characterized
KOPMK 53	from CML-my BC	ANAE− MPO−; CD13+ CD33+ CD41+	107	insufficiently characterized
MEG-J	from PB of 24 M with CML-BC	CD41+ CD42+ HLA-DR− PPO+; IL-11 mRNA+; responsive to IL-11	108	insufficiently characterized
P-320	from PB of AML M7	CD41a+ CD42+ CD71+ HLA-DR+; ANAE+ EBNA− MPO− PAS+; produces PDGF; polyploid	101	probably EBV+ B-LCL
S-1214	from PB of ALL	CD41a+ CD42+ CD71+ HLA-DR+; ANAE+ EBNA− MPO− PAS+; produces PDGF; polyploid	101	probably EBV+ B-LCL
TK91	from CML-BC	CD13+ CD33+ CD34+ CD36− CD41(+) GlyA− HLA-DR−	28	insufficiently characterized
TW14-8	from PB of 25 M with AML M6 (with Rothmund-Thomson syndrome)	CD13+ CD33+ CD34+ HLA-DR+; responsive to IL-5	109	insufficiently characterized

* For most cell lines, the insufficient characterization concerns the description of essential features, including clinical data, authentication, immunoprofile and/or cytogenetics.

A unique erythroid lineage-specific marker is GlyA which was detected on many cell lines (25 positive, 19 negative cell lines). The panel of megakaryocytic surface proteins is considerably larger and includes CD31, CD36, CD41, CD42 and CD61. These latter antigens are different types of the so-called platelet glycoproteins. As expected, all cell lines express at least one of these markers. The "von Willebrand Factor" antigen is also associated with cells committed to megakaryocytic differentiation, but was detected on only a few cell lines (on 9/18 tested, 50%). Another informative megakaryocyte-specific marker is the platelet peroxidase (PPO) which requires electron microscopic detection with specific antibodies: 18 (75%) cell lines are positive, 6 cell lines are negative. In light of the close relationship of immature erythroid and megakaryocytic cells with presumably a common immunologically and functionally definable precursor, it is not surprising that 8/25 (32%) cell lines tested for both markers are GlyA+ PPO+.

Expression of the progenitor cell marker CD34 (27/41 [65%] cell lines are positive) confirms the immaturity of the cell lines. This notion is underlined by the detection of HLA-DR on 30/41 (73%) cell lines. Finally, most cell lines examined express one or more surface adhesion molecules.

A typical immunoprofile of an erythroid-megakaryocytic cell line is: T-antigen negative, B-antigen negative, CD13+, CD14–, CD15–, CD33+, CD34+, CD41+, CD42+, CD61+, GlyA+, HLA-DR+, PPO+.

4. CYTOKINE-RELATED CHARACTERIZATION

Two cytokines uniquely associated with the erythroid and megakaryocytic cell lineages promote survival, proliferation and differentiation at various stages of maturation: EPO and TPO [18,20,111,112]. Appropriately, several cell lines express the receptors for EPO and TPO, the latter also known as MPL [20,113] (Table 3). With regard to cytokine production, PDGF mRNA expression and protein production is of particular interest and seen in several cell lines [16]. As most cell lines grow autonomously in culture, without the addition of external growth factors apart from those present in the FBS, it is no surprise that the majority of the cell lines do not respond to cytokine exposure, either with cellular proliferation or differentiation.

An exception to the above rule are the so-called growth factor-dependent leukemia cell lines (Appendix 3, p. 287). These cell lines will die by apoptosis within 1–3 days in the absence of specific growth factors, although FBS can delay this factor withdrawal-induced cell death. Twenty-two percent of the erythroid-megakaryocytic cell lines are constitutively growth factor-dependent, mainly on GM-CSF or IL-3. The cytokine dependency of some of these cell lines could be switched to TPO [42,80,89]. In addition, these

factor-dependent cell lines are proliferatively responsive to a large panel of other cytokines [89]. The differentiation-inducing effects of cytokines on erythroid-megakaryocytic cell lines are rather limited, reflecting their primary action in the enhancement of survival and proliferation.

5. GENETIC CHARACTERIZATION

Detailed cytogenetic karyotypes are available for 44 of the 49 erythroid-megakaryocytic cell lines (Table 4). As already seen in other leukemia-lymphoma cell lines, there are complex numerical and structural chromosomal aberrations. Among the many abnormalities, the occurrence of the so-called Philadelphia chromosome stands out. The majority of the erythroid-megakaryocytic cell lines have been established from patients with CML, a disease which is highly associated (>90%) with the unique translocation (9;22). This translocation and the resulting *BCR-ABL* fusion gene are seen in many cell lines [114–116] (Appendix 4). The t(3;21) and the inv(3) alterations causing the *EVI1-AML1* fusion gene and *EVI1* overexpression have been described for cell lines MOLM-1 and SKH1, respectively. Amplifications of the *MLL* and *ABL* genes in the cell lines UoC-M1 and K-562, respectively, are of further note.

Homozygous or heterozygous deletions of the tumor suppressor genes *P15INK4B* and *P16INK4A*, both mapped to chromosome region 9p21 [117,118], have been found in several cell lines (including F-36P, HEL, JK-1, K-562, LAMA-84, MEG-01, and UT-7). Their functional homologue genes *P18INK4C* and *P19INK4D* were not deleted or otherwise altered in any of the cell lines. Point mutations or other alterations of the *P53* tumor suppressor gene [119] were reported for cell lines CMK, CMY, F-36P, LAMA-84, MC3, MEG-01, and UT-7.

6. FUNCTIONAL CHARACTERIZATION

The doubling times of the cell lines listed in Table 5 are mostly in the range of 1–2 days. None of the cell lines tested is positive for EBV. In cytochemical staining, these cell lines showed the following results: 22/24 (91%) ACP+, 2/13 (15%) ALP+, 16/24 (66%) ANAE+, 9/23 (39%) ANBE+, 8/14 (57%) benzidine+, 1/24 (4%) CAE+, 0/37 MPO+, 29/33 (87%) PAS+, and 2/12 (16%) SBB+. Thus, the typical cytochemical profile of an erythroid-megakaryocyte cell line is ACP+ PAS+ ANAE± ANBE± benzidine± ALP– CAE– MPO– SBB–.

The majority of the cell lines have been subjected to induction of differentiation using pharmacological (AraC, DMSO, sodium butyrate, TPA) or physiological reagents (δ-ALA, hemin, retinoic acid, vitamin D3). Depending on the inducer used and the stage of differentiation at which the cells are arrested, the cells can be triggered to differentiate along the megakaryocytic, erythroid, or monocytic/macrophage cell axes. AraC, δ-ALA and hemin induce erythroid differentiation, whereas DMSO, sodium butyrate and retinoic acid promote either erythroid or megakaryocytic differentiation, presumably depending on the stringency of commitment to one cell lineage or the other. Besides vitamin D3, TPA is the most effective inducer of megakaryocytic differentiation. However, some TPA-treated cell lines can acquire monocytic/macrophage-associated features [120]. Bipotency with regard to induced differentiation along the erythroid or megakaryocytic lineages was reported for 16 cell lines (Table 5).

Nine cell lines have been reported to be heterotransplantable in nude or SCID mice (Appendix 5, p. 289). Specific functional features of erythroid cells are the expression of hemoglobin (detectable at the protein level by benzidine staining or isoelectric focusing) and of the various globin chains (at the mRNA or protein level). Of the various globin chains, the embryonic chains predominate, including Hb Gower I/II, Hb Bart's, Hb Portland, and fetal hemoglobin HbF. Rare cell lines express adult HbA. The various types of hemoglobin are composed of heme and different globin chains, i.e. α-, β-, γ-, δ-, ϵ- and ζ-globin. Specific morphological features of megakaryocytes are α-granules and demarcation membranes. Of the cell lines that were examined for these two specific parameters, 11/17 (64%) lines showed α-granules and/or demarcation membranes and 19/21 (90%) lines expressed hemoglobin or globin (Table 5). Finally, several cell lines express the GATA-1, GATA-2 and SCL transcription factors that are associated with both cell lineages.

7. UNCONFIRMED CELL LINES

Several cell lines with erythroid-megakaryocytic characteristics not listed in Table 1 have been reported in the literature (Table 6). However, these cell lines are either not sufficiently characterized, not immortalized (EST-IU), not of leukemic origin (B-403, P-320, S-1214), or are cross-contaminated (DAMI). For the insufficiently characterized cell lines (AML-HJ, KG-91, KOPMK 53, MEG-J, TK-91, TW14-8), essential descriptive parameters such as clinical data, authentication, immunoprofile, and karyotype have yet to be published.

8. APPENDICES

Appendix 1. Myeloid cell lines available from major cell banks

Cell line	Cell type	Cell bank[1]	Catalogue no.
AML-193	monocytic	ATCC	CRL 9589
CMK	erythroid-megakaryocytic	DSMZ	ACC 392
		IFO	IFO 50428/30
CTV-1	monocytic	DSMZ	ACC 40
EM-2	myelocytic	DSMZ	ACC 135
EM-3	myelocytic	DSMZ	ACC 134
EOL-1	myelocytic (eosinophil)	DSMZ	ACC 386
		ECACC	ECACC 94042252
		RIKEN	RCB0641
F-36E	erythroid-megakaryocytic	RIKEN	RCB 0776
F-36P	erythroid-megakaryocytic	RIKEN	RCB 0775
GDM-1	myelocytic	DSMZ	ACC 87
HEL	erythroid-megakaryocytic	ATCC	TIB 180
		DSMZ	ACC 11
		ECACC	ECACC 92111706
		JCRB	JCRB 0062
HL-60	myelocytic	ATCC	CCL 240
		DSMZ	ACC 3
		ECACC	ECACC 88120805
		IFO	IFO 50022
		JCRB	JCRB 0085
		RIKEN	RCB 0041
HNT-34	myelocytic	RIKEN	RCB 1296
JK-1	erythroid-megakaryocytic	DSMZ	ACC 347
JOSK-I	monocytic	DSMZ	ACC 155
JOSK-M	monocytic	DSMZ	ACC 30
K-562	erythroid-megakaryocytic	ATCC	CCL 243
		DSMZ	ACC 10
		JCRB	JCRB 0019
		RIKEN	RCB 0027
Kasumi-1	myelocytic	DSMZ	ACC 220
KG-1	myelocytic	ATCC	CRL 8031/CCL 246
		DSMZ	ACC 14
		JCRB	JCRB 0611
		RIKEN	RCB 1166
KG-la	myelocytic	ATCC	CCL 246.1
		DSMZ	ACC 421

Continued on next page

Appendix 1. (continued)

Cell line	Cell type	Cell bank[1]	Catalogue no.
KMOE-2	erythroid-megakaryocytic	DSMZ	ACC 37
KMT-2	monocytic	RIKEN	RCB 0712
KU-812	myelocytic (basophil)	DSMZ	ACC 378
		ECACC	ECACC 90071807
		IFO	IFO 50363
		JCRB	JCRB 0104
		RIKEN	RCB 0495
KU-812E	myelocytic (basophil)	ECACC	ECACC 90071803
		JCRB	JCRB 0104.1
		RIKEN	RCB 0496
KU-812F	myelocytic (basophil)	ECACC	ECACC 90071804
		JCRB	ICRB 0104.2
		RIKEN	RCB 0497
KY821	myelocytic	JCRB	JCRB 0105
LAMA-84	erythroid-megakaryocytic	DSMZ	ACC 168
M-07e	erythroid-megakaryocytic	DSMZ	ACC 104
MEG-01	erythroid-megakaryocytic	ATCC	CRL 2021
			ACC 364
IFO		DSMZ	IFO 50151
ML-2	monocytic	DSMZ	ACC 15
Mono Mac 1	monocytic	DSMZ	ACC 252
Mono Mac 6	monocytic	DSMZ	ACC 124
MUTZ-2	myelocytic	DSMZ	ACC 271
MUTZ-3	monocytic	DSMZ	ACC 295
MV4-11	monocytic	ATCC	ATCC 9591
		DSMZ	ACC 102
NB4	myelocytic	DSMZ	ACC 207
OCI/AML-2	monocytic	DSMZ	ACC 99
OCI/AML-5	myelocytic	DSMZ	ACC 247
P31/Fujioka	monocytic	JCRB	JCRB 0091
P39/Tsugane	monocytic	JCRB	JCRB 0092
PLB-985	monocytic	DSMZ	ACC 139
RC-2A	monocytic	DSMZ	ACC 6
SCC-3	monocytic	JCRB	JCRB 0115
SKM-1	monocytic	JCRB	JCRB 0118
SPI-801	erythroid-megakaryocytic	DSMZ	ACC 86
SPI-802	erythroid-megakaryocytic	DSMZ	ACC 92

Continued on next page

Appendix 1. (continued)

Cell line	Cell type	Cell bank[1]	Catalogue no.
TF-1	erythroid-megakaryocytic	ATCC	CRL 2003
		DSMZ	ACC 334
THP-1	monocytic	ATCC	TIB 202
		DSMZ	ACC 16
		JCRB	JCRB 0112
		RIKEN	RCB 1189
TMM	EBV+ B-LCL	DSMZ	ACC 95
U-937	monocytic	ATCC	CRL 1593
		DSMZ	ACC 5
		IFO	IFO 50038
		JCRB	JCRB 9021
		RIKEN	RCB 0435
UT-7	erythroid-megakaryoctic	DSMZ	ACC 137
YNH-1	myelocytic	RIKEN	RCB 1291

[1] ATCC – American Type Culture Collection; DSMZ – German Collection of Microorganisms and Cell Cultures; ECACC – European Collection of Animal Cell Cultures; IFO – Institute for Fermentation Osaka (now = HSRRB-Health Science Research Resources Bank; JCRB – Japanese Collection of Research Bioresources; RIKEN – Cell Bank.

Appendix 2. Myeloid cell lines growing in serum-free media

Cell line	Cell type	Culture medium
AML-193	monocytic	serum-free: IMDM + 2 ng/ml GM-CSF or 3 U/ml IL-3
GF-D8	myelocytic	serum-free: RPMI 1640 + 50 ng/ml GM-CSF or + 20% FBS]
MHH 225	erythroid-megakaryocytic	serum-free: RPMI 1640 [or + 10% FBS]
KU-812-F	myelocytic	serum-free: RPMI 1640 [or + 10% FBS]
MV4-11	monocytic	serum-free: IMDM + 5 ng/ml GM-CSF [or + 10% FBS without GM-CSF]
NKM-1	myelocytic	serum-free: RPMI 1640 [or + 10% FBS]
NOMO-1	monocytic	serum-free: RPMI 1640 [or + 10% FBS]
PLB-985	monocytic	serum-free: RPMI 1640 [or + 10% FBS]
T-33	erythroid-megakaryocytic	serum-free: RPMI 1640 [or + 10% FBS]

Appendix 3. Growth factor-dependent myeloid cell lines

Cell line	Cell type	Absolute dependency on cytokines
AML-193	monocytic	GM-CSF or IL-3
AS-E2	erythroid-megakaryocytic	EPO
ELF-153	erythroid-megakaryocytic	GM-CSF
F-36P	erythroid-megakaryocytic	EPO, GM-CSF or IL-3
FKH-1	myelocytic	G-CSF or GM-CSF
GF-D8	myelocytic	GM-CSF or IL-3
GM/SO	myelocytic	GM-CSF
GRW	erythroid-megakaryocytic	SCF
HML	erythroid-megakaryocytic	GM-CSF
HU-3	erythroid-megakaryocytic	GM-CSF, IL-3 or TPO
IRTA17	myelocytic	G-CSF, GM-CSF or SCF
M-07e	erythroid-megakaryocytic	GM-CSF, IL-3 or TPO
M-MOK	erythroid-megakaryocytic	GM-CSF or TPO
MB-02	erythroid-megakaryocytic	GM-CSF
MDS92	myelocytic	IL-3
M-TAT	erythroid-megakaryocytic	EPO, GM-CSF or SCF
MUTZ-2	myelocytic	5637 CM or SCF
MUTZ-3	monocytic	5637 CM, GM-CSF, IL-3 or PIXY-321
OCI/AML-1	myelocytic	5637 CM or G-CSF
OCI/AML-4	myelocytic	grows better with GM-CSF, IL-3 or SCF
OCI/AML-5	myelocytic	5637 CM or GM-CSF or IL-3
OHN-GM	myelocytic	GM-CSF
OIH-1	myelocytic	G-CSF, GM-CSF
SKNO-1	myelocytic	GM-CSF
TF-1	erythroid-megakaryocytic	GM-CSF, IL-3 or TPO
UCSD/AML1	myelocytic	GM-CSF
UG3	monocytic	GM-CSF or IL-3
UT-7	erythroid-megakaryocytic	EPO, GM-CSF, IL-3 or TPO
YNH-1	myelocytic	G-CSF, GM-CSF or IL-3

Appendix 4. Myeloid cell lines with translocations and fusion genes

Chromosomal abnormality	Fusion genes	Cell line	Cell type	Remarks
t(1;12)(q25;p13)	*ETV6-ARG*	HT93	myelocytic	
t(3;3)(q21;q26)	*EVI1*	HNT-34	myelocytic	EVI1 overexpression
		UCSD/AML1	myelocytic	EVI1 overexpression
inv(3)(q21q26)	*EVI1*	Kasumi-4	myelocytic	EVI1 overexpression
		MOLM-1	erythroid-megakaryocytic	EVI1 overexpression
		MUTZ-3	monocytic	
t(3;7)(q27;q22)	*EVI1*	Kasumi-3	myelocytic	EVI1 overexpression
t(3;21)(q26;q22)	*EVI1-AML1*	SKH1	erythroid-megakaryocytic	
t(4;11)(q21;q23)	*MLL-AF4*	KOCL-48	monocytic	
		MV4-11	monocytic	
t(6;9)(p23;q34)	*DEK-CAN*	FKH-1	myelocytic	
t(6;11)(q27;q23)	*MLL-AF6*	ML-2	monocytic	
		CTS	myelocytic	
t(8;21)(q22;q22)	*ETO-AML1*	Kasumi-1	myelocytic	
		SKNO-1	myelocytic	
t(9;11)(p22;q23)	*MLL-AF9*	IMS-M1	monocytic	
		Mono Mac 6	monocytic	
		THP-1	monocytic	
		UG3	monocytic	
ins(11;9)(q23;p22p23)	*MLL-AF9*	MOLM-13	monocytic	
t(9;22)(q34;q11)Philadelpha	*BCR-ABL*	AP-217	erythroid-megakaryocytic	b3-a2 fusion
		AR230	myelocytic	e19/e18-a2 fusion
		CML-C-1	myelocytic	
		EM-2	myelocytic	b3-a2 fusion
		GM/SO	myelocytic	b3-a2 fusion
		HNT-34	myelocytic	M-bcr/m-bcr fusion
		JK-1	erythroid-megakaryocytic	b2-a2 fusion
		JURL-MK1	erythroid-megakaryocytic	b3-a2 fusion
		K-562	erythroid-megakaryocytic	b3-a2 fusion
		Kasumi-4	myclocytic	b2-a2 fusion
		KBM-5	monocytic	b3-a2 fusion
		KBM-7	myelocytic	b2-a2 fusion
		KCL-22	myelocytic	b2-a2 fusion
		KH88	erythroid-megakaryocytic	b3-a2 fusion
		KOPM-28	myelocytic	b3-a2 fusion
		KOPM 30	myelocytic	el-a2 fusion
		KOPM 55	myelocytic	
		KT-1	myelocytic	b2-a2 fusion
		KU-812	myelocytic	b3-a2 fusion
		KYO-1	myelocytic	b2-a2 fusion
		LAMA-84	erythroid-megakaryocytic	b3-a2 fusion
		MC3	erythroid-megakaryocytic	b3-a2 fusion
		MEG-01	erythroid-megakaryocytic	b2-a2 fusion
		MEG-A2	erythroid-megakaryocytic	b3-a2 fusion
		MOLM-1	erythroid-megakaryocytic	b2-a2 fusion
		RM10	erythroid-megakaryocytic	M-bcr fusion
		RWLeu-4	monocytic	b2-a2 fusion
		SAM-1	erythroid-megakaryocytic	b3-a2 fusion
		SKH1	erythroid-megakaryocytic	b3-a2 fusion
		T-33	erythroid-megakaryocytic	b3-a2 fusion
		TS9;22	erythroid-megakaryocytic	b3-a2 fusion
		Y-1K	erythroid-megakaryocytic	b3-a2 fusion
		YN-1	erythroid-megskaryocytic	b3-a2 fusion
		YOS-M	myelocytic	b2-a2 fusion
		YS9;22	erythroid-megakaryocytic	b3-a2 fusion
t(10;11)(p14;q23)	*MLL-CALM*	U-937	monocytic	not *MLL-AF10*
t(12;22)(p13;q12)	*ETV6/TEL-MN1*	MUTZ-3?	monocytic	
		UCSD/AML1	myelocytic	
t(15;17)(q22;q21)	PML-RARA	Ei501	myelocytic	
		HT93	myelocytic	bcr3 fusion
		NB4	myelocytic	bcrl-2 fusion
		UF-1	myclocytic	
inv(16)(p13q22)	*CBFB-MYH11*	ME-1	monocytic	
t(i6;21)(p11;q22)	*TLS/FUS-ERG*	IRTA17	myelocytic	
		YNH-1	myelocytic	

Appendix 5. Heterotransplantable myeloid cell lines

Cell line	Cell type	Heterotransplantation
AML-CL[1]	myelocytic	into SCID mice
AML-PS[1]	myelocytic	into SCID mice
CHRF-288-11	erythroid-megakaryocytic	into nude mice
CML-C-1	myelocytic	into nude mice
ELF-153	erythroid-megakaryocytic	into nude mice
EM-2	myelocytic	into SCID mice
HL-60	myelocytic	into nude mice or SCID mice
HMC-1	mast cell	into nude mice
K-562	erythroid-megakaryocytic	into nude mice or SCID mice
KBM-3	monocytic	into nude mice or SCID mice
KBM-5	monocytic	into SCID mice
KBM-7	myelocytic	into nude mice
KCL-2	myelocytic	into newborn hamsters
KG-1	myelocytic	into nude or SCID mice
KMOE-2	erythroid-megakaryocytic	into nude mice
LAMA-84	erythroid-megakaryocytic	into estrone-treated nude mice
M-07e	erythroid-megakaryocytic	into SCID mice
MKPL-1	erythroid-megakaryocytic	into nude mice
OCIM2	erythroid-megakaryocytic	into SCID mice
OMA-AML-1	monocytic	into nude or SCID mice
P39/Tsugane	monocytic	into nude mice
PLB-985	monocytic	into nude mice
RWLeu-4	monocytic	into nude mice

[1] Cell lines can be maintained and serially transplanted, but do not grow long-term *in vitro*

Appendix 6. Cross-contaminated cell lines

Purported cell line	Purported cell type	Subclone of cell line	Actual cell type
DAMI	erythroid-megakaryocytic	HEL	erythroid-megakaryocytic
J-111	monocytic	HELA	cervix carcinoma
JOSK-I	monocytic	U-937	monocytic
JOSK-K	monocytic	U-937	monocytic
JOSK-M	monocytic	U-937	monocytic
JOSK-S	monocytic	U-937	monocytic
LR10.6	precursor B-cell	NALM-6	precursor B-cell
MOBS-1	monocytic	U-937	monocytic
MOLT-15	T-cell	CTV-1	monocytic
PBEI	precursor B-cell	NALM-6	precursor B-cell
SPI-801	T-cell	K-562	erythroid-megakaryocytic
SPI-802	T-cell	K-562	erythroid-megakaryocytic

Abbreviations

ACP – acid phosphatase
ADCC – antigen-dependent cell-mediated cytotoxicity
ALL – acute lymphoblastic leukemia
ALP – alkaline phosphatase
AML – acute myeloid leukemia
ANAE –α-naphthylacetate esterase
ANBE –α-naphthylbutyrate esterase
AraC – cytosine arabinoside
ATCC – American Type Culture Collection
ATRA – all-trans retinoic acid
AUL – acute undifferentiated leukemia
β-TG –β-thromboglobulin
baso – basophil
BC – blast crisis (of CML)
BCGF – B-cell growth factor
BCP – B-cell precursor
bFGF – basic fibroblast growth factor
BM – bone marrow
BMT – bone marrow transplantation
CAE – naphthol AS-D chloroacetate esterase
cALL – common acute lymphoblastic leukemia
CLL – chronic lymphocytic leukemia
CM – conditioned medium
CML – chronic myeloid leukemia
CMML – chronic myelomonocytic leukemia
CP – chronic phase (of CML)
CSF – colony-stimulating factor or cerebrospinal fluid
cyIg – cytoplasmic immunoglobulin
D – at diagnosis of disease
δ-ALA –δ-aminolevulinic acid
DMEM – Dulbecco's modified essential medium
DMSO – dimethylsulfoxide
DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen
dt – doubling time
EBNA – Epstein-Barr virus nuclear antigen
EBV – Epstein-Barr virus
ECACC – European Collection of Animal Cell Cultures
EDF – erythroid differentiation factor
EGF – epidermal growth factor
ELISA – enzyme-linked immunoassay
eosino – eosinophil
EPO – erythropoietin
ery – erythroid
F – female
FAB – French–American–British morphological AML/ALL/MDS classifications
FBS – fetal bovine serum
FL – FLT3 ligand
FN – fibronectin
G-CSF – granulocyte CSF
GLC –β-glucuronidase
GlyA – glycophorin A
GM-CSF – granulocyte-macrophage CSF

HS – horse serum
hu – human
IFN – interferon
Ig – immunoglobulin
IGF – insulin-like growth factor
IGH/K/L – immunoglobulin heavy/kappa light/lambda light chain gene
IL – interleukin
IMDM – Iscove's modified Dulbecco's medium
jCML – juvenile chronic myeloid leukemia
JCRB – Japanese Cancer Research Resources Bank
LCL – lymphoblastoid cell line
LGL – large granular lymphocytes
LIF– leukemia inhibitory factor
lym – lymphoid
M–male
M1 – (immature) myeloblastic AML
M2 – myeloblastic AML
M3 – promyelocytic AML
M4 – myelomonocytic AML
M5 – monocytic AML
M6 – erythroid AML
M7 – megakaryocytic AML
macro – macrophage
M-CSF – macrophage CSF
MDR – multiple drug resistance
MDS – myelodysplastic syndromes
meg – megakaryocytic
MEM – minimum essential medium
MIP – macrophage inflammatory protein
MLR – mixed lymphocyte reaction
mono – monocytic
MPO – myeloperoxidase
MPD – myeloproliferative disorder
MSE – monocyte-specific esterase
my – myeloid
NBT – nitroblue tetrazolium
NCS – newborn calf serum
neutro – neutrophil
NGF – nerve growth factor
NHL – Non-Hodgkin's lymphoma
NK – natural killer
OSM – oncostatin M
PAS – periodic acid Schiff
PB – peripheral blood
PDGF – platelet-derived growth factor
PE – pleural effusion
PF-4 – platelet factor-4
Ph – Philadelphia chromosome
PPO – platelet peroxidase
R – at relapse of disease
RAEB – refractory anemia with excess of blasts
RAEBT – refractory anemia with excess of blasts in transformation
RARS – refractory anemia with ring sideroblasts
RIKEN – Riken (Japanese cell bank)

RIA – radioimmunoassay
RPMI–RPMI 1640 medium
RT-PCR – reverse transcriptase-polymerase chain reaction
SBB – Sudan black B
SCF – stem cell factor
sIg – surface immunoglobulin
T – during therapy of disease
t-AML/MDS – therapy-related AML/MDS
TCRA/B/G/D - T-cell receptor $\alpha, \beta, \gamma, \delta$-chain
TGF – transforming growth factor
TNF – tumor necrosis factor
TPA – phorbol ester 12-0-tetradecanoyl phorbol-13 acetate
TPO – thrombopoietin
TRAP – tartrate-resistant acid phosphatase
Tu – tumor
Vit. D3 – vitamin D3
vWF – von Willebrand factor

References

1. Bessis M (ed.) *Blood Smears Reinterpreted*, Springer, Berlin, 1977.
2. Castoldi GL, Beutler E. In: Zucker-Franklin D, Greaves MF, Grossi CE, Marmont AM (eds.), *Atlas of Blood Cells. Function and Pathology*, 2nd edn., Fischer, Stuttgart, pp 47–156, 1988.
3. Zucker-Franklin D. In: Zucker-Franklin D, Greaves MF, Grossi CE, Marmont AM (eds.), *Atlas of Blood Cells. Function and Pathology*, 2nd edn., Fischer, Stuttgart, pp 619–693, 1988.
4. Bennett JM, Catovsky D, Daniel MT et al. *Br J Haematol* 33: 451–458, 1976.
5. Bennett JM, Catovsky D, Daniel MT et al. *Ann Int Med* 103: 460–462, 1985.
6. Bennett JM, Catovsky D, Daniel MT et al. *Br J Haematol* 87: 746–754, 1994.
7. Hassan HT, Freund M. *Leukemia Res* 19: 589–594, 1995.
8. Hassan HT, Drexler HG. *Leukemia Lymphoma* 20: 1–15, 1995.
9. Hoffman R. *Blood* 74: 1196–1212, 1989.
10. McDonald TP, Sullivan PS. *Exp Hematol* 21: 1316–1320, 1993.
11. Romeo PH, Prandini MH, Joulin V et al. *Nature* 344: 447–449, 1990.
12. Ohyashiki K, Ohyashiki JH, Shimamoto T et al. *Leukemia Lymphoma* 23: 431–436, 1996.
13. Ito E, Kasai M, Toki T et al. *Leukemia Lymphoma* 23: 545–550, 1996.
14. Long MW, Heffner CH, Williams JL et al. *J Clin Invest* 85: 1072–1084, 1990.
15. Nakahata T, Okumura N. *Leukemia Lymphoma* 13: 401–409, 1994.
16. Papayannopoulou T, Raines E, Collins S et al. *J Clin Invest* 79: 859–866, 1987.
17. Tani T, Ylänne J, Virtanen I et al. *Exp Hematol* 24: 158–168, 1996.
18. Kaushansky K. *Blood* 86: 419–431, 1995.
19. Kaushansky K, Broudy VC, Grossman A et al. *J Clin Invest* 96: 1683–1687, 1995.
20. Drexler HG, Quentmeier H. *Leukemia* 10: 1405–1421, 1996.
21. Fraser JK, Tan AS, Lin FK et al. *Exp Hematol* 17: 10–16, 1989.
22. Debili N, Coulombel L, Croisille L et al. *Blood* 88: 1284–1296, 1996.
23. Mossuz P, Prandini MH, Leroux D et al. *Leukemia Res* 21: 529–537, 1997.
24. Miyazaki Y, Kuriyama K, Higuchi M et al. *Leukemia* 11: 1941–1949, 1997.
25. Bonsi L, Grossi A, Strippoli P et al. *Br J Haematol* 98: 549–559, 1997.

26. Witte DP, Harris RE, Jenski LJ et al. *Cancer* 58: 238–244, 1986.
27. Fugman DA, Witte DP, Jones CLA et al. *Blood* 75: 1252–1261, 1990.
28. Toba K, Kishi K, Koike T et al. *Exp Hematol* 24: 894–901, 1996.
29. Komatsu N, Suda T, Moroi M et al. *Blood* 74: 42–48, 1989.
30. Sato T, Fuse A, Eguchi M et al. *Br J Haematol* 72: 184–190, 1989.
31. Sato S, Ohnuma N, Tanabe M et al. *Leukemia Lymphoma* 36: 397–404, 2000.
32. Miura N, Sato T, Fuse A et al. *Int J Mol Med* 1: 559–563, 1998.
33. Mouthon MA, Freund M, Titeux M et al. *Blood* 84: 1085–1097, 1994.
34. Hassan HT, Hanauske AR, Lux E et al. *Int J Exp Pathol* 76: 361–367, 1995.
35. Chiba S, Takaku F, Tange T et al. *Blood* 78: 2261–2268, 1991.
36. Zipursky A, Vanek W, Grunberger T et al. *Blood* 82: 122a, 1993.
37. Martin P, Papayannopoulou T. *Science* 216: 1233–1235, 1982.
38. Cheng T, Erickson-Miller CL, Li C et al. *Leukemia* 9: 1257–1263, 1995.
39. Ma F, Koike K, Higuchi T et al. *Br J Haematol* 100: 427–435, 1998.
40. Morgan D. *Blood* 82: 373a, 1993.
41. Morgan D, Class R, Soslau G et al, *Exp Hematol* 25: 1378–1385, 1997.
42. Drexler HG, Zaborski M, Quentmeier H. *Leukemia* 11: 541–551, 1997.
43. Okuno Y, Suzuki A, Ichiba S et al. *Cancer* 66: 1544–1551, 1990.
44. Di Noto R, Luciano L, Lo Pardo C et al. *Leukemia* 11: 1554–1564, 1997.
45. Lozzio CB, Lozzio BB. *Blood* 45: 321–334, 1975.
46. Yasanuga M, Ryo R, Sawada YKS et al. *Ann Hematol* 68: 145–151, 1994.
47. Furukawa T, Koike T, Ying W et al. *Leukemia* 8: 171–180, 1994.
48. Okano H, Okamura J, Yagawa K et al. *J Cancer Res Clin Oncol* 102: 49–55, 1981.
49. Seigneurin D, Champelovier P, Mouchiroud G et al. *Exp Hematol* 15: 822–832, 1987.
50. Champelovier P, Valiron O, Michèle J et al. *Leukemia Res* 18: 903–918, 1994.
51. Avanzi GC, Lista P, Giovinazzo B et al. *Br J Haematol* 69: 359–366, 1988.
52. Avanzi GC, Brizzi MF, Giannotti J et al. *J Cell Physiol* 145: 458–464, 1990.
53. Itano M, Tsuchiya S, Minegishi N et al. *Exp Hematol* 23: 1301–1309, 1995.
54. Morgan DA, Gumucio DL, Brodsky I. *Blood* 78: 2860–2871, 1991.
55. Okabe M, Kunieda Y, Nakane S et al. *Leukemia Lymphoma* 16: 493–503, 1995.
56. Ogura M, Morishima Y, Ohno R et al. *Blood* 66: 1384–1392, 1985.
57. Ogura M, Morishima Y, Okumura M et al. *Blood* 72: 49–60, 1988.
58. Abe A, Emi N, Kato H et al. *Leukemia* 9: 341–349, 1995.
59. Higashitani A, Yokoyama S, Shimizu Y et al. *J Jpn Pediatr Soc* 99: 920–928, 1995.
60. Hassan HT, Grell S, Borrmann-Danso U et al. *Hematol Oncol* 12: 61–66, 1994.
61. Hassan HT, Petershofen E, Lux E et al. *Ann Hematol* 71: 111–117, 1995.
62. Takeuchi S, Sugito S, Uemura Y et al. *Leukemia* 6: 588–594, 1992.
63. Matsuo Y, Adachi T, Tsubota T et al. *Human Cell* 4: 261–264, 1991.
64. Tsuji-Takayama K, Kamiya T, Nakamura S et al. *Human Cell* 7: 167–171, 1994.
65. Minegishi N, Minegishi M, Tsuchiya S et al. *J Biol Chem* 269: 27700–27704, 1994.
66. Tsuyuoka R, Takahashi T, Suzuki A et al. *Stem Cells* 13: 54–64, 1995.
67. Papayannopoulou T, Nakamoto B, Kurachi S et al. *Blood* 72: 1029–1038, 1988.
68. Hirata J, Sato H, Takahira H et al. *Leukemia* 4: 365–372, 1990.
69. Skinnider LF, Pabello P, Zheng CY. *Acta Haematol* 98: 26–31, 1997.
70. Kamesaki H, Michaud GY, Irving SG et al. *Blood* 87: 999–1005, 1996.
71. Uozumi K, Otsuka M, Ohno N et al. *Leukemia* 14: 142–152, 2000.
72. Mitani K, Ogawa S, Tanaka T et al. *EMBO J* 13: 504–510, 1994.
73. Tange T, Nakahara K, Mitani K et al. *Cancer Res* 48: 6137–6144, 1988.
74. Kitamura T, Tojo A, Kuwaki T et al. *Blood* 73: 375–380, 1989.

75. Kitamura T, Tange T, Terasawa T et al. *J Cell Physiol* 140: 323–334, 1989.
76. Ohyashiki K, Ohyashiki JH, Fujieda H et al. *Leukemia* 8: 2169–2173, 1994.
77. Alien RJ, Smith SD, Moldwin RL et al. *Leukemia* 12: 1119–1127, 1998.
78. Komatsu N, Nakauchi H, Miwa A et al. *Cancer Res* 51: 341–348, 1991.
79. Komatsu N, Yamamoto M, Fujita H et al. *Blood* 82: 456–464, 1993.
80. Komatsu N, Kunitama M, Yamada M et al. *Blood* 87: 4552–4560, 1996.
81. Komatsu N, Kirito K, Shimizu R et al. *Blood* 89: 4021–4033, 1997.
82. Endo K, Harigae H, Nagai T et al. *Br J Haematol* 85: 653–662, 1993.
83. Drexler HG, Dirks W, MacLeod RAF et al. (eds.) *DSMZ Catalogue of Human and Animal Cell Cultures,* 7th edn., DSMZ, Braunschweig, 1999.
84. Micallef M, Matsuo Y, Takayama K et al. *Hematol Oncol* 12: 163–174, 1994.
85. Blom T, Nilsson G, Sundström C et al. *Scand J Immunol* 44: 54–61, 1996.
86. Schumann RR, Nakarai T, Gruss HJ et al. *Blood* 87: 2419–2427, 1996.
87. Okabe M, Kunieda Y, Shoji M et al. *Leukemia Res* 19: 933–943, 1995.
88. Sandrock B, Hudson KM, Williams DE et al. *In Vitro Cell Dev Biol* 32A: 225–233, 1996.
89. Drexler HG, Zaborski M, Quentmeier H. *Leukemia* 11: 701–708, 1997.
90. Quentmeier H, Zaborski M, Graf G et al. *Leukemia* 10: 297–310, 1996.
91. Ogawa S, Hirano N, Sato N et al. *Blood* 84: 2431–2435, 1994.
92. Nakamaki T, Kawamata N, Schwaller J et al. *Br J Haematol* 91: 139–149, 1995.
93. Aguiar RCT, Sill H, Goldman JM et al. *Leukemia* 11: 233–238, 1997.
94. Shiohara M, Spirin K, Said JW et al. *Leukemia* 10: 1897–1900, 1996.
95. Ogawa S, Kurokawa M, Tanaka T et al. *Oncogene* 13: 183–191, 1996.
96. Berthier R, Mossuz P, Valiron O et al. *Blood* 84: 154a, 1994.
97. Shimamoto T, Ohyashiki K, Ohyashiki JH et al. *Blood* 86: 3173–3180, 1995.
98. Kamel-Reid S, Letarte M, Sirard C et al. *Science* 246: 1597–1600, 1989.
99. Kaku M, Yagawa K, Nakamura K et al. *Blood* 64: 314–317, 1984.
100. Scheid C, Fuchs M, Winter S et al. *Blood* 86: 778a, 1995.
101. Morgan DA, Brodsky I. *J Cell Biol* 100: 565–573, 1985.
102. Greenberg SM, Rosenthal DS, Greeley TA et al. *Blood* 72: 1968–1977, 1988.
103. MacLeod RAF, Dirks WG, Reid YA et al. *Leukemia* 11: 2032–2038, 1997.
104. Roth BJ, Sledge GW Jr, Straneva JE et al. *Blood* 72: 202–207, 1988.
105. Sledge GW Jr, Giant M, Jansen J et al. *Cancer Res* 46: 2155–2159, 1986.
106. Kollia P, Politou M, Tassiopoulou A et al. *Blood* 90: 156b, 1997.
107. Kojika S, Sugita K, Inukai T et al. *Leukemia* 10: 994–999, 1996.
108. Kobayashi S, Teramura M, Sugawara I et al. *Blood* 81: 889–893, 1993.
109. Winkler U, Rieder H, März W et al. *Blood* 90: 236b, 1997.
110. Drexler HG. *Leukemia* 1: 697–705, 1987.
111. Barber DL, D'Andrea AD. *Semin Hematol* 29: 293–304, 1992.
112. Youssoufian H, Longmore G, Neumann D et al. *Blood* 81: 2223–2236, 1993.
113. Graf G, Dehmel U, Drexler HG. *Leukemia Res* 20: 831–838, 1996.
114. Drexler HG, MacLeod RAF, Borkhardt A et al. *Leukemia 9:* 480–500, 1995.
115. Drexler HG, Borkhardt A, Janssen JWG. *Leukemia Lymphoma* 19: 359–380, 1995.
116. Drexler HG, MacLeod RAF, Uphoff CC. *Leukemia Res* 23: 207–215, 1999.
117. Siebert R, Willers CP, Opalka B. *Leukemia Lymphoma* 23: 505–520, 1996.
118. Drexler HG. *Leukemia* 12: 845–858, 1998.
119. Drexler HG, Fombonne S, Matsuo Y et al. *Leukemia* 14: 198–206, 2000.
120. Koeffler HP. *Blood* 62: 709–721, 1983.

Chapter 9

Non-Hodgkin's B-Lymphoma Cell Lines

Ramzi M. Mohammad, Frances W.J. Beck and Ayad M. Al-Katib
Department of Hematology and Oncology, Wayne State University and Karmanos Cancer Institute, Lande Research Building Room 317, 550E Canfield, Detroit, MI 48201, USA. Tel: +1-313-577-7919; Fax: +1-313-577-7925; E-mail: mohammad@karmanos.org

1. INTRODUCTION

The non-Hodgkin's lymphomas (NHL) comprise a group of B- or T-lymphocytic malignancies that most commonly originate in lymph nodes. These lymphomas are thought to represent lymphocyte populations that are arrested at a certain stage of the differentiation pathway [3,72,77].

There are several different classification schemes for B-NHL [9,18,30, 37,52,59,63]. Until the 1960s, the classification of the NHL included the terms reticulum cell sarcoma, lymphosarcoma, and giant follicular lymphoma [10]. Later, Rappaport classified lymphomas based on the cell growth and morphology [9,10,47,89]. Other classification systems have been introduced to include types of NHL which did not fit the Rappaport classification. The most widely used classification in the United States is that of Lukes-Collins [60], which was based on the immunologic profile of the lymphoma cell. A number of other classification schemes were reported in Europe [18,31,52]. Recently, two additional classifications, the International Working Formulation (WF) and the Revised European-American Lymphoma (REAL) have been developed [11,35,36,94]. This chapter will use the most recent NHL classification, the REAL, but will include, where applicable, the older classifications, including Rappaport, Lukes-Collins and the International Working Formulation.

In Table 1 we present the classification of non-Hodgkin's B-lymphoma according to the Revised European American Lymphoma (REAL) system and compare the previous classification of the International Working Formulations and the Rappaport classification to this present and newest classification. In Table 2, we list the chromosomal abnormalities frequently detected in NHL. Data listed in Tables 3–5 detail the general characteristics of individual B-NHL cell lines based on REAL classification.

J.R.W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol. III, 295–320.

Table 1. Classifications of non-Hodgkin's B-lymphoma according to the Revised European–American Lymphoma (REAL), Working Formulation (WF) and Rappaport

Revised European–American lymphoma (REAL)		Working formulation (WF)	Rappaport
(1)	Precursor B-lymphoblastic lymphoma/leukemia (B-LBL)	Lymphoblastic	Lymphoblastic
(2)	B-cell chronic lymphocytic leukemia/prolymphocytic leukemia/Small lymphocytic lymphoma	Small lymphocytic consistent with CLL Small lymphocytic, plasmacytoid	Well-differentiated lymphocytic diffuse
(3)	Lymphoplasmacytoid lymphoma	Small lymphocytic, plasmacytoid Diffuse, mixed small and large cell	Well-differentiated lymphocytic, plasmacytoid Diffuse mixed lymphocytic and histiocytic
(4)	Mantle cell lymphoma	Small lymphocytic Diffuse, small cleaved cell Follicular, small cleaved cell Diffuse, mixed small and large cell Diffuse, large cleaved cell	Intermediately or poorly differentiated lymphocytic, diffuse or nodular
(5)	Follicular center lymphoma, follicular		
	– Grade I	Follicular, predominantly small cleaved cell	Nodular, poorly differentiated, lymphocytic
	– Grade II	Follicular, mixed small and large cell	Nodular, mixed lymphocytic & histiocytic
	– Grade III	Follicular, predominantly large cell	Nodular histiocytic
(6)	Follicular center lymphoma, diffuse, small cell (provisional)	Diffuse, small cleaved cell Diffuse, mixed small and large cell	Diffuse lymphocytic Poorly differentiated
(7)	Extranodal marginal zone B-cell lymphoma (low grade B-cell lymphoma of MALT type)	Small lymphocytic Diffuse, small cleaved cell Diffuse, mixed small and large cell	Not specifically listed, diffuse mixed lymphocytic and histiocytic
	Nodal marginal zone B-cell lymphoma (provisional)	Small lymphocytic Diffuse, small cleaved cell Diffuse, mixed small and large cell Unclassified	Nodular or diffuse

Continued on next page

Table 1. (continued)

Revised European–American lymphoma (REAL)		Working formulation (WF)	Rappaport
(8)	Splenic marginal zone B-cell lymphoma (provisional)	Small lymphocytic Diffuse, small cleaved cell	Not specifically listed, well-differentiated lymphocytic (WDL) or WDL-plasmacytoid
(9)	Diffuse large B-cell lymphoma	Diffuse, large cell Large cell immunoblastic	Diffuse histiocytic
(10)	Burkitt's lymphoma	Small non-cleaved cell, Burkitt's	Undifferentiated lymphoma, Burkitt's type
(11)	High-grade B-cell lymphoma, Burkitt-like (provisional)	Small non-cleaved cell, non-Burkitt's Diffuse, large cell Large cell immunoblastic	Undifferentiated; non-Burkitt's; kind not listed

Table 2. Chromosomal abnormalities frequently detected in NHL

Chromosome No.	Type of abnormality	Histological subtypes
1	q-Armbreak	Diffuse large cell
2	q-Armbreak	Diffuse large cell
3	Trisomy	Follicular mixed
	t(3;14)	Diffuse large cell lymphoma
6	q-Armbreak	Diffuse large cell, immunoblastic
		Follicular lymphomas
7	Trisomy	Follicular large cell
8	Trisomy,	Follicular mixed, small non-cleaved
	t(2;8)	
	t(8;14)	
	t(8;22)	
11	t(11;14)	Small lymphocytic, mantle cell
12	Trisomy	Small lymphocytic
14	q-Armbreak	Most subtypes, follicular lymphomas
		Diffuse large cell
17	I(17q)	Follicular lymphomas, diffuse large cell
18	t(14;18)	Follicular lymphomas, diffuse large cell
	Trisomy	Diffuse large cell, follicular lymphoma
21	Trisomy	Diffuse large cell

The establishment of human EBV-negative (non-transformed) B-NHL cell lines has been difficult. Fifty-nine well-characterized immortal B-NHL cell lines will be described in this chapter. The oldest continuous B-NHL cell line is the Burkitt's lymphoma cell line Raji, reported in 1964 by Pulvertaft [88], but is not listed here because it is EBV+. The oldest established EBV-negative cell line is SKW-4, established from a diffuse histiocytic lymphoma in 1966 [81].

2. IMMUNOPHENOTYPE

Approximately 80% of all lymphoid tumors are of B-cell origin, while the remaining 20% are of T-cell origin [27]. The use of a large number of monoclonal antibodies directed against specific antigens known to be associated with certain stages of B-NHL development has greatly contributed to the identification and classification of NHL [6,41,86,97].

Table 3. EBV-negative non-Hodgkin's B-lymphoma cell lines: clinical characterization

Cell line[a]	Donor age[b]/sex	Diagnosis[c]	Specimen site est.	Year	Culture medium[d]	Source[e]
(1) Precursor B-lymphoblastic lymphoma						
Karpas 1106	23 F	Mediastinal lymphoblastic B-NHL	Pleural and ascitic fluid	1983	RPMI 1640 with 10% FBS	76
(2) Chronic lymphocytic leukemia (CLL)						
WSU-CLL	66 M	CLL	PB mononuclear cells	1995	RPMI 1640 with 10% FBS	74
TANOUE	11 M	B cell leukemia	PB mononuclear cells	1995	RPMI 1640 with 10% FBS	24
(3) lymphoplasmacytoid lymphoma						
FM	43 M	DML	ND	1988	RPMI 1640 with 20% FBS	28
NU-DHL-1	ND	DHL	Lymph node	1983	RPMI 1640 with 10% FBS	100
SK-DHL-2	39 M	DHL	Peritoneal fluid (ascitic)	1983	RPMI 1640 with 20% FBS and 40% autologous peritoneal fluid	82
SK-DHL-2A	39 M	DHL	Peritoneal fluid (ascitic)	1983	RPMI 1640 with 20% FBS and 40% autologous peritoneal fluid	82
SK-DHL-2B	39 M	DHL	Peritoneal fluid (ascitic)	1983	RPMI 1640 with 20% FBS and 40% autologous peritoneal fluid	82
SKW-4	ND, M	DHL	Pleural fluid	1966	RPMI 1640 with 10% FBS	81
SU-DHL-1	10 M	DHL	Pleural fluid	1977	RPMI 1640 with 20% FBS and 10% human serum	25
SU-DHL-2	73 F	DHL	Pleural fluid	1977	RPMI 1640 with 20% FBS and 10% human serum	25
SU-DHL-3	35 M	DHL	Peritoneal fluid	1977	RPMI 1640 with 20% FBS and 10% human serum	25

Continued on next page

Table 3. (continued)

Cell line[a]	Donor age[b]/sex	Diagnosis[c]	Specimen site est.	Year	Culture medium[d]	Source[e]
(3) lymphoplasmacytoid lymphoma (continued)						
SU-DHL-4	38 M	DHL	Peritoneal fluid	1977	RPMI 1640 with 20% FBS and 10% human serum	25
SU-DHL-5	17 F	DHL	Lymph node	1977	RPMI 1640 with 20% FBS and 10% human serum	25
SU-DHL-6	43 M	DHL	Peritoneal fluid	1977	RPMI 1640 with 20% FBS and 10% human serum	25
SU-DHL-7	47 F	DHL	Pleural fluid	1977	RPMI 1640 with 20% FBS and 10% human serum	25
SU-DHL-10	25 M	DHL	Pleural fluid	1977	RPMI 1640 with 20% FBS and 10% human serum	25
(4) Mantle cell lymphoma (ML)						
HF-4a	ND	ML	PB mononuclear cell	1993	DMEM with 10% FBS	48
JeKo-1	78 F	Retroperitoneal mass	PB mononuclear cell	1998	RPMI 1640, Ham's F-12, HBSS (2:1:1)	46
SP-53 mantle cell	58 F	Intermediate lymphocytic lymphoma	PB mononuclear cell	1988	RPMI 1640 with 20% FBS	14
(5) Follicular follicular-Grade I						
CJ	54 F	Nodular small cleaved lymphoma	Lymph node	1989	RPMI 1640 with 20% FBS	28
Follicular follicular-Grade II						
WSU-FSCCL	30 M	FSCCL	PB mononuclear cell	1993	RPMI 1640 with 20% FBS	73
FL-18	68 M	FSCCL	Lymph node	1982	RPMI 1640 with 10% FBS	17
ONHL-1	58 M	FSCCL	Bone marrow mononuclear cells	1980	RPMI 1640 with 10% FBS	64

Continued on next page

Table 3. (continued)

Cell line[a]	Donor age[b]/sex	Diagnosis[c]	Specimen site est.	Year	Culture medium[d]	Source[e]
(5) Follicular follicular-Grade III						
U-937	37 M	Histiocytic lymphoma	Pleural effusion	1976	RPMI 1640 with 10% FBS	DSMZ-93
JOSK-M	37 M	Histiocytic lymphoma	PB mononuclear cell	1984	RPMI 1640 with 10% FBS	DSMZ-84
WSU-NHL	46 F	NHL	Pleural fluid	1988	RPMI 1640 with 20% FBS	DSMZ-71
(6) Follicular center lymphoma						
BALM-3	63 F	DPDL	Pleural fluid (effusion)	1979	RPMI 1640 10% FBS and antibiotics	58
BALM-4	63 F	DPDL	Pleural fluid (effusion)	1979	RPMI 1640 10% FBS and antibiotics	58
BALM-5	63 F	DPDL	Pleural fluid (effusion)	1979	RPMI 1640 10% FBS and antibiotics	58
(7) Extranodal marginal zone						
No cell lines						
(8) Splenic marginal zone						
No classified cell lines						
(9) Diffuse large cell lymphoma (DLCL)						
DS	70 F	Large immunoblastic B-cell lymphoma	Bone marrow	1993	Serum free culture medium	50
HOB1	24 M	Immunoblastic B-lymphoma	Gingival lesions	1989	RPMI 1640 with 10% FBS	38
a. HOB1/VCR1.0				1993	RPMI 1640 with 10% FBS	51
b. HOB1/ADR				1995	RPMI 1640 with 10% FBS	12
WSU-DLCL	32 F	Diffuse histiocytic lymphoma	Pleural fluid	1991	RPMI 1640 with 10–20% FBS	75
WSU-DLCL2	40 M	Diffuse large cell lymphoma	Pleural fluid	1996	RPMI 1640 with 10% FBS	2
HBL-1	65 M	Diffuse large cell lymphoma	Pleural effusion	1984	RPMI 1640 with 10% FBS	1
HBL-2	65 M	Diffuse large cell lymphoma	Pleural effusion	1984	RPMI 1640 with 10% FBS	1
HF-1		Diffuse large non-cleaved cell lymphoma	Lymph node	1993	DMEM with 10% FBS	48
KAL-1	37 M	Diffuse large cell lymphoma	Pleural effusion	1988	RPMI 1640 with 10% FBS	43
LNPL	18 M	Diffuse large cell lymphoma		1980	RPMI 1640 with 10% FBS	16
OZ	32 M	Diffuse large cell lymphoma	Bone marrow mononuclear cells	1997	RPMI 1640 with 20% FBS	79

Continued on next page

Table 3. (continued)

Cell line[a]	Donor age[b]/sex	Diagnosis[c]	Specimen site est.	Year	Culture medium[d]	Source[e]
(10) Burkitt's lymphoma (BL)						
BJAB	5 F	Burkitt's lymphoma	Tumor tissue	1979	RPMI 1640 with 10% FBS	DSMZ-68
BL-41	8 M	Burkitt's lymphoma	Tumor tissue	1987	RPMI 1640 with 5–10% FBS	DSMZ
BL-70	16 M	Burkitt's lymphoma	Tumor tissue	1985	RPMI 1640 with 10% FBS	DSMZ
CA-46	young, M	Burkitt's lymphoma	Ascites fluid	1980	RPMI 1640 with 20% FBS	ATCC-DSMZ-61
CW 678	ND	Burkitt's lymphoma	ND		RPMI 1640 with 10% FBS	7
DG-75	young, ND	Burkitt's lymphoma	ND	1981	RPMI 1640 with 10% FBS	69
MANCA	young, ND	Burkitt's lymphoma	ND	1985	RPMI 1640 with 10% FBS	33
Ramos (RA-1)	3 M	Burkitt's lymphoma		1973	RPMI 1640 with 10% FBS	ATCC
Sc-1	67 M	Burkitt's lymphoma	Ascitic fluid	1977	RPMI 1640 with 10% FBS	95
ST486		Burkitt's lymphoma	Ascitic fluid	1977	RPMI 1640 with 20% FBS	ATCC
WSU-BL	41 M	Burkitt's lymphoma	Peritoneal fluid (ascites)	1989	RPMI 1640 with 10% FBS	
(11) High-grade B-cell lymphoma						
MC 116	ND, M	Undifferentiated lymphoma	Pleural effusion	1980	RPMI 1640 with 20% FBS	ATCC
Others						
U-698-M	7 M	B cell lymphoma	Involved tonsil	1974	RPMI 1640 with 10% FBS	DSMZ 81
Karpas 422	72 F	Non-Hodgkin's lymphoma	Pleural fluid	1989	RPMI 1640 with 20% FBS	22
HF-1		Nodular and diffuse Centroblastic lymphoma	Lymph node	1993	DMEM with 10% FBS	48

Table 3. (continued)

Cell line[a]	Donor age[b]/sex	Diagnosis[c]	Specimen site est.	Year	Culture medium[d]	Source[e]
Others (continued)						
MHH-PREB-1	5 M	Non-Hodgkin's lymphoma	Lymph node	1994	RPMI 1640 with 7.5–10% FBS	DSMZ
MN-60	20 M	B cell leukemia	PB	1982	Ham's F10 with 10% FBS	DSMZ-90

[a] Cell line names are given as listed in the original literature.
[b] Age of patient at the time of establishment.
[c] Diagnosis is indicated as given in the original reference.
[d] Cell line might also grow in other culture media.
[e] Availability from cell bank as indicated (ATCC, DSMZ, author, or not listed).

Abbreviations: BM – bone marrow, PB – peripheral blood, CLL – chronic lymphocytic leukemia, DML – diffuse mixed lymphoma, DHL – diffuse histiocytic lymphocytic lymphoma, LIB – large cell immunoblastic lymphoma, ML – mantle cell lymphoma, FSCCL – follicular small cleaved cell, NHL – nodular histiocytic (follicular) lymphoma, DPDL – diffuse poorly differentiated lymphocytic lymphoma, DLCL – Diffuse large cell lymphoma, BL – 'Burkitt's lymphoma', NPDL – nodular poorly differentiated lymphocytic lymphoma, ATCC – American Tissue Culture Collection, DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, ND – not determined.

Table 4. EBV-negative non-Hodgkin's B-lymphoma cell lines: genetic characterization

Cell line	Primary ref.	Doubling time	EBV status	Karyotype
(1) Precursor B-lymphoblastic lymphoma				
Karpas 1106	*Blood* 84:3422; 1994	20–24 hr	Negative	49, X, del(2)(p11.2;p13.3), der(3)t(2;3)(p13.3;p25 1)+I(9p), ins(12;?)(q13.1;q13.3), del(14)(q11.2;q13.1), del(15)(q11.2;q15.3), der(18)t(x;13;18)(q28;q21.3;q12.1) −20 del(20)(q13.1;q13.3)X2, der(X)t(X;13;18)(q28;q21.3;q12.1)+ix(p)
(2) Chronic lymphocytic leukemia (CLL)				
WSU-CLL	*Leukemia* 10:130; 1996	18–24 hr	Negative	45, X, del(3)(p14;p24), t(4;12;12)(q31;q22;p13), t(5;12)(q31;p13), add(16)(q24) X2, t(18;21)(q12;p12)
TANOUE	*Leukemia Res* 19:249; 1995	40 hr	Negative	human hyperdiploid karyotype with 12% polyploidy; 47/48X, −Y/XYqh+, +7, +14, dup(1)(q21.1/21.2;q23.1/23.2), t(2;4)(q2??2;q2?5), del(6)(q27), t(8;14)(q24;q32)
(3) Lymphoplasmacytoid lymphoma				
FM	*Blood* 75:1311; 1989	ND	ND	t(14;18), +3, +9, +12, del 7
NU-DHL-1	*Blood* 63:140; 1984	ND	Negative	ND
SK-DHL-2	*Cancer Genet Cytogenet* 12:39; 1984	15 hr	Negative	t(8;14)
SK-DHL-2A	*Cancer Genet Cytogenet* 12:39; 1984	18 hr	Negative	diploid 46, XY t(8q;14q)
SK-DHL-2B	*Cancer Genet Cytogenet* 12:39; 1984	18 hr	Negative	tetraploid 82-92, XXYY t(8q;14q), t(8q;14q)
SKW-4	*Hematol Oncol* 1:277; 1983	ND	Negative	t(6p;1q), t(1p;2p), t(?;14q), t(?;7q), 7p+ t(q;11q)
SU-DHL-1	*Cancer* 42:2379; 1978	ND	Negative	83, XXYY, −1, −2, −3, −4, −5, −7, −8, −9, −10, −11, +12, −13, −13, −13, −14, −15, −16, −17, −21, +8mar, 6q−, t(y;?), t(Y;?)
SU-DHL-2	*Cancer* 42:2379; 1978	ND	Negative	51, XX, +4, +9, +11, +13, −22, +2mar,6q−, 18q−
SU-DHL-3	*Cancer* 42:2379; 1978	ND	Negative	47, XY, −4, +12, −14, +2mar, 2p−, t(11;?), t(13;?), 18q−
SU-DHL-4	*Cancer* 42:2379; 1978	ND	Negative	47, XY, +8, t(3;?), t(14;18)(q32;q21)
SU-DHL-5	*Cancer* 42:2379; 1978	ND	Negative	47, XX, +12, 2q−, 6q−
SU-DHL-6	*Cancer* 42:2379; 1978	ND	Negative	47, X, −4, +6, +7, −8, −17, −22, +i(17q), +3mar, 6p−, 6q−, 7q−, 9p−, 18q−, t(11;?)(q25;?), t(14;18)(q32;q21)
SU-DHL-7	*Cancer* 42:2379; 1978	ND	Negative	44, XX, −10, −12, −13, −18, −21, −22, +4mar, 2q−, 6q−, t(1;X)(p11;q28), t(14;?)(q32;?)
SU-DHL-10	*Cancer* 42:2379; 1978	ND	Negative	96, XXYY, −14, −14, +6mar, t(7;?), t(11;Y)(q23;q11), t(11;Y)(q23;q11), 18q−, 18q−
(4) Mantle cell lymphoma (ML)				
HF-4a	*Eur J Haematol* 52: 65, 1994		ND	47–52, XX, +X, +1, t(1;8)(p21;q24), −2, del(3)(q21), +add(7)(q36), t(14;18) t(1;6), t(1;8), t(3;19)
JeKo-1	*Br J Haematology* 102:1323; 1998	33 hr	Negative	41, XO (85%):+add(1)(p13), add(1)(q12), −2, add(3)(q27), add(5)(p13), −6, add(7)(q22), −8, −9, add(9)(q34), add(10)(p15), add(11)(p11), −12, −13, −14, −14, −16, −16, −20, add(21)(p13), −22, +mar1, +mar2, +mar3, +mar4, +mar5, +mar6, devoid of t(11;14)
SP-53 mantle cell	*Cancer* 64:1248; 1989	ND	Negative	46, XX (64%) t(11;14)(q13;q32)
(5) Follicular-Grade I				
CJ	*Blood* 75:1311; 1990		Negative	51, XX, +X, −4, +12, +del(2)(p21), +del(2)(q32), +del(3)(q21), +del(3)(q21), der(4)t(1,4)(q23;q35), t(8;22)(q24;q11), del(10)(q22;q24), t(14;18)(q32;q21)

Continued on next page

Table 4. (continued)

Cell line	Primary ref.	Doubling time	EBV status	Karyotype
(5) Follicular-Grade II				
WSU-FSCCL	*Cancer Genet Cytogenet* 70:62; 1993	26 hr	Negative	46–47, t(14;18)(q32;q21), t(8;11)(q24;q21)
FL-18	*Blood* 70:1619, 1987	26 hr	Negative	49, XY, +7, +12, −17, +del(X)(p11), del(6)(p11), dir ins (13;8) q(14;q22;q24) t(8;22)(q24;q13), t(14;18)(q32;q21)+der(17), t(17;?)(q23;?)
ONHL-1	*Int J Cancer* 46:1107; 1990	25 hr	Negative	46, X, −Y, −3, −8, −9, −10, −11, −13, −14, −15, −16, −18, −20, t(2;12)(p11.2;q24.1), +der(3)t(3;9;10)(p21.1;p22;q22), del(5)(q22;q31.1), +del(6)(q15;q22.2), +der(8)t(8;?)(p23.1;?), +der(9)t(3;9;10)(p21.1;p22;q22), +der(11)t(11;?)(q25;?), +der(14)t(14;?)(q32.3;?), t(15,16)(q21.1;q22), +der(15)t(15;?)(p13;?), +der(16)t(16;?)(p13.1;?), t(17;17)p13;q21.1), +der(18)t(18;?)(q21;?), +der(20)t(20;?)(q13.3;?), +der(21)t(8;21)(q13;p13), +mar1, +masr2
(5) Follicular-Grade III				
U-937	*Int J Cancer* 17:565; 1976	30–40 hr	Negative	*human flat-moded hypotriploid karyotype; 63(58-69)XXY, −2, −4, −6, +7, −9, −20, −21, +3mar,* t(1;12)(q21;p13), der(5)t(1;5)(p22;q35), add(9)(p22), t(10;11)(p14;q23), i(11q), i(12p), add(16)(q22), add(19)(q13); carries t(10;11)(seen in AML M5); t(1;5) resembles variant of t(2;5)(histiocytic lymphoma)
JOSK-M	*Cancer Res* 46: 3067, 1986	ND	Negative	hyperdiploid with 4% polyploidy; 55 (51-75)<2n>XXY, +X, +3, +7, +t(1;5;7)(p21-22;q35;p11), der(3), t(1;3)(q21;q26-27), der(6)t(6;?), der(10)t(10;?)(p13;?), del(11)(q22-23), der(12)(12;?)(p13;?)
WSU-NHL	*Leukemia Res* 12:833; 1988	57 hr	Negative	45, XX, t(14;18)(q32;q21)
(6) Follicular center lymphoma				
BALM-3	*Int J Cancer* 24:572; 1979	36–48 hr	Negative	52, XX, +7, +8, +8, t(14;18)(q32;q21), +19, +M1, +M2
BALM-4	*Int J Cancer* 24:572; 1979	60–72 hr	Negative	52, XX, +7, +8, +8, t(14;18)(q32;q21), +19, +M1, +M2
BALM-5	*Int J Cancer* 24:572; 1979	60–72 hr	Negative	52, XX, +7, +8, +8, t(14;18)(q32;q21), +19, +M1, +M2
(9) Diffuse large cell lymphoma (DLCL)				
DS	*Leukemia* 8:1164; 1994	24 hr	Negative	48, XX, t(1;6)(q12;q26), add(2)(q37), +del(7)(q21;q32), t(8;14)(q24;q32)+ inv(12)(p12.3;q24), der(13)(q12;p12), t(14;18)(q32;q21), del(20)(p11;p13), +ace
HOB1	*Br J Cancer* 61:655; 1990	22 hr	Negative	hypodiploid 45 (22–73) with multiple abnormalities: t(2;4)(p21>cen>qter::q26), t3;4;18)(p25;q21;q21), del(2)(p12p25), t(8,14), +13, +20, +17, +21
(a) HOB1/VCR1.0	*Cancer Lett* 73:105; 1993			
(b) HOB1/ADR	*FEBS Lett* 373:285; 1995			
WSU-DLCL	*Cancer* 69:1468; 1992	20 hr	Negative	70–85, XXXXX, 14q+, t(q8;q10), structural abnormalities of 3;4
WSU-DLCL2	*Clinical Cancer Res* 4:1305; 1998	18 hr	Negative	48, XY, t(1;2)(p36.1;q37), der(3)t(3;7)(q13;p15), t(4;14)(q27;132), +I(7p), der(7)t(3;7)(q21;q11.2), +8, t(14;18)(q32;q21), der(15)(q26.1), del(16)(q22), del(17)(q25)
HBL-1	*Cancer* 61:483; 1988	18–24 hr	Negative	44, X, t(6; 14; 16), t(12;7), t(14;17), t(16;17)
HBL-2	*Cancer* 61:483; 1988	18–24 hr	Negative	46, X, t(6; 9; 11), t(11;14)(q13;q32), t(11;?), t(14;15), t(18;9), t(9;22)
HF-1	*Eur J Haematol* 52:65; 1994	ND	ND	ND
KAL-1	*Cancer Res* 51: 5392; 1991	20 hr	Negative	46, XY, dup (1)(q21;q32), t(8;22)(q24;q11)
LNPL	*Cancer Res* 42:1368; 1982	25 hr	Negative	t(7;2) and t(8;14)
OZ	*Hematological Oncology* 15:109; 1997	36 hr	Negative	47, XY, del(2)(q35), der(8)t(8;12)(p21;q13.3), add(10)(p11.12), +add(12)(q24), t(14;18)(q32;q21), add(17)(p11)

Continued on next page

Table 4. (continued)

Cell line	Primary ref.	Doubling time	EBV status	Karyotype
(10) Burkitt's lymphoma (BL)				
BJAB	*Biomedicine* 22:276; 1979	ND	Negative	human pseudodiploid karyotype with 1.5% polyploidy; 46(44-46)XX, +7, −9, der(7)t(7;?)(p21;?), der(8)t(2;8)(p12;q24)
BL-41	*IARC Scientific Publ* 59:309; 1985	30 hr	Negative	human hyperdiploid karyotype with 7% polyploidy; 48(42-49)XY, +7, −13, +2mar, add(8)(q24), t(8;14)(q24;q32), der(15)t(13;15)(q13;p11), add(17)(p12), subclonal rearrangements at 1q23, 7p22, 11q13
BL-70	*IARC Scientific Publ* 60:309; 1985	44 hr	Negative	human hyperdiploid karyotype with 4% polyploidy; 47(42-49)XY, +7, inv(1)(p21;q21), del(2)(q33), t(8;14)(q24;q32), t(12;22)(q21.1;q13.2)
CA-46	*J Natl Cancer Inst* 64:477; 1980	16 hr	Negative	human near-diploid karyotype with about 3% triploidy; 46(45-48)<2n>X/XY, dup(1)(q21;q32), dup(7)(q12;q22), t(8;14)(q24;q32); additional rearrangements were present in a sideline, viz. t(6;13)(p21;q32) and del(11)(p11); a supernumerary dmin was present in about 25% metaphases
CW 678	*J Immunol* 129: 1336, 1982	ND	Negative	t(8;14)
DG-75	*Haematol Blood Transf* 26:322; 1981	ND	Negative	t(8;14)
MANCA	*Nature* 314:366; 1985 *JNCI* 37:547; 1966	22 hr	Negative	t(8;14)
Ramos (RA-1)	*Intervirology* 5:319; 1975	18–24 hr	Negative	human hypodiploid 45
Sc-1	*NJC* 39:89; 1987			49, XY, t(14;17)(q32;q21), +3, +7, +8
ST486	*Magrath JNCI* 64:465; 1980 *J Immunol* 129:1336; 1982	19 hr	Negative	human 48, XX, +7, +t(7;17)(7qter–7q22::17qter–17pter); t(8;14)(8pter–8q23::18q11–18qter)
WSU-BL	*Cancer* 64:1041; 1989	19hr	Negative	53, XY, t(8;14)(q24;q32) with 1q+, 2q+, +7, +13, +14q+, +18, +19q+, +21
(11) High-Grade B-cell lymphoma				
MC 116	*Magrath* 64: 1980	26 hr	Negative	human 45XO/46XY, dupl (q21;32), t(8;14)(8pter–8q23:: 14q32–14qter; 14 pter–14q32::8q23–8qter), del10
Others				
U-698-M	*Int J Cancer* 13:808; 1974	48 hr	Negative	human hyperdiploid karyotype with 5.5% polyploidy; 49(44-50)XY, +3, +7, −14, +mar, dup(1)(q43q21.2), der(2)t(2;3)(p16;p11), add(3)(p11), del(6)(q15;q22), del(9)(p22), dup(11)(q23;q13), add(13)(p12), add(16)(q24); carries large submetacentric dup(1) marker
Karpas 422	*Blood* 75:709; 1990		Neg./Pos.	46, XX, t(2;10)(p23;q22.1), t(4;11)(q21.3;q23.1), t(4;16)(q21.3;p13.1), t(14;18)(q32.1;q21.3)
HF-1	*Eur J Haematol* 52:65; 1994			52, XX, +X, t(2;8)(p12;q24), +?i(5)(p10), +?i(5)(p10), +?i(5)(p10), del(7)(q35), +add(7)(q32), add(9)(q34), +12, t(14;18)(q32;q21), +21[20]
MHH-PREB-1	not published	20–40 hr	Negative	human hyperdiploid karyotype with 12% polyploidy; 48<2n>XYY, +21, ?ins(2;2)(q21;p15p22), t(8;14)(q24;q32)
MN-60	*Leukemia Res* 6:685; 1982		Negative	human near-diploid karyotype; 46(45-47)<2n>XY, dup(1)(q21;q41), del(6)(q21), t(8;14)(q24;q32), i(13q)

ND — not determined.

Table 5. EBV-negative non-Hodgkin's B-lymphoma cell lines: immunophenotypic characterization

Cell line	Immunophenotype	Additional comments
(1) Precursor B-lymphoblastic Lymphoma		
Karpas 1106	CD3−, CD5−, CD10−, CD19+, CD20+, CD23−, CD37+, FMC7+, IgM−, IgD−, IgG+, IgA−, Ig lambda+, Ig kappa−	Bcl-2−
(2) Chronic lymphocytic leukemia (CLL)		
WSU-CLL	CD3−, CD5−, CD10+, CD19+, CD20+, CD22+, CD37+, CD45R+, HLA-DR+, sm/cyIgG+, sm/cylamda+	Bcl-2+, P53−, C-MYC+
TANOUE	CD3−, CD10+, CD13−, CD19+, CD20+, CD34−, CD37+, HLA-DR+, sm/cyIgG−, sm/cyIgM+, sm/cykappa(+), sm/cylambda+	
(3) Lymphoplasmacytoid lymphoma		
FM	CD3−, CD19+, CD20+, HLA-DR+, sm/cyIgM+, sm/cykappa+	
NU-DHL-1	CD3+, CD20+, CD19−, CD10−, sm/cylambda+, HLA-DR+, sm/cykappa−	
SK-DHL-2	CD3−, CD20+, CD21−, CD19+, CD10+, HLA-DR+, sm/cyIgM+, sm/cylambda+	
SK-DHL-2A	CD3−, CD20+, CD21−, CD19+, CD10+, HLA-DR+, sm/cyIgM+, sm/cylambda+	
SK-DHL-2B	CD3−, CD20+, CD21−, CD19+, CD10+, HLA-DR+, sm/cyIgM+, sm/cylambda+	
SKW-4	CD3−, CD20+, CD19+, CD10−, sm/cyIgM+, HLA-DR+, sm/cykappa+	
SU-DHL-1	CD3−, CD19−, CD10−, negative T-cell markers, smIgM−, smIgE−, smIgA−, smkappa−, smlambda−	
SU-DHL-2	CD3−, CD19−, CD10−, negative T-cell markers, smIgM−, smIgE−, smIgA−, smkappa−, smlambda−	
SU-DHL-3	CD3−, CD19−, CD10−, negative T-cell markers, smIgM−, smIgE−, smIgA+, sm/cykappa+, smlambda−	

Continued on next page

Table 5. (continued)

Cell line	Immunophenotype	Additional comments
(3) Lymphoplasmacytoid lymphoma (continued)		
SU-DHL-4	CD3−, CD19−, CD10−, negative T-cell markers, smIgM−, smIgE+, smIgA−, sm/cykappa+, smlambda−	
SU-DHL-5	CD3−, CD19−, CD10−, negative T-cell markers, smIgM+, smIgE−, smIgA−, smkappa−, sm/cylambda+	
SU-DHL-6	CD3−, CD19−, CD10−, negative T-cell markers, smIgM+, smIgE−, smIgA−, smkappa−, sm/cylambda+	
SU-DHL-7	CD3−, CD19−, CD10−, negative T-cell markers, smIgM−, smIgE+, smIgA+, smkappa−, smlambda+	
SU-DHL-10	CD3−, CD19−, CD10−, negative T-cell markers, smIgM−, smIgE+, smIgA−, smkappa−, smlambda+	
(4) Mantle cell lymphoma (ML)		
HF-4a	CD19+, CD20+, CD22+, CD39+, CD45+, smIgG+, HLA-DR+, sm/cykappa+, sm/cyIgM+, sm/cyIgD+	Bcl-2+
JeKo-1	CD3−, CD5+, CD10−, CD19+, CD20+, CD23−, sm/cyIgM+	
SP-53 mantle cell	CD2−, CD3−, CD4−, CD5+, CD10−/+, smIgM+, sm/cylamda+, sm/cykappa−, sm/cymu+, Leu-12+, OKIa1+	
5) Follicular-Grade I		
CJ	CD2−, CD3−, CD19+, CD20+, CD10+, sm/cyIg+, HLA-DR+, Tac(IL-2R)−	
(5) Follicular-Grade II		
WSU-FSCCL	CD2−, CD3−, CD4−, CD5−, HLA-DR+, CD10+, CD19+, CD20+, leu-10+, CD22+, CD37+, CD38+, CD11b−, Cdw13−, CD11c−/+, CDw14−, MY9, sm/cyIgM+, sm/cykappa+, sm/cylambda−, sm/cyIgD−, sm/cyIgD−	Bcl-2+

Continued on next page

Table 5. (continued)

Cell line	Immunophenotype	Additional comments
(5) Follicular-Grade II (continued)		
FL-18	CD2−, CD3, HLA-DR+, CD10+, CD19+, CD20+, CD22+, CD38+, OKT9+, sm/cykappa+ and Mu+	
ONHL-1	CD2−, HLA-DR+/−, CD20+, CD19−, CD24+, CD10−, PCA-1−, CD38−, sm/cykappa−/+, sm/cylamda−	
(5) Follicular-Grade III		
U-937	CD3−, CD13+, CD14−, CD15+, CD19−, CD33+, CD34−, CD68+	
JOSK-M	CD3−, CD13+, CD14−, CD15+, CD19−, CD33+, CD34−	
WSU-NHL	CD2−, CD3−, CD19+, CD20+, CD10+, CD21−,Leu-10+, Leu12+, Leu14+, Leu16+, BL1+, BL4+, BL7+, HLA-DR+, sm/cyIgG+, sm/cylambda+	
(6) Follicular center lymphoma		
BALM-3	sm/cyIg+, Ia antigen weakly positive, CALLA-negative	
BALM-4	sm/cyIg+, Ia antigen weakly positive, CALLA-negative	
BALM-5	sm/cyIg+, Ia antigen weakly positive, CALLA-negative	
(9) Diffuse large cell lymphoma (DLCL)		
DS	CD2−,CD3−, CD4−, CD5−, CD13−,CD15−, CD19+, CD10+, CD22−, CD33−, CD37+, CD45+, HLA-DR+,TdT−, lambda rearranged	Bcl-2+, C-MYC+
HOB1	CD2−, CD3−, CD4−, CD8−, CD10−, HLA-DR+, CD21−, CD20+, CD19+, Leu 14, TdT−, negative Igs	
(a) HOB1/VCR1.0	CD2−, CD3−, CD4−, CD8−, CD10−, HLA-DR+, CD21−, CD20+, CD19+, Leu 14, TdT−, negative Igs	

Continued on next page

Table 5. (continued)

Cell Line	Immunophenotype	Additional comments
(9) Diffuse large cell lymphoma (DLCL) (continued)		
(b) HOB1/ADR	CD2−, CD3−, CD4−, CD8−, CD10−, HLA-DR+, CD21−, CD20+, CD19+, Leu 14, TdT−, negative Igs	
WSU-DLCL	CD2−, CD3−, CD4−, CD5−, CD8−, CD19+, CD20+, CD21−, CD22+, BL4+, BL7+, HLA-DR+, Leu-10+, CD22+/−, CD37+, CD38−, CD10−/+, sm/cyIgM+, sm/cykappa+, sm/cylambda−, sm/cyIgG−	
WSU-DLCL2	CD2−, CD3−, CD4−, CD5−, CD8−, CD10+, CD19+, CD20+, CD21−, CD22+,CD37+, CD45R−/+, HLA-DR+, Leu-10+, sm/cyIgG+, sm/cylambda+, sm/cykappa−	Bcl-2+, P53+, C-MYC+
HBL-1	CD3−, CD20+, CD24+, HLA-DR+, sm/cyIgM+, sm/cykappa+	
HBL-2	CD3−, CD20+, CD24+, HLA-DR+, sm/cyIgM(D)+, sm/cylambda+	
HF-1	CD19+, CD20+, CD22+, CD39+, CD45+, smIgG+, HLA-DR+, sm/cykappa+, sm/cyIgM−, sm/cyIgD−	Bcl-2+
KAL-1	CD3−, CD20+, CD19+, CD10+, HLA-DR+, sm/cyIgM+, sm/cylambda+	
LNPL	CD10+, HLA-DR+, sm/cykappa+, mu+	
OZ	CD2−, CD3−, CD4−, CD5−, CD10+, CD13−, CD19+, CD20−, CD24+, CD33−, CD38+, HLA-DR+, smIg-mix−	Bcl-2+, p26+
(10) Burkitt's lymphoma (BL)		
BJAB	CD3−, CD10+, CD13−, CD19+, CD37+, HLA-DR+, sm/cyIgG−, sm/cyIgM+, sm/cykappa+, sm/cylambda−	
BL-41	CD3−, CD10+, CD13−, CD19+, CD20+, CD37+, HLA-DR+, sm/cyIgM+, sm/cyIgG−, sm/cykappa+, sm/cylambda−	

Continued on next page

Table 5. (continued)

Cell Line	Immunophenotype	Additional comments
(10) Burkitt's lymphoma (BL) (continued)		
BL-70	CD3−, CD10−, CD13−, CD19+, CD20+, CD37+, HLA-DR+, sm/cyIgM+, sm/cyIgG−, sm/cykappa+, sm/cylambda−	
CA-46	CD3−, CD10+, CD13−, CD19+, CD37+, HLA-DR+, sm/cyIgM+, sm/cyIgG−, sm/cykappa+	
DG-75	CD3−, CD19+, CD20+, SmIg+, cyIg+, HLA-DR+, CD10+, TdT−	
MANCA	CD19+, CD20+, CD22, CD10+, Leu10+, Leu12+, Leu14+, Leu16+, BL1+, BL4+, BL7+, HLA-DR+, sm/cyIgM+, sm/cylambda+	
Ramos (RA-1)	CD3−, CD19+, CD20+, HLA-DR+, sm/cykappa+, cymu+	
Sc-1	HLA-DR+, CD19+, CALLA+ (CD10+), CD20+, FMC7−, OKT10+, sm/cylamda+	
ST486	CD3−, CD19+, CD20+, HLA-DR+, sm/cyIgM+, sm/cykappa+	
WSU-BL	HLA-DR+, B1+, B4+, CALLA+, sm/cyIgM+, sm/cylamda+, BL3+, BL4+	Bcl-2+
(11) High-Grade B-cell lymphoma		
MC 116	CD3−, CD19+, CD20+, HLA-DR+, smIgM+, sm/cylamda+, C mu+	
Others		
U-698-M	CD3−, CD13−, CD19+, CD37+, HLA-DR+	
Karpas 422	CD19+, CD37+, sm/cyIgM+, smIgG+, smIgD+, CDw52+	
HF-1	CD19+, CD20+, CD22+, CD39+, CD45+, smIgG+, HLA-DR+, sm/cykappa+,	Bcl-2+
MHH-PREB-1	CD3−, CD10+, CD13+, CD19+, CD20+, CD37+, HLA-DR+, sm/cyIgG−, sm/cyIgM+, sm/cykappa−, sm/cylambda+	
MN-60	CD3−, CD10+, CD13−, CD19+, CD37+, HLA-DR+, sm/cyIgG−, sm/cyIgM+	

(+), strong, definite protein expression (more than 50% positive) moderate expression (20–50% positive); (−/+), weak expression (less than 20% positive).

The cell surface antigens expressed during B-cell differentiation are represented in the B-NHL cell lines. These are CD21 (B2) [96], BL4 [40] and BL7 [13]. CD11c and CD22 are two markers that were first reported in 1982 [92]. While CD11c is expressed on monocytes and CD22 on B-lymphocytes, the co-expression of the two antibodies was initially thought to be specific for hairy cell leukemia (HCL). Since then, however, a new subset of non-Hodgkin's lymphoma, the monocytoid B-cell lymphoma (MBCL), has been described that also co-expresses CD11c and CD22 [23,78]. Both HCL and MBCL also express acid phosphatases (AcP). However, such expression can be inhibited by tartrate (tartrate sensitive) in MBCL but not in HCL [45].

The most common forms of NHL are follicular small cleaved cell lymphoma (REAL; Mantle cell lymphoma, 40%) and follicular mixed small cleaved and large cell lymphomas (both now classified as follicular center lymphomas, 20-40%). These lymphomas are high grade, express the B-cell antigens CD 19, CD20 and CD22, and are CD5-negative. The expression of other antigens varies among cell lines representing B-NHL. More than 80% of splenic lymphoma with villous lymphocytes (SLVL) are CD24+ and FMC7+ and express membrane CD22 [65]. Mantle cell NHL is almost always CD5+ and CD43+ [98] and cell lines isolated from these patients and some SLVL exhibit overexpression of cyclin D1, unlike other B-cell NHLs [15]. B-NHL cell lines established from patients with various grades of B-cell malignancy are presented in Tables 3–5.

3. GENETIC CHARACTERIZATION

The first detection of chromosomal abnormality in NHL was the demonstration of the reciprocal translocation of genetic material between chromosomes 8 and 14. This translocation is frequent in patients with diffuse small non-cleaved cell lymphoma of the Burkitt's type [56,62]. Translocation (8;14)(q24;q32) is associated with small non-cleaved cell lymphoma of Burkitt's type; t(14;18)(q32;q21) with follicular B-NHL and trisomy 12 and t(11;14)(q11;q32) with small lymphocytic lymphoma (Table 2). There are correlations between the cytogenetic findings and the prognosis of patients with NHL [54].

4. CLINICAL CHARACTERISTICS

The histopathological diversity of the NHL is also reflected in its clinical characteristics. The natural history of NHL, clinical management and treatment philosophy varies among different types of NHL. One advantage of the

Working Formulation is the grouping of NHL into three categories based on their clinical behavior.

5. CLASSIFICATION OF EBV-NEGATIVE B-NHL CELL LINES ACCORDING TO THE REVISED EUROPEAN AND AMERICAN LYMPHOMA (REAL) SYSTEM

Fifty-nine well-characterized (EBV-) B-NHL cell lines are listed in Tables 3–5. The tables show stage, cell line name, donor age, sex, specimen site, year established, culture medium, source, reference, doubling time, EBV status, karyotype, and immunophenotype. For most cell lines, where there is insufficient information, this concerns descriptions of the essential features and data collected on immunophenotypic, genetic and clinical characteristics. As a result, we are limited in allocating such cell lines to the proper NHL stage or category.

To represent the immunophenotypes, the notations and abbreviations are as follows: +, over 90% of the cases positive; +/–, over 50% of the cases positive; –/+, less than 50% of the cases positive; –, less than 10% of the cases positive; IgH-R and IgL-R, Ig heavy/light chain genes rearranged; smIg, surface Ig; cyIg, cytoplasmic Ig; CD, cluster of differentiation.

5.1. Precursor B-Lymphoblastic Lymphoma/Leukemia (B-LBL)

Example: Karpas 1106. Rappaport: lymphoblastic and formerly diffuse poorly differentiated lymphocytic [PDL]). Lukes-Collins: not defined. Working Formulation: lymphoblastic.

Children are more commonly affected than adults by this disease. B-LBL accounts for less than 20% of lymphoblastic lymphoma. The Karpas 1106 cell line was established from a 23-year-old patient with mediastinal lymphoblastic B-NHL [76].

The tumor cells are characteristically CD 19+ CD79a+ CD22+ CD20 –/+ CD10+/– HLA-DR+ smIg- cyMu–/+ CD34+/–, and may express CD13 and/or CD33 [20,44,87]. Ig heavy chain genes are usually rearranged: light chain genes may be rearranged [49]. Karpas 1106 is a B-NHL lymphoblastic cell line with the immunophenotype CD5–, CD10–, CD19+, IgG+, and lambda+ (Table 5).

Cytogenetic abnormalities in B-LBL are variable [53,000]. Karpas 1106 has complex chromosomal abnormalities.

5.2. B-Cell Chronic Lymphocytic Leukemia (B-CLL)/Prolymphocytic Leukemia (B-PLL)/Small Lymphocytic Lymphoma (B-SLL)

Examples: TANOUE and WSU-CLL. Rappaport: well-differentiated lymphocytic, diffuse. Lukes-Collins: small lymphocyte B, B-CLL. Working Formulation: small lymphocytic, consistent with CLL.

The majority of the cases occur in adults, although TANOUE was established from an 11 year old. The B-CLL comprises >90% of CLL in United States and Europe. Most patients have bone marrow (BM) and peripheral blood (PB) involvement and tumors can invade nodes, spleen, and liver. Extra-nodal infiltrates may also be evident [94].

WSU-CLL and TANOUE lines were established from peripheral blood (PB) and are relatively slow-growing cells with doubling time up to 40 hours (Table 4). There is a shortage of B-PLL and B-SLL cell lines.

Typical tumor cells of B-CLL have faint smIgM, are smIgD+/–, (cyIg –/+), B-cell-associated antigen CD19+ CD20+ CD79a+ CD5+ CD23+ CD11c–/+ and CD10– [34,91,103]. WSU-CLL and TANOUE cell lines are CD5- and CD10+ (Table 5). Cases of B-PLL may be CD5-, have strong smIg and more often express CD22 [8]. Ig heavy and light chain genes are rearranged.

5.3. Lymphoplasmacytoid Lymphoma/Immunocytoma

Examples: Nu-DHL-1, SK-DHL-2, SKW-3, SU-DHL, FM. Rappaport: well-differentiated lymphocytic, plasmacytoid, diffuse mixed lymphocytic and histiocytic. Lukes-Collins: plasmacytic-lymphocytic. Working Formulation: small lymphocytic, plasmacytoid, diffuse mixed small and large cells.

These tumors involve BM, lymph nodes and spleen, and less frequently PB or extranodal sites. Most of the patients have a serum spike of monoclonal IgM. 17 cell lines are listed, most of which were established from patients with diffuse histiocytic lymphocytic (DHL) lymphoma, aged between 17 and 73 years. Generally, the disease is well represented by the cell lines.

The cells have surface and in some types, cytoplasmic Ig, usually of IgM type. Cells usually lack IgD, but are B-cell-associated antigens+ (CD 19, 20, 22, 79a). CD5– CD10– CD43+/– and CD11c may be faintly positive in some cases [34,91,103]. A lack of CD5 and the presence of strong cyIg are useful to distinguish them from B-CLL. The Ig heavy and light chain genes are rearranged, although no specific abnormality is known.

The main cytogenetic abnormalities involve chromosomes 8 and 14.

5.4. Mantle Cell Lymphoma

Examples: JeKo-1, SP-53, HF-4. Rappaport: intermediate or poorly differentiated lymphocytic, diffuse or nodular (ILL/IDL/PDL). Lukes-Collins: small cleaved follicular center cell (FCC). Working Formulation: small cleaved cell, diffuse or nodular; rarely diffuse mixed or large cleaved cell.

The tumor occurs in older adults with a high male to female ratio. Sites involved include lymph nodes, spleen, BM, PB (from which JeKo-1 and SP-53 were derived) and extra nodal sites such as the gastrointestinal tract [83]. The growth pattern of mantle cell lymphoma is usually diffuse or vaguely nodular. The well-defined follicles characteristic of follicular lymphomas are rarely seen.

The tumor cells are smIgM+, usually IgD+, $\lambda > \kappa$, B-cell–antigen+; CD5+ CD10–/+ CD23– CD43+ CD11c–. The absence of CD23 is useful in distinguishing mantle cell lymphoma from B-CLL. CD5 is useful in distinguishing mantle cell lymphoma from follicle center and marginal zone lymphomas. Two of the three cell lines are CD5+ and have a low level of CD10 expression. The JeKo-1 cell line is CD23– and smIgM+.

t(l 1;14) involves the Ig heavy chain locus at bcl-1 locus on the long arm of chromosome 11, and this translocation is present in the JeKo-1 and SP-53 cell lines.

5.5. Follicle Center Lymphoma, Follicular

Examples: CJ; (grade I); WSU-FSCCL, FL-18, ONHL-1 (grade II); WSU-NHL, JC (grade III). Rappaport: nodular PDL mixed lymphocytic-histiocytic, or histiocytic. Lukes-Collins: small cleaved, large cleaved or large non-cleaved FCC, follicular. Working Formulation: follicular, small cleaved, mixed, or large cell.

The terms follicular lymphoma, grades I, II and III are analogous to the terms used to classify other tumor types. The pattern of growth can be follicular or follicular and diffuse, and is of prognostic significance.

The cell lines are CD19–, CD34–, CD 13+ and CD33+ for grade I and CD10+ and CD19+ for grade-III.

Cytogenetic abnormalities mostly involve chromosomes 10, 11 and 14.

5.6. Follicle Center Lymphoma, Diffuse

Examples: BALM-3, BALM-4, BALM-5. Rappaport: diffuse poorly differentiated lymphocytic. Lukes-Collins: diffuse small cleaved FCC. Working Formulation: diffuse small cleaved cell.

Follicle center lymphoma, diffuse, affects predominantly adults, with equal male/female incidence [94]. This disease accounts for 40% of adult NHL in the United States [53].

BALM-3,4 and 5 are slow-growing cell lines with doubling time from 36 to 72 hours.

The tumor cells are usually smIg+ (smIgM+/– IgD>IgG>IgA), B-cell-associated antigen +, CD10+/– CD5– CD23–/+ CD11c–. BALM-3, 4 and 5 cell lines show sm/cyIg+ and CD 10–. A lack of CD5 and CD43 is useful in distinguishing follicle center lymphoma from mantle cell lymphoma, and the presence of CD 10 can be useful in distinguishing it from marginal zone cell lymphomas.

All the cell lines have t(14;18). This translocation, involving a rearrangement of Bcl2, is present in 70 to 95% of the cases [39,66]:

5.7. Extranodal and Nodal Marginal Zone B-Cell Lymphoma

Example: none. Rappaport: (not specifically listed) well-differentiated lymphocytic (WDL) or WDL-plasmacytoid, IDL, ILL, PDL, mixed lymphocytic-histiocytic (nodular or diffuse). Lukes-Collins: small lymphocyte B, lymphocytic-plasmacytic, small lymphocyte B, monocytoid. Working Formulation: (not specifically listed) SLL (some CLL, some plasmacytoid), small cleaved or mixed small and large cell (follicular or diffuse).

There are two major clinical presentations. (1) Extranodal marginal zone lymphomas (low grade marginal zone or MALT type) are tumors of adults. Many of the patients have a history of autoimmune disease such as Sjogren's syndrome or Hashimoto's Thyroiditis and (2) Nodal marginal zone lymphomas. There are no representative cell lines for this type of B-NHL.

Typically, the tumor cells express smIg (M>G or A), lack IgD, and about 40% are cyIg+. B-cell-associated-antigens are expressed and the tumors are CD5– CD10– CD23- CD43–/+ CD11c+/–.

No rearrangements of bcl-2 or bcl-1 are seen [85]. Trisomy 3 chromosome or t(l 1;18) have been reported [4,26].

5.8. Splenic Marginal Zone B-Cell Lymphoma

Example: none. Rappaport: (not specifically listed) well-differentiated lymphocytic (WDL) or WDL-plasmacytoid. Lukes-Collins: small lymphocyte B, lymphocytic-plasmacytic, small lymphocyte B, monocytoid. Working Formulation: (not specifically listed) SLL.

Patients with this B-NHL have BM and PB involvement. The course of the disease is indolent, and splenoctomy may be followed by prolonged remission. There are no cell lines derived from this disease.

Tumor cell antigen expression is similar to that of extra-nodal and nodal marginal zone B-cell lymphomas.

No molecular genetic changes have been identified, as the tumors are not well studied.

5.9. Diffuse Large B-Cell Lymphoma

Examples: HBL-1, KAL-1, LNPL, WSU-DLCL, WSU-DLCL2. Rappaport: diffuse histiocytic, occasionally diffuse mixed lymphocytic-histiocytic. Lukes-Collins: large cleaved or large non-cleaved FCC, B-immunoblastic. Working Formulation: diffuse large cell cleaved, non-cleaved or immunoblastic; occasionally diffuse mixed small and large cell.

Large B-cell lymphomas constitute 30–40% of adult NHLs. Patients typically present with a rapidly enlarging, often symptomatic, mass at a single nodal or extranodal site. LCL are aggressive but potentially curable [94].

The tumor cells are smIg+/–, cyIg–/+, B-cell-associated-antigens+ CD45+/– CD5–/+ CD10–/+ [21]. Most of the cell lines listed under this stage are CD5– and CD10+/– with B-cell-associated antigens+.

The *bcl*-2 gene is rearranged in about 30% of these tumors [57,99]. C-*myc* is reported to be rearranged in some cases [102]. The cell lines carry t(8;14) and t(14;18).

5.10. Burkitt's Lymphoma

Examples: BJAB, BL-41, CA-46, WSU-BL. Rappaport: undifferentiated lymphoma, Burkitt's type. Lukes-Collins: small non-cleaved FCC. Working Formulation: small non-cleaved cell, Burkitt's type.

Burkitt's lymphoma is most common in children, and 7 of the 11 cell lines were derived from tumors in children. Adult cases are often associated with immune deficiency. The tumor is very aggressive, but potentially curable.

The established cell lines provide a good representation of Burkitt's lymphoma. The cell doubling time is short, less than 24h in 6 of the 12 cell lines in which it was measured.

The tumor cells are smIgM+, B-cell-associated-antigens+ CD10+ CD5– CD23– [29]. The cell lines are smIg+/– (may have cyIg), B-cell-associated-antigens+ (11/11 of cell lines tested).

Most cases have a translocation of c-*myc* from chromosome 8 to the Ig heavy chain region on chromosome 14 (t(8;14), which is present in 7 of 11 cell lines. Less commonly, c-*myc* is translocated to light chain loci on 2 [t(2,8) or 22 t(8,22)]. Epstein-Barr virus genomes can be demonstrated in the tumor cells in most Burkitt's lymphoma arising in Africans and in 25% to 40% of cases associated with acquired immune deficiency syndrome [5,32,67].

5.11. High Grade B-Cell Lymphoma, Burkitt's-like

Example: MC116. Rappaport: undifferentiated; non-Burkitt's. Lukes-Collins: small non-cleaved FCC. Working Formulation: small non-cleaved cell, non-Burkitt's.

Tumors in this category are relatively uncommon and occur mostly in adults, sometimes with a history of immunosuppression. Cases in children appear to behave similarly to classic Burkitt's tumor [42], whereas in adults they appear to be highly aggressive.

Tumor cells are smIg +/– (may have cyIg), B-cell associated antigens+ CD5–, and usually CD 10+ [29].

C-*myc* is rearranged in approximately 30% of cases [101].

ACKNOWLEDGEMENTS

This work was supported by Grant CA79837 from the US National Cancer Institute and from the Leukemia Society of America grant No. 6323-99. The authors would like to thank Dr. Ishtiaq Ahmad for his technical help.

References

1. Abe M, Nozawa Y, Wakasa H et al. *Cancer* 61: 483, 1988.
2. Al-Katib A, Smith MR, Kamanda WS et al. *Clinical Cancer Res* 4: 1305, 1998.
3. Al-Katib A, Mohammad RM. In: Valeriote, FA, Nakeff A, Valdivieso M (eds.), *Basic and Clinical Applications of Flow Cytometry*, Kluwer Academic Publishers, Boston, 1992.
4. Auer IA, Gascoyne RD, Connors JM et al. *Annals of Oncology* 8: 979, 1997.
5. Ballerini P, Gaidano G, Gong J et al. *Blood* 81:166, 1993.
6. Battifora H, Trowbridge IS. *Cancer* 51: 816, 1983.
7. Benjamin D, Magrath IT, Maguire R et al. *J Immunol* 129: 1336, 1982.
8. Bennett J, Catovsky D, Daniel M-T et al. *J Clin Pathol* 42: 567, 1989.
9. Brown TC , Peters MV, Bergsagel DE et al. *Br J Cancer* 31 (Suppl II): 174, 1975.
10. Byme Jr GE. *Cancer Treat Rep* 61: 935, 1977.
11. Chan JK, Banks PM, Cleary ML et al. *Am J Clin Pathol* 103: 543, 1995.
12. Chao CC. *Cancer Lett* 73: 105, 1993.
13. Cossman J, Neckers LM, Arnold A et al. *N Engl J Med* 307: 1251, 1982.
14. Daibata M, Kubonishi I, Eguchi T et al. *Cancer* 64: 1248, 1989.
15. De Boer CJ, Schuuring E, Dreef E et al. *Blood* 86: 2715, 1995.
16. Dillman RO, Handley HH, Royston I. *Cancer Res* 42: 1368, 1982.
17. Doi S, Ohno H, Talsumi E et al. *Blood* 70: 1619, 1987.
18. Dorfman RF. In: *The Reticuloendothelial System*, International Academy of Pathology, No. 16, Baltimore, Williams & Wilkins, p262, 1975.
19. Dorfman RF. *Lancet* 1: 1295, 1974.

20. Drexler H, Thiel E, Ludwig W-D. *Leukemia* 5: 637, 1991.
21. Doggett R, Wood G, Horning S et al. *Am J Pathol* 115: 245, 1984.
22. Dyer M, Fischer P, Nacheva E et al. *Blood* 75: 709, 1990.
23. Efremidis AP, Haubenstock H, Holland JF et al. *Blood* 66: 953, 1985.
24. El-Sonbaty et al. *Leukemia Res* 19: 249, 1995.
25. Epstein AL, Leu YR, Kim H et al. *Cancer* 42: 2379, 1978.
26. Fiun T, Isaacson P, Wotherspoon *A. J Pathol* 170: 335, 1995.
27. Foon KA, Todd RF. *Blood* 68: 1, 1986.
28. Ford R, Goodacre A, Ramirez I et al. *Blood* 75: 1311, 1990.
29. Garcia C, Weiss L, Warnke R. *Hum Pathol* 17: 454, 1986.
30. Gaul EA, Mallony TB. *Am J Pathol* 18: 381, 1942.
31. Gerard-Marchant R et al. *Lancet 2:* 406, 1974.
32. Hamilton-Dutoit S, Pallesen G, Franzmann M et al. *Am J Pathol* 138: 149, 1991.
33. Hann SR, Thompson CB, Eisenman RN. *Nature* 314: 366, 1985.
34. Harris N, Bhan A. *Hum Pathol* 16: 829, 1985.
35. Harris NL, Jaffe ES, Stein H et al. *Blood* 84: 1361, 1994.
36. Harris NL, Jaffe ES, Stein H et al. *Blood* 85: 857, 1995.
37. Henry K, Bennett MH, Farrer-Brown G. In: Mathe C, Seligmann M, Tubiana M (eds.), *Recent Results in Cancer Research,* New York, Springer-Verlag, pp 38–56, 1978.
38. Ho Y-S, Sheu L-F, Ng J-A et al. *Br J Cancer* 61: 655, 1990.
39. Hockenbery D, Zutter M, Hickey W et al. *PNAS USA* 88: 6961, 1991.
40. Hokland P, Ritz J, Schlossman SF et al. *J Immunol* 135: 1746, 1985.
41. Hsu S-M. *Am J Clin Pathol* 80: 429, 1983.
42. Hutchison R, Murphy S, Fairclough D et al. *Cancer* 64: 23, 1989.
43. Ichinose I, Nakano S, Ohshima KI et al. *Cancer Res* 51: 5392, 1991.
44. Janossy G, Bollum F, Bradstock K et al. *Blood* 56: 430, 1980.
45. Janossy G, Bollum FJ, Bradstock KF et al. *J Immunol* 123: 1525, 1979.
46. Jeon HJ, Kirn CW, Yoshino T, et al. *Br J Haematol* 102: 1323, 1998.
47. Jones SE, Fuks Z, Bull M et al. *Cancer* 31: 806, 1973.
48. Knuutila S, Klefstrom J, Szymanska J et al. *Eur J Hematol* 52: 65, 1994.
49. Korsmeyer S, Hieter P, Pavetch J et al. *Proc Natl Acad Sci USA* 78: 7096, 1981.
50. Lambrechts AC, Dorssers LCJ, Hupkes PE et al. *Leukemia* 8: 1164, 1994.
51. Lee WP. *FEBS Lett* 373: 285, 1995.
52. Lennert K , Mohri N, Stein H et al. *Br J Haematol* 31 (Suppl): 193, 1975.
53. Lennert K , Feller A. *Histopathology of Non-Hodgkin's Lymphomas*, 2nd edn., Springer-Verlag, New York, 1992.
54. Lenoir GM, Preud'homme JL, Bernheim A et al. *Nature* 298: 474, 1982.
55. Lenoir et al. *IARC Scientific Publ* 60: 309, 1985.
56. Levine EG, Arthur DC, Frizzer G et al. *Blood* 66: 1414, 1985,
57. Lipford E, Wright J, Urba W et al. *Blood* 70: 1816, 1987.
58. Lok MS, Koshiba H, Han T et al. *Int J Cancer* 24: 572, 1979.
59. Lukes RJ , Butler JJ. *Cancer Res 26:* 1063, 1966.
60. Lukes RJ, Collins RD. *Cancer* 34: 1488, 1974.
61. Magrath IT, Freeman CB, Pizzo P et al. *J Natl Cancer Inst* 64: 477, 1980.
62. Manolova Y, Manolova G, Kieler J et al. *Hereditas* 90: 5, 1979.
63. Mathe G et al. In: *WHO International Histological Classification of Tumors*, No. 59, 813, 1981.
64. Matsumara I, Tomaki T, Katagiri S et al. *Int J Cancer* 46: 1107, 1990.
65. Matutes E, Morilla R, Owusu-Ankomah K et al. *Blood* 83: 1558, 1994.

66. McDonnell T, Deane N, Platt F et al. *Cell* 57: 79, 1989.
67. Meeker T, Shiramizu B, Kaplan L et al. *Blood* 81: 166, 1993.
68. Menezes J, Bourkas AE. *Biomedicine* 31: 2, 1979.
69. Minowada J. *Haematol Blood Trans* 26: 322, 1981.
70. Mohamed AN, Mohammad RM, Koop BF et al. *Cancer* 64: 1041, 1989.
71. Mohamed AN, Al-Katib A., *Leukemia Res* 12: 833, 1988.
72. Mohammad RM, Al-Katib A. *Leukemia Res* 17: 1, 1993.
73. Mohammad RM, Mohamed AN, Smith MR et al. *Cancer Genet Cytogenet* 70: 62, 1993.
74. Mohammad RM, Mohamed AN, Hamdan MY et al. *Leukemia* 10: 130, 1996.
75. Mohammad RM, Mohamed AN, KuKuruga M et al. *Cancer* 69: 1468, 1992.
76. Nacheva E, Dyer MJS, Metivier C et al. *Blood* 84: 3422, 1994.
77. Nadler LM. In: Wilson JD, Braunwald E, et al (eds.), *Harrison's Principles of Internal Medicine* 12th edn., Vol. 2, McGraw-Hill, New York, 1991.
78. Nadler LM, Anderson KC, Marti G et al. *J Immunol* 131: 244, 1983.
79. Nagai M, Fujtta M, Ohmori M et al. *Hematol Oncol* 15: 109, 1997.
80. Nilsson K, Sundstrom C. *Int J Cancer* 13: 808, 1974.
81. Nilsson K, Klareskog L, Ralph P et al. *Hematol Oncol* 1: 277, 1983.
82. Nishikori M, Hansen H, Jhanwar S et al. *Cancer Genet Cytogenet* 12: 39, 1984.
83. O'Briain D, Kennedy M, Daly P et al. *Am J Surg Pathol* 13: 691, 1989.
84. Ohta M, Furukawa Y, Ide C et al. *Cancer Res* 46: 3067, 1986.
85. Pan L, Diss T, Cunningham D et al. *Am J Pathol* 135: 7, 1989.
86. Preud'homme JL, Seligmann M. *Blood* 40: 777, 1972.
87. Pesando J, Ritz J, Lazarus H et al. *Blood* 54:1240, 1979.
88. Pulvertaft *Lancet* 1: 238, 1964.
89. Rappaport H. In: *Atlas of Tumor Pathology,* sec 3, fasc 8, Washington DC, US Armed Forces Institute of Pathology, 1966.
90. Roos G, Adams A, Giovanella B, et al. *Leukemia Res* 6: 685, 1982.
91. Stein H, Lennert K, Feller A et al. *Adv Cancer Res* 42: 67, 1984.
92. Sugawara I. *Cellular Immunol* 72: 88, 1982.
93. Sundstrom C, Nilsson K. *Int J Cancer* 17: 565, 1976.
94. *The Non-Hodgkin's Lymphoma Classification Project*: National Cancer Institute. *Cancer* 49: 2112, 1982.
95. Th'ng, KH. *Int J Cancer* 39: 89, 1987.
96. Wang CY, Al-Katib A, Lane Cl et al. *J Exp Med* 158: 1757, 1983.
97. Warnke RA, Gatter KC, Falini B et al. *N Engl J Med* 309: 1281, 1983.
98. Weisenburger DD, Armitage JO. *Blood* 87: 4483, 1996.
99. Weiss L, Warnke R, Sklar J et al. *N Engl J Med* 317: 1185, 1987.
100. Winter JN, Variakojis D, Epstein AL. *Blood* 63: 140, 1984.
101. Yano T, van Krieken J, Magrath I et al. *Blood* 79: 1282, 1992.
102. Yunis J, Mayer M, Amesen M. *N Engl J Med* 320: 1047, 1989.
103. Zukerberg L, Medeiros L, Ferry J et al. *Am J Clin Pathol* 100: 373, 1993.

Chapter 10

Mature T-Cell Malignancies

Martin J.S. Dyer
Academic Hematology and Cytogenetics, Haddow Laboratories, Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK. Tel: +44-208-722-4254; Fax: +44-208-770-1208; E-mail: mdyer@icr.ac.uk

1. INTRODUCTION

Malignancies of mature, post-thymic T-cells are rare in comparison with their B-cell counterparts and are highly heterogenous. They continue to pose major clinical problems both in terms of diagnosis and management. This is in part due to their rarity, but also to the fact that diagnosis requires detailed immunophenotypic and genotypic analyses to demonstrate lineage, clonality and stage of differentiation. In many cases, these data are not available. More extensive study of the pathogenesis of the various types of malignancy remains hampered by the lack of suitable cell lines.

The purpose of this chapter is to review some of the functions of mature T-cells and advances in our understanding of the different forms of mature T-cell malignancy, to describe some of the derived cell lines and to place these in the current scheme of classification.

2. FUNCTIONS OF MATURE T-CELLS

T-lymphocytes derive from hematopoietic precursor cells within the bone marrow, which initially migrate to the thymus. Here, the T-cell receptor for antigen (TCR) proteins are first expressed following *TCR* gene rearrangement. A complex process of both positive and negative selection of antigen- and self-reactive T-cells occurs through interaction of the T-cell precursors with thymic stromal and antigen presenting cells. Two different T-cell lineages can be identified on the basis of their expression of TCR proteins composed of either $\mathrm{TCR}\alpha/\beta$ or $\mathrm{TCR}\gamma/\delta$ heterodimers. These lineages have different functions and tissue distributions. In clinical specimens, affiliation

J.R.W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol. III, 321–337.

to either lineage can be ascertained either by the use of monoclonal antibodies (MAB) specific for constant epitopes within the TCR proteins or by using DNA methods to detect clonal rearrangements within the *TCR* genes. The *TCRδ* gene segments are located entirely within the *TCRα* complex and rearrangement of *TCRα* results in complete deletion of the *TCRδ* sequences. Mature T-cells of the **TCRγ/δ** lineage comprise about 5% of total peripheral blood T-cells and malignancies of this lineage are uncommon and have some distinct properties, as discussed below.

On emerging from the thymus, mature T-cell subpopulations express a panoply of surface membrane proteins that reflect their functions. The functions of many of these proteins have now been identified. Some of those that have been used clinically are shown in Table 1 (reviewed in Barclay et al. 1998). These molecules can be used to differentiate the malignancies of T-cell precursors (T-cell lymphoblastic leukemias and lymphomas) from the various malignancies of post-thymic T-cells and from malignancies of other related lineages, notably malignancies of natural killer (NK) cells. Some forms of T-cell malignancy may co-express both T-cell and NK lineage markers. Clinically, the most widely utilized of these proteins are the CD4 and CD8 molecules, which broadly divide mature T-cells into those which mediate B-cell "help" and those which mediate T-cell cytolysis respectively. In contrast to thymic malignancies that are often CD4/CD8 double positive, mature post-thymic T-cells usually express only one or other of these molecules, although in some instances, notably in T-cell prolymphocytic leukemia, co-expression of CD4 and CD8 may be observed. Assessment of the expression of the nuclear enzyme terminal deoxynucleotidyl transferase (TdT) may be necessary to distinguish malignancies of T-cell precursors from those of mature T-cells.

Mature T-cells migrate to a number of peripheral lymphoid sites, including spleen and lymph nodes, but also to more "specialized" sites such as the skin and intestinal epithelia; T-cells in these sites may differ from those elsewhere. They are competent to perform a number of different effector functions including mediation of:

- B-cell "help" to produce specific antibodies (predominantly a function of CD4+ subpopulation).
- Cytolysis of virally infected/bacterially infected cells as well as allogeneic and malignant cells (predominantly a function of CD8+ subpopulation).
- Stimulation of monocytes/macrophages in the inflammatory response.

These subjects are discussed in detail in Paul [37].

Table 1. Clinically utilized T-cell and NK differentiation antigens

CD No.	Cellular distribution	Functions
1	Cortical thymocytes	Antigen presentation
2	Thymocytes and mature T-cells	Adhesion molecule – binds CD58
3[a]	Thymocytes and mature T-cells	Component of the antigen receptor
4	Thymocytes/mature T-cell subsets	Component of TCR/coreceptor for MHC class II
5	Thymocytes, T-cells, some B-cells	T-cell activation – binds CD72
7	Thymocytes and mature T-cells	Unknown
8	Thymocytes/mature T-cell subsets	Component of TCR/Coreceptor for MHC class I
25	Immature thymocytes/activated T-cells	Component of IL-2R
28	Thymocytes and mature T-cells	T-cell activation – binds B7.1/2
52	All lymphocytes	Unknown
56	NK cells	Adhesion molecule – NCAM isoform
57	NK cells, some mature T-cells	Unknown
TdT	Lymphoid precursors	Adds in non-templated nucleotides during recombination
HLA-DR	Mature/activated T-cells	Antigen presentation

[a] Surface CD3 expression seen in most mature T-cell malignancies *in vivo*, whereas expression is limited to the cytoplasm in malignancies of T-cell precursors.

3. CLASSIFICATION OF MATURE T-CELL MALIGNANCIES

Malignancies of mature T-cells can be objectively diagnosed with MAB specific for T-cell differentiation antigens and PCR or DNA blot methods for the detection of clonal *TCR* gene rearrangements. Using these techniques, a number of distinct clinical entities have been recognised. These can be divided into those that present with a primarily leukemic picture and those that present with primarily lymph nodal or extra-nodal infiltration (Table 2). The interested reader is referred to a monograph [28] and to several papers [4,20,39].

Given the distinctiveness of the individual diseases, the cell lines are described within the most appropriate clinical entity. Only those diseases from which cell lines have been derived are mentioned in detail below. T-cell prolymphocytic leukemia is also discussed as this disease, despite its highly aggressive nature, has repeatedly failed to yield cell lines, and the lack of such lines remains a major deficiency.

Table 2. Classification of mature T-cell malignancies

(A) Predominantly leukemic	
Adult T-cell leukemia/lymphoma	(ATLL)
T-cell prolymphocytic leukemia	(T-PLL)
T-cell large granular lymphocytic leukemia	(T-LGL)
NK/T-cell leukemia/lymphoma	
(B) Predominantly nodal	
Nodal peripheral T/NK cell lymphoma unspecified	
Anaplastic large cell lymphoma	
Angioimmunoblastic T-cell lymphoma	
(C) Predominantly extranodal	
Mycosis fungoides/Sézary syndrome	(MF/SS)
Enteropathy-associated intestinal T-cell lymphoma	
Primary cutaneous CD30+ve lymphoproliferative disorders	
Subcutaneous panniculitis-like T-cell lymphoma	
NK/T-cell lymphoma – nasal type	
Hepatosplenic T-cell lymphoma	
Extranodal peripheral T-cell lymphoma unspecified	

3.1. Mature T-Cell Leukemias

A comparison of the typical immunophenotypes of the mature T-cell leukemias is shown in Table 3. Distinction from malignancies of T-cell precursors is usually made from the cytological appearances in conjunction with expression of surface membrane CD3 and HLA-DR and absence of CD1 and TdT.

Table 3. Comparison of the common immunophenotypes of mature, post-thymic T-cell leukemias

Disease	sCD3	TCRα/β	CD5	CD7	CD4+/CD8−	CD4+/CD8+	CD4−/CD8+	CD25
T-PLL	++	++	++	++	++	+	−	−
T-LGL	++	++	++	++	−	−	++	−
ATLL	+/−	+/−	++	+/−	++	−	−	++
MF/SS	++	++	++	+/−	++	−	−	−

3.1.1. Adult T-Cell Leukemia/Lymphoma (ATLL)

This disease is intimately associated with the human retrovirus, HTLV-1, although the role of the virus in the etiology of the disease, like EBV in the pathogenesis of Burkitt lymphoma, remains obscure. ATLL is clustered within regions in which HTLV-1 is endemic, including south-western Japan and the Caribbean basin. All cases show clonal HTLV-1 proviral integration and have serological evidence for HTLV-1 infection. About 60% of patients present with a leukemic form of the disease, whilst the remainder present with lymphomatous disease. Hypercalcemia is common at presentation. A characteristic feature is high expression of CD25, a component of the IL-2 receptor.

Many cell lines used in the study of HTLV-1 infection and its role in neoplastic transformation of T-cells were produced by the co-culture of uninfected lymphocytes with virus-producing cells (for example, Miyoshi et al. [31]). These cell lines, which include MT-2, are not discussed further. There is also in the literature a large number of cell lines derived directly from patients with leukemia and lymphoma, predominantly from Japanese patients (see for example Nakao et al. [34] and references therein; Morita et al. [33]; Sagawa et al. [41]). Most of these are poorly characterized. In contrast, the HUT-102 cell line has been extensively studied. This cell line was derived from the primary culture of lymph node cells from a patient with ATLL, initially in the presence of IL-2. The origin of this cell line and the HUT-78 cell line and their uses in the isolation and characterization of the HIV and HTLV-1 have been reviewed recently (Bunn and Foss [7], both cell lines originally described in Gazdar et al. [16]).

The ATLL cell line MU is of some interest [24]. Despite being derived from the peripheral blood of a patient with ATLL in leukemic phase, the cell line fails to express any of the anticipated range of T-cell differentiation antigens *in vitro*. The only evidence that this cell line was derived from the leukemic cells is the presence of identical *TCRβ* rearrangement in both the primary cells and the derived cell line. There is also another interesting cell line, KHM-3S, derived from a Japanese patient with small cell lung cancer, which nevertheless expresses CD25 and CD56, but is otherwise negative for all other hematopoietic markers such as CD45 [26]. This cell line shows monoclonal proviral integration, but most surprisingly, clonal *TCR* rearrangements. The tropism of HTLV-1 is not limited to T-cells alone and the cell of origin of both cell lines is not obvious. The presence of the clonal *TCRβ* rearrangements would suggest a T-cell origin but the complete lack of expression of all T-cell differentiation antigens is unusual.

In contrast, an rIL2-dependent cell line from a patient with apparent ATLL (WHN2) has been described which, despite retaining the phenotype, karyo-

type and clonal *TCR* gene rearrangements of the primary tumor, failed to demonstrate any clonal HTLV-1 proviral integration [21].

3.1.2. T-cell Prolymphocytic Leukemia (T-PLL)

T-PLL is a very aggressive disease which typically presents in the sixth or seventh decade with extremely high white cell counts (up to 10^{12}/litre), organomegaly, usually splenomegaly and lymphadenopathy [21,28]. The disease is often completely resistant to intensive chemotherapy but frequently remits with the humanized Mab, CAMPATH-1H [38]. A disease with identical cytology, immunophenotype, karyotype and molecular features has been seen in adult patients with ataxia-telangiectasia [45].

Cytogenetically, T-PLL is characteristically associated with rearrangements of chromosome 14 involving the *TCRα/δ* locus at 14q11.2 and the *TCL1* locus at chromosome 14q32.1, centromeric of the *IGH* locus, which is located at the telomere at 14q32.3. These rearrangements take the form of either t(14;14)(q11.2;q32.1)or inv(14)(q11.2;q32.1). They are not, however, specific for T-PLL, and identical translocations and inversions have been reported in T-cell precursor ALL. Cases which lack rearrangements of the *TCL1* locus may exhibit rearrangement of the *MTCP1* gene, a protein homolog of *TCL1*, on chromosome Xq28 as either t(X;14)(q28;q11.2) or involving the *TCRβ* locus on 7q35 as t(X;7)(q28;q35). Acquired abnormalities within the ataxia telangiectasia or *ATM* gene are common in T-PLL, and homozygous mutations and structural rearrangements within the gene may be seen in most cases of sporadic T-PLL [46,47].

Despite the aggressive nature of the disease, there are no cell lines available from T-PLL. SKW-3 was derived from a 68-year-old Japanese man with some form of mature T-cell leukemia, but whether or not this truly represents T-PLL cannot be determined. There is no reference available that describes the establishment of this line. Initially, SKW-3 co-expressed CD4 and CD8, as is seen in some cases of T-PLL, but with further *in vitro* growth, expression of these molecules has been lost (Larsen et al. [25] and references therein). This cell line is of some interest as it has a t(8;14)(q24.1;q11.2) chromosomal translocation involving the MYC oncogene on chromosome 8 with the *TCRδ/α* locus on chromosome 14 [15,29,44]. This translocation is therefore a variant of the *MYC* translocations involved in Burkitt and other forms of mature B-cell malignancy that involve *MYC* with the various immunoglobulin loci. Chromosome abnormalities of chromosome 8, but not usually translocations involving *MYC*, are seen commonly in T-PLL.

A related cell line may be the Kit-225 cell line, which was also derived from an elderly Japanese man with T-cell lymphocytosis [19]. This cell line is rIL2 dependent and has a complex karyotype with, like SKW-3, a breakpoint on chromosome 3q27. Both Kit-225 and SKW-3 are HTLV-1 negative.

We have examined some of the derived T-cell precursor ALL/lymphoblastic lymphoma cell lines with either inv(14) or t(14;14) to determine if any of these might have been derived from patients with T-PLL. SUP-T11 (Chapter 4) for example was derived from a 78-year-old man in whom the diagnosis of T-PLL might be anticipated. However, this cell line has the phenotype of TCP-ALL. HT-1 is another TCP-ALL cell line with inv(14) [1]. It has been claimed that it is possible to grow T-PLL cells in immunodeficient mice although, despite repeated attempts, we have been unable to grow xenografts. The development of a T-cell lymphoproliferative disorder similar to T-PLL in mice transgenic for the *TCL1* oncogene may allow further advances in our understanding of this disease.

3.1.3. T-Cell Large Granular Lymphocytic Leukemia (T-Cell LGL)

This disease is characterized by the cytological appearances of the malignant cells within the blood and marrow. There are T-cell (sCD3+ve) and NK (sCD3-ve) forms of the disease. Patients present with only mild lymphocytosis and often with cytopenias. Lymphadenopathy is uncommon. Clonality studies are therefore essential to demonstrate this leukemia. The disease usually follows an indolent course, although the NK form may be more aggressive. There has been no consistent cytogenetic abnormality detected.

No typical human T-cell LGL cell lines have been reported, although cell lines with an NK-LGL phenotype exist (Chapter 4). Interestingly, 50% of the Fisher strain of laboratory rats develop T-cell LGL and cell lines have been derived. The nature of the genetic abnormalities underlying this remains unknown. One IL-2 dependent cell line (EBT-8) has been derived from a patient with Epstein–Barr viral-associated disease. Although described as LGL and although the phenotype of the cells is consistent with this diagnosis, the presence of EBV in the cells is not characteristic.

3.1.4. NK/T-Cell Leukemia/Lymphoma

These malignancies co-express differentiation antigens of both T-cell and NK lineages. Some of these are EBV positive and are mentioned below. Others are EBV negative and often pursue an aggressive and leukemic pattern. They can be distinguished from malignancies of the NK lineage by the presence of clonal *TCR* gene rearrangements. A cell line (MTA) from one such patient has been described and may be useful in determining the pathogenesis of this disease [13]. This cell line co-expresses CD2, sCD3, CD4 and CD56 and has clonal *TCR* rearrangements.

3.1.5. Sézary-Cell-Like Leukemia

This disease is very rare and related to T-PLL. No cell lines are known to exist, although the HUT-78 cell line, with its highly complex karyotype, may have been derived from such a patient.

3.2. Nodal T-Cell Lymphomas

3.2.1. Nodal Peripheral T-NHL – Unspecified

This is something of a "waste-basket" for T-cell lymphomas that do not readily fit into a more specific category and, unfortunately, is the largest diagnostic group. There are no consistent immunophenotypic, cytogenetic or molecular features. CD4 expression is more common than CD8, but expression of this and other T-cell differentiation antigens can change during the course of the disease; loss or lack of expression of CD7 is common. Most of the described T-cell lines fall into this category, although it should be noted that the description of the cell lines is in nearly all cases inadequate in one or more aspects and limited to a single report.

3.2.2. Anaplastic Large Cell Lymphoma (ALCL)

ALCL are characterized by chromosomal translocations involving the *ALK* gene that encodes a tyrosine kinase and is located on chromosome 2p23. The most common translocation is the t(2;5)(p23;q35), which involves *ALK* with the nucleophosmin or *NPM* gene on chromosome 5. Unlike most other translocations that are specific for one hematopoietic lineage and often for one specific disease, *ALK* translocations are seen in lymphomas of both B and T-cell lineages. There are several *ALK*+ve T-cell lines available: these are discussed in Chapter 11. It is noteworthy that the cell line HPB-MLp-W, derived from a patient with a poorly characterized T-NHL, expresses CD30. This cell line does not appear to show the karyotypic changes typical of ALCL, but it has not been studied with modern cytogenetic methods. Whether it expresses ALK has not been established.

3.3. Extranodal T-Cell Lymphomas

3.3.1. Mycosis Fungoides/Sézary Syndrome

This is primarily a cutaneous disorder. Mycosis fungoides often presents with localized skin lesions and pruritus. Sézary syndrome may be considered as the leukemic manifestation of Mycosis fungoides, although there may also be lymph nodal involvement. Transformation to large-cell T-NHL may occur in about 10% of patients. Most, if not all cases are CD4+ve and lack expression of CD25. Association of MF/SS with retroviruses has been claimed, but has not been confirmed in large studies.

The HUT-78 cell line was derived from a patient with typical Sézary syndrome and exhibits a number of interesting features. It is the only malignant lymphoid cell line to express large amounts of the CAMPTH-1 (CD52) antigen. This molecule, whose functions are unknown, is expressed at very high levels ($> 5 \times 10^5$ molecules per cell comprising about 2% of the cell surface) on nearly all malignant lymphocytes of both T-cell and B-cell lineages and has been used as a target for antibody therapy. However, for reasons which remain obscure, the protein is lost during *in vitro* culture in most cell lines with the exception of HUT-78, which retains levels of expression comparable to those seen *in vivo*. HUT-78 is therefore useful in modelling therapy with CD52 antibodies.

HUT-78 exhibits an extermely complex karyotype which has not yet been adequately studied [9]. Nevertheless, molecular studies have shown a number of interesting abnormalities, although none are characteristic of "regular" Sézary syndrome. Firstly, HUT-78 exhibits a potent synergistic combination of deregulated *MYC* expression and p53 mutation [10] that may explain the rapid growth of the cells. The *MYC* expression derives from a chromosomal translocation t(2;8)(q34;q24) involving the *MYC* oncogene with a novel locus, TCL4 on chromosome 2q34 [14]. Both the *MYC* translocation and the p53 mutation (analogous to that seen in Burkitt lymphoma) appear to be unusual events in the pathogenesis of MF/SS and indeed of all T-cell malignancies. The possible involvement of the *TCL4* gene in other T-cell malignancies remains to be determined.

HUT-78 also exhibits carboxy-terminal deletion of two molecules involved in signal transduction. The *NFκB2/Lyt-10* gene, which maps to chromosome 10q24, was cloned through its direct involvement in the t(10; 14)(q24;q32) seen in a subset of aggressive B-cell lymphomas (reviewed in Neri et al. [35]). HUT-78 exhibits a carboxy-terminal truncation of this molecule [48]. *c-CBL*, a ring-finger gene that maps to human chromosome 11q23.3 has also been shown to undergo deletion through rearrangement with unknown sequences [6]. Neither genetic rearrangement appears to be common. Further molecular genetic dissection of the chromosomal abnormalities of HUT-78 is warranted.

3.3.2. Enteropathy-Associated Intestinal T-Cell NHL

This disease is strongly associated with celiac disease (gluten-sensitive enteropathy) and appears to arise from T-cells present within the mucosa which are predominantly CD8+ve/CD103+ve. Disease is usually localized to the small bowel and patients may present with perforation or obstruction. At least one cell line, (OCI-Ly 17) has been derived from a patient with this disease, but it is poorly characterized and the clonal relationship with the primary tumor was not demonstrated.

3.3.3. Hepatosplenic T-NHL

Historically, this disease was described as a disease of T-cells of the TCRγ/δ lineage although cases of the α/β lineage have now been reported [11]. This disease occurs mostly in young males, with marked hepatosplenomegaly occurring in the absence of significant lymphadenopathy. There is a characteristic immunophenotype: CD2+, CD3+, CD7+, CD56+ with expression of TCRγ/δ proteins. Isochromosome 7q(iso(7q)) has been suggested to be a recurrent event [42,43]. One cell line (HPB-MLp-W) with this cytogenetic abnormality, on the background of a highly complex karyotype, has been reported. However, from the clinical data presented it is very unlikely that the cell line was derived from a case of hepatosplenic T-NHL.

3.4. EBV-Related T-Cell Lymphomas

Although in lymphocytes EBV has been classically associated with infection of B-cells, it is now clear that EBV can infect T-cells through low level expression of CD21 on these cells. In some cases this can give rise to EBV-associated lymphomas. Syndromes include:

- Post-transplant T-cell lymphoproliferative disorders, although these remain rare in contrast with their B-cell counterparts [17].
- Nasal T-cell/NK lymphomas are strongly associated with EBV. These occur primarily in the Orient. There is one recently described cell line (HANK-1), although from the presence of the germline *TCR* genes it is likely that the cell line belongs to the NK rather than the T-cell lineages [22].
- Severe chronic active EBV infection leading to EBV-positive T-NHL [22].

EBV positive normal T-cell lines have been described. The EBV-associated LGL cell line EBT-8 has been mentioned above. Details of the EBV-positive DEGLIS cell line which co-expresses T-cell and B-cell lineage antigens and exhibits a most unusual combination of *TCR* and *IG* gene rearrangements are given in the tables. The cell of origin of this apparently unique cell line is not known, but the authors suggest that it arose in a "cell broadly committed to the lymphoid lineage".

4. CELL LINES DERIVED FROM PATIENTS WITH MATURE T-CELL MALIGNANCIES

There are at least two problems in discussing cell lines derived from patients with mature T-cell malignancies. Firstly, the classification presented above is recent, and therefore the precise categorization of cell lines established

Table 4. Mature T-cell malignant cell lines: clinical characterization

Cell line	Patient age/sex	Diagnosis	Treatment status	Specimen Site	Authenti-cation	Year est.	Culture medium	Availability	Primary ref.
HUT 78	53 M	Sézary syndrome	NK	PB	NO	1977	RPMI 1640 10% FCS	ATCC	16
HUT 102	26 M	ATLL leukemic phase	NK	LN	NO	1978	RPMI 1640 10% FCS	ATCC	16
KARPAS 384	48 M	T-NHL NOS— subcutaneous → leukemic transformation	PROGR	PB	YES	1987	RPMI 1640 10% FCS	Author	12
SKW-3	61 M	Mature T-cell leukemia	NK	PB	NO	NK	RPMI 1640 10% FCS	DSMZ	Unknown
SMZ-1	46 M	T-NHL NOS/stage IV/ systemic lupus erythematosis (SLE)	DIAGN	ASCITES	NO	1992	RPMI 1640 15% FCS	Author	30
ST-4	12 M	T-NHL NOS	DIAGN	LN	NO	1984	RPMI 1640 10% FCS	Author	2
PFI-285	13 M	?T-NHL NOS/stage IV	2ND REL	PB	NO	Unknown	RPMI 1640 10% FCS	Author	
OC-LY 17	72 M	T-NHL NOS/celiac disease	DIAGN	PB	NO	1987	IMDM 20% human plasma	Author	8
MU	37 M	ATLL leukemic phase	NK	PB	YES	1986	RPMI 1640 20% FCS	Author	24
HPB-MLp-W	50 F	T-NHL NOS/stage IV/CD30+ve	DIAGN	LN	NO	1986	RPMI 1640 10% FCS	Author	32
EBT-8	36 M	T-cell LGL(?) EBV+ve	NK	PB	NO	1994	RPMI 1640 10% FCS + 40U/mL rIL2	Author	3
T-34	73 M	T-NHL NOS	1ST REL	LN	YES	1986	RPMI 1640 20% FCS	Author	36
HANK-1	46 F	Nasal type T/NK NHL retroperitoneal mass	DIAGN	LN	YES	1995	Cosmedium 20% human plasma+ 100U/ml rIL2	Author	22
DEGLIS	68 M	Mediastinal mass	DIAGN	LN	YES	1990	Iscoves's 10% FCS	Author	40
KIT-225	62 M	Mature T-cell leukemia	DIAGN	PB	YES	1985	RPMI 1640 10% FCS + 10U/mL rIL2	Author	19

Table 5. Mature T-cell malignant cell lines: immunophenotypic characterization

Cell line	T-cell marker	B-cell marker	Myelomonocytic marker	Non-lineage/ activation markers
HUT 78	CD3+, CD4+, CD8−	NT	NT	CD25− HLA-DR+
HUT 102	CD4+, CD8−	NT	NT	CD25+, HLA-DR+
KARPAS 384	CD1a−, CD2−, sCD3+, CD4−, CD5−, sCD7−, cCD7+, CD8−, TCRα/β−, TCRγ/δ−	CD10−, CD19−, CD20−	NT	CD25−, CD38−, CD45+, CD45RO−, CD52−, HLA-DR+
SKW-3	CD2+, CD3−, CD4−, CD5+, CD6+, CD7+, CD8−, TCRα/β−	NT	CD13−	
SMZ-1	CD1b−, CD2+, sCD3+, CD4+, CD5−, CD7+/−, CD8−, CD28+/−	CD10−, CD19−, CD20−	CD13−, CD14−, CD15−, CD16−	CD25−, CD71+, HLA-DR+
ST-4	CD1+, CD2−, sCD3−, CD4−, CD7+, CD8−	CD10−	NT	CD25−, HLA-DR−
PFI-285	CD1+/−, CD2+, sCD3−, CD4+/−, CD5+, CD7+, CD8+/−	CD10−, CD19−	CD15−, CD16−, CD36−	CD25−, HLA-DR−
OC-LY 17	CD2+, sCD3−, CD4+, CD5−, CD7−, CD8−,	CD10−, CD19−, CD20−, sIg−	NT	
MU	CD1−, CD2−, CD3−, CD7−, CD8−	CD10−, CD19−, CD20−, sIg−	CD11−, CD13+, CD14−, CD15−, CD16	CD25+/−, CD34−, HLA-DR+/−
HPB-MLp-W	CD1a−, CD2+, sCD3−, cCD3+, CD4+, CD5−, CD7−, CD8+, TCRα/β− TCRγ/δ−	CD9−, CD10−, sIg−	CD13−, CD14−, CD15−, CD16−	CD25+, CD30+, HLA-DR+
EBT-8	CD2+, sCD3+, CD4−, CD8+, TCRα/β+, TCRγ/δ− NK markers CD56, CD57−	CD19−, CD20−, CD21−, CD23−	NT	CD25+, HLA-DR+
T-34	CD1a−, CD2+/−, sCD3+, CD4+/−, CD8−,	CD10−, CD19−, sIg−	NT	CD25+/−

Continued on next page

Table 5. (continued)

Cell line	T-cell marker	B-cell marker	Myelomonocytic marker	Non-lineage/ activation markers
HANK-1	CD1−, CD2+, CD3− CD4−, CD5−, CD7+, CD8−, cCD3ε+ NK markers CD56+, CD57−,	CD10−	NT	CD25−, HLA- DR+
DEGLIS	CD1−, CD2+, cCD3+/−, CD4+, CD5−, CD7+, CD8− TCRα/β−	CD10−, CD19+, CD20+, CD21−, CD22−, CD23+, CD37+	CD11−, CD14−, CD15−, CD16− CD68+	CD25−, CD30+, CD70+, CD45+, CD45RO− TdT+
KIT-225	SCD3+, CD4+, CD6+, CD8−	NT	NT	CD25+, HLA-DR+

Table 6. Mature T-cell malignant cell lines: genetic characterization

Cell line	Cytogenetic karyotype	Chromosomal rearrangements of potential interest	Antigen receptor gene rearrangements	Ref.
HUT 78	*Extremely complex* – see Chen [9]	*MYC/TCL4* rearrangement Truncation of *NFκB2* and *c-CBL*		
HUT 102	Poorly characterized	Unknown		
KARPAS 384	47, XO, +20, t(1;2)(q11;q35), t(2;1;14)(q35;q11- q32.1;q22.1), t(7;14)(p13;q11.2), inv(7)(p13;q22.1), int deletion(12)(q24.1q24.3)+ marker	14q11.2 = location of the *TCRα/δ* locus. t(7;14)(p13;q11.2) does not involve the *TCRγ* locus	Belongs to the TCR*γ/δ* lineage (a) 3 TCR Jδ3 rearrangements presumably marking involvement in 14q11.2 translocation (b) *TCRVγ9/JγP/Cγ1* rearrangement	12
SKW-3	43-48 XY, +8, –14, t(3;3)(q11;q27), der(8)t(8;14)(q24;q11)x2, t(8;11)(p21;p12), der(12)t(12;?)(q24;?), der(14)t(8;14)(q24;q11)	*MYC* translocated to the *TCRα/δ* locus		15
SMZ-1	47 XY, t(6;14)(p21.1;q24), +8, del(9)(p13q22), der(9)t(1;9)(p12;p13), –10, der(17)(?::p11- q23::?), +21	Unknown		
ST-4	47 X -Y, +X, t(2;6)(q21;q23)+der(2)t(2;6)(q21;q23)			
PFI-285	Normal karyotype in 29/44 metaphases – remainder = tetraploid only with no structural changes			
OC-LY 17	Not determined			
MU	*Clonal evolution* 54 XY, –1, +7, +8, –14, +19, +20, +21, + der(1)t(1;?)(q36:?), + del(6)(q13q25), +der (14)t(14;?)(q32;?)+ two markers	Del(6q) and abnormalities of 14q32 commonly seen in T-cell NHL	Identical *TCRCβ* rearrangements seen in patient material and cell line	
HPB-MLp-W	88, XX, +3q, +3q, –8p, iso(7q), +8 markers	Unknown		
EBT-8	48 XY, +2, der(3)t(3;3)(p25;q21), del(11)(q23), +17, der(22)t(1;22)(q11;p11) Del(11)(q23) similar to that seen in T-PLL and may involve the *ATM* locus	Unknown EBV monoclonal integration		

Continued on next page

Table 6. (continued)

Cell line	Cytogenetic karyotype	Chromosomal rearrangements of potential interest	Antigen receptor gene rearrangements	Ref.
T-34	*Extremely complex* Multiple abnormalities of chromosomes # 1, 2 , 3, 8, 11, 12, 13, 14, 15, 16, and 19 Del(11)(q21q23) similar to that seen in T- PLL and may involve the *ATM* locus	8- to 16-fold *MYC* amplification		
HANK-1	48 XX, +2, del(16q13), +21	Unknown		
DEGLIS	46 XY, –2, +der(2)(?::2p23-2q37::?), –4, + der(4)t(4;?)(q34;?), iso(6p), +del(6p22), –8, +der(8)t(8;?)(q24;?), t(13;15)(p11;p11), t(21;22)(p11;p11)	Possible breaks within *ALK* locus at 2p23 and *MYC* locus at 8q24	*IGJH* and *TCRCβ* rearrangements	
KIT-225	*Complex* 47 XY, –5, –6, –14, +19, ins inv(1)(pter-p36.3::p34.1->p31.2::p36.3->p34.1::p31.2->p22.1::q12-> p22.1::q12-qter) inv(3)(p26q27), +der(5)t(5;7)(p15.3;q22), +der(6)t(6;?)(q21;?), +der(14)t(6;14)(q21;p12)	Unknown – abnormalities of chromosome 1p22 described in Sézary syndrome		

several years ago may not be possible. Thus, there may be some confusion between cell lines derived from patients with T-cell precursor ALL, T-cell lymphoblastic lymphoma and post-thymic T-cell leukemia. Although immunophenotypic studies may allow clarification, changes associated with *in vitro* culture may obscure the precise derivation. Some T-cell malignancies lose surface antigen expression with *in vitro* culture (see for example Ohno et al. [36]). As with B-cell malignancies, loss of surface antigen receptor expression due to ongoing rearrangements or mutations at the *TCR* loci may result in loss of surface CD3 expression and may cloud the issue. Secondly, many of the cell lines listed have been inadequately studied both in terms of expression of T-cell differentiation antigens and *TCR* gene rearrangements. The latter demands the use of a series of probes to both the constant and joining gene segments; a single probe is insufficient. Thus, in many cases, it is not possible to determine the precise stage of T-cell differentiation from which the cells are derived. In the case of PFI-285 and ST-4 it seems likely from the immunophenotype (CD1 positivity and lack of HLA-DR expression) that the cell lines were derived from a lymphoblastic lymphoma/ T-cell precursor ALL rather than a mature T-cell malignancy.

Finally, most of the cell lines described have been derived from Oriental patients where the pattern of T-cell disease differs substantially from that in the Occident, are the subject of only a single report and their availability in some instances is not known. There remains a requirement for cell lines from many of the recently recognised diseases of mature T-cells.

References

1. Abe M et al. *Cancer* 69: 1235–1240, 1992.
2. Arione R et al. *Cancer Res* 48: 1312–1318, 1988.
3. Asada H et al. *Leukemia* 8: 1415–1423, 1994.
4. Ascari S et al. *Annals Oncol* 8: 583–592, 1997.
5. Barclay AN et al. *The Leucocyte Antigen Factsbook*, 2nd edn., Academic Press, London, 1997.
6. Blake TJ and Langdon WY. *Oncogene* 7: 757–762, 1992.
7. Bunn PA and Foss FM. *J Cell Biochem Supplement* 24: 12–23, 1996.
8. Chang H et al. *Leukemia Lymphoma* 8: 97–107, 1992.
9. ChenTR. *J Natl Cancer Inst* 84: 1922–1926, 1992.
10. Cheng J and Haas M. *Mol Cell Biol* 10: 5502–5509, 1990.
11. Cooke CB et al. *Blood* 88: 4265–274, 1996.
12. Dyer MJS et al. *Leukemia* 7: 1047–1053, 1993.
13. Emi N et al. *Int J Hematol* 69: 180–185, 1999.
14. Finger Let al. *Proc Natl Acad Sci USA* 85: 9158–9162, 1988.
15. Finver SN et al. *Proc Natl Acad Sci USA* 85: 3052–3056, 1988.
16. Gazdar AF et al. *Blood* 55: 409–417, 1980.
17. Hanson MN et al. *Blood* 88: 3626–3633, 1996

18. Helgestad J et al. *Eur J Haematol* 44: 9–17, 1990.
19. Hori T et al. *Blood* 70: 1069–1072, 1987.
20. Jaffe ES, Krenacs L and Raffeld M. *Annals Oncol* 8 (Suppl. 2): S17–S24, 1997.
21. Kagami Y et al. *Jpn J Cancer Res* 84: 371–378, 1993.
22. Kagami Y et al. *Br J Haematol* 103: 669–677, 1998.
23. Kanegane H et al. *Blood* 91: 2085–2091, 1998.
24. Koizumi S et al. *J Natl Cancer Inst* 84: 690–693, 1992.
25. Larsen CJ et al. *Leukemia* 2: 247–248, 1988.
26. Matsuzaki H et al. *Jpn J Cancer Res* 83: 450–457, 1992.
27. Matutes E et al. *Blood* 78: 3269–3274, 1991.
28. Matutes, E. *T-cell Lymphoproliferative Disorders: Classification, Clinical and Laboratory Aspects*, Harwood Academic Publishers, 1999.
29. McKeithan TW et al. *Proc Natl Acad Sci USA* 83: 6636–6640, 1986.
30. Miyanishi S and Ohno H. *Cancer Genet Cytogenet* 59: 199–205, 1992.
31. Miyoshi I et al. *Nature* 294: 770–771, 1981.
32. Morikawa S et al. *Leukemia Res* 15: 381–389, 1991.
33. Morita M et al. *Blood* 77: 1766–1775, 1991.
34. Nakao Y et al. *J Natl Cancer Inst* 78: 1079–1086, 1987.
35. Neri A et al. *Leukemia Lymphoma* 23: 43–8, 1996.
36. Ohno H et al. *Cancer Res* 48: 4959–4963, 1988.
37. Paul WE (ed.) *Fundamental Immunology*, 4th edn., Lippincott-Raven, Philadelphia/New York, 1998.
38. Pawson R et al. *J Clin Oncol* 15: 2667–2672, 1997.
39. Pileri SA et al. *Annals Oncol* 9: 797–801, 1998.
40. Al Saati T et al. *Blood* 80: 209–216, 1992.
41. Sagawa K et al. *Kurume Med J* 42: 149–160, 1995.
42. Salhany KE et al. *Blood* 89: 3490–3491, 1997.
43. Salhany KE et al. *Hum Pathol* 28: 674–685, 1997.
44. Shima EA et al. *Proc Natl Acad Sci USA* 83: 3439–3443, 1986.
45. Taylor AMR et al. *Blood* 87: 423–438, 1996.
46. Vorechovsky I et al. *Nat Genet* 17: 96–99, 1997.
47. Yuille MAR et al. *Oncogene* 16: 789–796, 1998.
48. Zhang J et al. *Oncogene* 9: 1931–1937, 1994.

Chapter 11

Hodgkin's Disease

Andrea Staratschek-Jox, Jürgen Wolf and Volker Diehl
Department of Internal Medicine I, HS16, University of Cologne, D-50924 Cologne, Germany. Tel: +49-221-478-4400; Fax: +49-221-478-5455

1. INTRODUCTION

The microscopic appearance of Hodgkin's disease (HD) tissue is a small number of lymphoma cells, the so-called Hodgkin and Reed-Sternberg (H-RS) cells, surrounded by a non-neoplastic cellular environment consisting mostly of T-lymphocytes [47].

The first cell line (L428) was derived from a pleural effusion obtained from a 37-year-old woman with relapse of nodular sclerosing Hodgkin's disease [52]. The L428 cell line was considered to be of H-RS cell origin for the following reasons: the clonal cell population expressed the H-RS cell-associated clusters of differentiation CD 15 and CD30, cytogenetic analysis revealed a grossly aberrant karyotype, the cell line did not harbor EBV and tumor development was observed in nude mice after intracranial inoculation. These four criteria are used to judge whether the continuous cell line was established from the HD cells. The establishment of such lines is a rare event and only 17 cell lines have been described. One of these, known as Co or Cole, is cross-contaminated and is in fact the T-ALL derived cell line CCRF-CEM [15].

A cell line that grows out from a culture of HD affected tissue or effusion does not, as a rule, represent an H-RS cell population, since other cells present can give rise to a continuous cell line. For only one cell line, L1236 [69] was derivation from H-RS cells unequivocally demonstrated by amplification of identical Ig gene rearrangements from the cell line and from single H-RS cells microdissected from a section of a bone marrow biopsy from the patient [31]. For all of the other cell lines, there is no such authentication.

From one cell line (SBH-1), the histology of the lymphoma tissue was not available [6], and thus there is no proof that the patient suffered from HD. Another cell line, (HKB-1), was derived from a recurrence of HD in a patient who initially presented with a large cell anaplastic lymphoma at the same

J.R.W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol. III, 339–353.

site [66]. Consequently, the cell line may have been derived from the ALCL, rather than the HD.

The clinical characterization, immunophenotype, cytokine expression, chromosomal aberrations and growth characteristics of the 16 cell lines which may represent HD cells are summarized in Tables 1–5 and discussed below.

2. CLINICAL CHARACTERIZATION

Two further cell lines (L591, L540) were established from HD by the same group that developed L428 [8]. The three lines were derived from pleural effusions or bone marrow aspirate obtained from young women with progressive HD of nodular sclerosis subtype. A further 13 HD derived cell lines have been reported, 11 of which were derived from young patients suffering from nodular sclerosis HD, and in the other 2 cases, the line was derived from HD of mixed cellularity. This reflects the incidence of the histological HD subtypes among young adults, with most having nodular sclerosis HD [2]. Like the first three cell lines, most of the subsequent lines grew from HD-affected material obtained from pretreated patients during relapse or progressive disease. Eleven cell lines were established from either pleural effusion, pericardial effusion or peripheral blood (see Table 1).

3. IMMUNOPHENOTYPE

The vast majority of H-RS cells and the HD cell lines express CD30. This antigen is also expressed on activated or transformed (with human T-lymphotrophic virus-1 or Epstein–Barr virus) T and B lymphocytes [55], activated [45] and differentiated macrophages [1], and on the tumor cells of anaplastic large cell lymphoma (ALCL), which can also be called Ki-1-lymphoma [43]. This reaction pattern makes CD30 antibodies a valuable diagnostic tool. The CD30 antigen is a 120 kDa, membrane-bound, phosphorylated glycoprotein with a non-phosphorylated, 84 kDa, intracellular apoprotein and a 90 kDa degradation residue released into the supernatant [21]. Additionally, an independently synthesized 57 kDa intracellular molecule has the same antigenicity. The gene coding for CD30 has been cloned and identified as a member of the TNF-receptor superfamily [16]. The CD30 ligand has also been cloned [57]. The interaction of CD30 with its ligand is thought to be involved in the regulation of apoptosis and proliferation of activated lymphatic cells.

Table 1. Hodgkin's disease derived cell lines: clinical characterization

Cell line	Patient age/sex	Diagnosis/staging	Treatment status	Specimen site	Culture medium	Cell of origin IG−, TCR rearrangement	Primary ref.
HuT_{11}	6/F	HD, mixed cellularity; IIA, first diagnosis	no treatment	lymph node	McCoy's medium 5A, 10%FCS	B-cell Cγ, Cκ expression	50
L428	37/F	HD, nodular sclerosis, IVB, relapse	mustine, oncovin, procarbazine, prednisone	pleural effusion	RPMI 1640, 10%FCS, 4 mmol/L L-glutamine	B-cell JH R/D, Cκ G/G, Cλ G/G [59]	52
L540	20/F	HD, nodular sclerosis, IVB		bone marrow	RPMI 1640, 10%FCS, 4 mmol/L L-glutamine	T-cell JH G/G, Cκ G/G, Cλ G/G,TcRα R, TCRβ R, TCRγ R/R [59]	8
L591	31/F	HD, nodular sclerosis/IVB		pleural effusion	RPMI 1640, 10%FCS, 4 mmol/L L-glutamine	B-cell JH R/D, [59]	8
SU/RH-HD1	12/M	HD, nodular sclerosis		spleen	RPMI-1640, 15%FCS, 0.3% L-glutamine		44
DEV	51/M	HD, nodular sclerosis, IV, relapse	radiation	pleural effusion	RPMI 1640, 10%FCS	B-cell expression of IGA2	46
KM-H2	32/M	HD, mixed cellularity, IV, relapse	prednosine, vinblastine, cyclophosphamide	pleural effusion	RPMI 1640, 10%FCS,	B-cell JH R/D	29
HDLM-2	74/M	HD, nodular sclerosis		pleural effusion	RPMI 1640, 10%FCS	T-cell JH G/G, Cκ G/G, TCRβ R/R, TCRγ R/R [14]	12
Sup-HD1	34/M	HD, nodular sclerosis, IV, relapse	procarbazine, melphalan, velban/adriamycin, bleomycin, velban, dacarbazine, aggressive chemotherapy and irradiation in relapse	pleural effusion	McCoy 5A, 10% FCS, 10ng/ml insulin like growth factor	B-cell JH R/D, Cκ R/R, Cλ G, TCRβ R	38

Continued on next page

Table 1. (continued)

Cell line	Patient age/sex	Diagnosis/staging	Treatment status	Specimen site	Culture medium	Cell of origin IG−, TCR rearrangement	Primary ref.
ZO	26/F	HD, nodular sclerosis, IV, progressive disease	mustine, oncovin, procarbazine, prednisone	pericardial effusion	RPMI 1640, 20% FCS, add lymphocult	B-cell JH R/R, Cκ R/R, Cλ G	49
HO		HD, nodular sclerosis		lymph node	RPMI 1640, 10%FCS	T-cell JH G, TCRγ R, TCRCβ1 R/R, TCRCβ2G/G	26
HD-70	69/M	HD, nodular sclerosis, IV, first diagnosis	cyclophosphamide, vincristine, procarbazine, prednisolone/ doxorubicin, bleomycin, vincristine, dexamethasone	peripheral blood	RPMI 1640, 20%FCS, 10% human cord blood serum	B-cell JH R/R, Cκ R/G, TcRα G, TCRβ G, TCRγ G	30
HD-Myz	29	HD, nodular sclerosis, IVB, relapse	combined chemo- and radiotherapy	pleural effusion	RPMI 1640, 20% FCS, 2 mM L-glutamine	non-B- non-T-cell JH G, TCRβ G/G	3
SBH-1	78/F	HD, no histology available, pleural effusion contained H-RS cells	no treatment	pleural effusion	RPMI 1640, 10%FCS	B-cell JH R/R, Cκ R/D, Cλ R/R	6
L1236	34/M	HD, mixed cellularity, IV, relapse	radiation, 3 cycles COPP/ABVD. High dose chemotherapy autologous bone marrow transplantation	peripheral blood	RPMI 1640, 10%FCS, 4 mmol/L L-glutamine	B-cell JH R/R, Cκ R/G, Cλ G/G	69
HKB-1	14/F	initial diagnosis: large cell anaplastic lymphoma developing on the basis of a preexisting HD, in relapse: relapse HD, nodular sclerosis, IV	combined chemo- and radiotherapy	intrapulmonary tumor biopsy during relapse	RPMI 1640, 10% FCS, glutamine	B-cell JH R, TCR G	66

R – rearranged, G – germline, D – deleted. Cell lines are listed in order of publication.

Table 2. Hodgin's disease derived cell lines: immunophenotypical characterization

Cell line	T/NK cell marker	B-cell marker	Myelomonocytic marker	Other markers	CD30/CD15	Ref.
HuT_{11}		Cγ+, Cα−, Cμ−, Cδ−, Cε−, Cκ+, Cλ−				58
L428	CD1−, CD2−, CD3−, CD4−, CD5−, CD6−, CD7−, CD8−, CD16−, Leu-7−, Leu-19−	IgM−, IgA−, IgG− Cκ−, Cλ−, CD19−, CD20−, CD21−, CD22−, CD24−	CD11a−, CD11b−, CD11c−, CD13−, CD14−, CD33−	CD9−, CD10−, CD34−, CD71+, Ki-67+, HLA-A, B, C−, HLA-DP+, HLA-DQ+, HLA-DR+,	CD30+, CD15+	8,14,17
L591	CD2+, CD3−, CD4−, CD8−	CD19+, CD20+, IgM−, IgA−, IgG−, Cκ−, Cλ−,		HLA-DR+	CD30+, CD15+	8,53
L540	CD2+, CD3−, CD4+, CD5−/+, CD7−, CD8−	IgM−, IgD−, IgG−, IgA−, Cκ−, Cλ−, CD22−, CD20−, CD19−	CD11b+, CD11c−, CD14+	Ki-M1 (+), CD10−, HLA-DR+	CD30+, CD15+	17,49
SU/RH-HD1	CD5−, CD8−, CD4−,	Leu-10−, IgG+, IgM−,		Leu-7−, HLA-I+, HLA-DR+	CD15−, CD30−	10,44
DEV	Leu7−, OKT1−, CD3−, CD4−, OKT5−, CD1a−, CD8−, OKT11−	Cκ−, Cλ−, Cα+, CD20+, CD24−, B2−	OKM1−, OKM2−	CD10−, HLA-DR−, CD71+,	CD30+, CD15+	46,49
KM-H2	CD1−, CD2−, CD3−, CD4−, CD5−,CD6−, CD7−, CD8− CD16−, Leu-7−, Leu-19−,	CD19−, CD20−, CD21−/+, CD22−, CD24−	CD11b−, CD11c−, CD13−, CD14−, CD33−	CD9+, CD10−, CD11a−, CD10−, CD34−, CD71+, HLA class A,B,C+, HLA-DQ+, HLA-DP+, HLA-DR+, Ki-67+	CD30+, CD15+	14,29,49
HDLM-2	CD1−, CD2+, CD3−, CD4−, CD5−, CD6−, CD7−, CD8−, CD16−, Leu-7−, Leu-19-	CD19−, CD20−, CD21−, CD22−, CD24−	CD11b−, CD13−, CD14−, CD33−	CD9−, CD10−, CD11a−, CD34−, CD71+, HLA-A,B,C+, HLA-DP+, HLA-DQ+, HLA-DR+, Ki-67+	CD30+, CD15+	14

Continued on next page

Table 2. (continued)

Cell line	T/NK cell marker	B-cell marker	Myelomonocytic marker	Other markers	CD30/CD15	Ref.
Sup-HD1	CD2−, CD3−, CD4−, CD5−, CD7−, CD8−, CD16−, CD56−	CD19−, CD20−, CD21−, CD22−, CD37−	CD11c (+), CD14−, CD68−		CD30−/CD15+	38
Zo	CD2−, CD3−, CD4+, CD7−, CD8−	CD19−, CD20−, CD21−	CD11c−	HLA class I+, HLA class II+, CD45−	CD30+, CD15+	49
Ho	CD1−, CD2−, CD3+, CD4+, CD7+, CD8−, CD38 (+)	CD19−, CD22−, CD23−, Cκ−, Cλ−,			CD30+, CD15−	26
HD-70	CD1a−, CD2−, CD3−, CD4−, CD5−, CD8−, CD45RO−	CpCκ+, CpCα+, CpCλ−, CpCδ−, CpCγ−, CpCμ−, CD19−, CD20−, CD21−, CD24−, CD38−,	CD13−, CD14−, CD33−, CD36−, Leu-M2−, Leu M3−		CD30+, CD15+	30
HD-Myz	CD1a−, CD2−, CD3−, CD3−, CD4−, CD5−, CD7−, CD8−	CD19−, CD20−, CD21−, CD22−, CD23−, CD37−, CD38−, CD39−, IgG−, IgD−, IgM−	CD11b−, CD13+, CD14−, CD68+	CD10+, CD29+, CD33−, CD34−, CD40−, CD56−, CD71+, CD76−, CD77−, HLA-DR+, MHC I+, CD95−	CD15−, CD30−	3
SBH-1	CD3−, CD5−, CD7−	CD19+, CD20+, CD22+	CD13−, CD33−, CD14−	CD10−, CD45+, CD71+, EMA−, HLA-DR−	CD30+, CD15+	6
L1236	CD3−, CD5−, CD8−, CD45−, CD45Ro−, TCRγ delta−, CD16−	CD23+, CD19−, CD20−, CD38−, s-Igκ−, s-Igλ−	CD33−, CD14−	CD34−, CD54+, CD58+, CD71+, CD80+, CD86+, HLA-DP+, HLA-DR+, CD10−	CD30+, CD15+	69
HKB-1	CD3−, CD4−, CD8−, CD16−, CD56−	CD19+, CD20+, CD21−, CD23+	CD14−		CD30+, CD15−	66

Table 3. Hodgkin's disease derived cell lines: cytokine related characterization

Cell line	Cytokine receptor expression	Cytokine production	Ref.
L428	IL-2R+, IL-6R+	TGFβ+, TNF+, IL-1+, IL-4+, IL-6+, TARC+, IL-13+, IL-5+	7,17,19,28,34,39,40,42,64
L591	IL-2R+, IL-6R+	IL-1+, IL-6+, TNF+, TARC+	10,19,28,40,53,64
L540	IL-2R+, IL-6R−	IL-1+, IL-6+/−, TNF+, TARC+	7,17,19,28,40,64
SU/RH-HD-1	IL-1+		10,44
DEV	IL-2R+		53
KM-H2	IL-2R+, IL-6R−	TNFα+, TNFβ+, M-CFS+, IL-6+, IL-13+, IL-5+	19,22,23,28,34,49
HDLM-2	IL-2R+, IL-6R+	IL-6+, IL-13+	13,28,34
Sup-HD1	IL-2R+	INFγ+, IL-2−	38
Zo	IL-2R+		49
Ho	IL-2R−	IL-6−, TNF+	19,26
HD-70	IL-2R−		30
HD-Myz	IL-1R+, IL-2R−, IL-3R(α-chain)−, IL-4R−, IL-6R+, IL-7R−, IL-8R+	IL-1α+, IL-1β+, IL-2−, IL-3−, IL-4−, IL-5+, IL-6+, IL-7+, IL-8+, IL-10+, GM-CSF−, TGFβ−, TNFα−, TNFβ−	3
SBH-1	IL-1R−, IL-2R+, IL-3R−, IL-4R+, IL-6R+, IL-7R+,TNFR+, G-CSFR−	TGFβ+, TGFα+, IL-1α−, IL-1β−, IL-2−, IL-3−, IL-4−, IL-5−, IL-6−, IL-7−, IL-9−, G-CSF−, SCF−, γIFN−	6
L1236	IL-2R−	IL-6+, IL-8+, IL-10+, INFγ+, TNFα+, TGFβ+, GM-CFS+, IL-2−, IL-4−, IL-7−, TARC+	64,69
HKB-1	IL-2R+	IL-1β−, IL-2−, IL-6+, IL-10−, IL-12−, INFγ−	66

Table 4. Hodgkin's disease derived cell lines: genetic characterization

Cell line	Representative karyotype	Ref.
L428	48, −9, −13, +12, dup(1)(p22p32), t(2p25:?), +del(2)(q33), +t(6q23;?), dup(7)(q22q36orq11, 2q32), t(9p24;?), del(11)(q21), +del(12)(q15), t(13p12;?), t(9p12;?), del(11)(q21), del(12)(q15), t(13p12;?), t(14q32;?), del(21)(q21)	18
L540	66, +2, +3, +4, +7, +9, +11, +12, +13, +17, +18, +19, +20, +del(1)(p22), +t(2;10?)(q33;q11.2), +del(5)(q15), t(8q24.1orq23.2;?), +t(11q23;?), +del(11)(q21), +t(12q22;?), +(15;1?)(p12;p31.2or22.3?), +t(21p12;?)	18
L591	46, dup(7)(q32q36), t(14q32.1;?)	18
DEV	48, XXY, −2, +12, +mar, t(3;14)(3;22), −del 3, t(3;7)	46
KM-H2	50th passage: 44, X, −Y, −5, −7, −11, −15, −15, −16, −16, −17, −17, +20, −22, 2q+, 4q+, 5p+, 6p+, 7q+, 10p+, 14p+, +8mar	29
HDLM-2	36 chromosomes, non-random chromosome aberrations: 1p32, 3q13, 3q27, 6q23, 7p14, 9p11, 9p12, 11p11, 12q24, 19p13, 22q11	11
Sup-HD1	44, X, Y−, −1, −2, −4, −7, −9, −13, dup(1)(p13q32), +der(1)t(1;1;6)(q44->q25::p34->q32::p25), del(2)(p23p25), +der(2)t(2;7)(p25;p15), del(5)(p13p15.3), xder(7), dic(4;7)(q31;p15), del(8)(p21p23), t(8;22)q22;q13), del(11)q23q25), t(11;?, 11;?)(p15;?;q23;?), t(14;?)(p11.2-q11.2;?), del(21)q21q22.3), +2mar	38
Zo	53, XX, iso 1q, t(1;13), iso 2p, −3, 4q−, 6p−, t(7;17), −15, 16p+, 16q−, +17, +20, +5 markers	49
HD-70	73, Y−, +1, +1, +2, +3, +5, +7, +9, +10, −13, −13, +16, +19, +20, +21, +ins(6;?)(p21;?), +der(7)t(7;?)(q22;?), +der(8)t(8;14)(q24?;q32, +der(11)t(11;1)(p13;q13), +der(11)t(11;1)(p13, q13), +der(11)t(11;1)(p13;q13), +der(11)t(11;?), +der(12)t(12;?)(p13;?)(qcen->qzer), +der(17)t(17;?)(p13;?), +2mar1, +mar2, +mar3, +mar4, +mar5	30
SBH-1	common chromosomal aberrations: del(3)(p11;p25), del(4)(p12p15), del (4)(q21q28), del(6)(q21q28), +7, +dup(8)(q13q22), dup(9)(p13q22), del(11)(q23), +7, +dup(8)(q13q22), dup(9)(p13q22), del(11)(q23), add(12)p13), i(15)(q10), t(14;18)(q32;q21)	6
L1236	65, +5, +der(1)t(1;14)(p34;?), +der(1)t(1;8)(p22;?), +der(1)dup(1)(q21q44)add(1)(p31-32), +dup(2)(p15p23), +dup(2)(p15p23)+der(3)t(3;16)(p25;?), +der(4)t(4;8)(q31;?), +der(6)dup(6)(p11p25), +der(6)t(1;6)(p34;q15), del(7)(q11), der(7)t(7;17)(p22;?), +der(7)t(7;/)(p22;q22), +der(7), der(8), del(10)(q11), add(11)(p13), +del(11)(q13), +der(12), +del(12)(q22), +del(12)(q15), der(14)t(1;14)(p34-35;q22), del(14)(q22-24), +der+(15)T(15;29)(q22;q11), +der(16), del(17)(q23), del(17)(q23), +der(19)t(7;19)(?;p13), add(20)t(15;20)(q22;q11), +add(20)t(15;20)(q22;q11), +mar1, +mar2	69
HKB-1	46, X, der(X)add(X)(p11)add(q21), t(1;13)(q2?5;q21), add(2)(p23), t(6;14)(p21;q22-23), t(8;14)(q24;q32)	66

Table 5. Hodgkin's disease derived cell lines: functional characterization

Cell line	Doubling time	EBV status	Cytochemistry	Heterotransplantation into mice	Ref.
HuT_{11}	12 hr	EBV−	acid phosphatase+, alkaline phosphatase−, α-naphthol esterase+, leucine aminopeptidase+, peroxidase−	hamster: s.c: tumor development in 100% of hamsters	50
L428	42–46 hr	EBV−	PAS−, peroxidase+/−, α-naphthylesterase+, acid phosphatase+, naphthol-chloracetate esterase−	nude mice: i.c: tumor development in 2/2 mice, s. c.: no tumor development, Scid mice: s.c.: tumor development in 3/5 mice, i.v.: tumor development in 1/10 mice	8, 32, 51, 52, 65
L540		EBV−	naphthol-chlorate esterase−, peroxidase−, acid α-naphthyl acetate esterase+, alkaline phosphatase−, acid phosphatase+	nude mice: i.c.: tumor development in 24/25 mice, s.c.: tumor development in 23/25 mice, Scid mice: i.v.: tumor development in 4/10 mice	8, 32, 51
L591		EBV+	naphthol-chlorate esterase−, peroxidase, α-naphthyl acetate esterase+, alkaline phosphatase−, acid phosphatase+	nude mice: s.c: no tumor development, i.c.: tumor development in 100% of mice, Scid mice: s.c.: tumor development in 1/5 mice, i.p.: no tumor development	8, 65
SU/RH-HD1		EBV−	peroxidase−, non-specific esterase+	nude mice: i.c: tumor development, s.c.: tumor development in 1/14 mice	10, 44
DEV	73 hr	EBV−	acid phosphatase+, alkaline phosphatase−, 5-nucleotidase−, aminopeptidase−, peroxidase−, α-naphthylesterase−	Scid mice: s.c.: tumor development in 3/5 mice, i.v.: no tumor development	46, 65

Continued on next page

Table 5. (continued)

Cell line	Doubling time	EBV status	Cytochemistry	Heterotransplantation into mice	Ref.
KM-H2	60 hr	EBV−	peroxidase−, acid phosphatase+, alkaline phosphatase−, α-NAE+, CAE−	nude mice: s.c.: no tumor development, Scid mice: s.c.: tumor development in 3/5 mice, i.v.: tumor development in 1/10 mice	29, 32, 65
HDLM-2	72−129 hr	EBV−	terminal deoxynucleotide transferase−	Scid mice s.c.: tumor development in 1/5 mice, i.v. no tumor development	13,65
Sup-HD1	72−96 hr	EBV−	adenosine deaminase+, nucleoside phosphorylase+, terminal deoxynucleotidyl transferase−	nude mice: s.c.: no tumor development, i.p.: no tumor development	38
Zo	growth: slowly	EBV−		nude mice: s.c. tumor development	49
Ho		EBV−			26
HD-70		EBV−	acid phosphatase+, α-naphthyl butyrate esterase+, periodic acid-Schiff+, peroxidase−, sudan black−, AS-D chloroacetate esterase−, alkaline phosphatase−	newborn hamster: i.p.: tumor development in 5/5 hamsters	30
HD-Myz		EBV−		Scid mice: i.v.: tumor development, s.c.: tumor development	3
SBH-1	48−60 hr		peroxidase−, sudan black−, acid phosphatase+, α-naphthyl acetate+	Scid mice: i.v.: tumor development, i.p.: tumor development	6
L1236		EBV−		Scid mice: i.v.: tumor development in 2/3 mice, s.c.: tumor development in 3/3 mice	69
HKB-1		EBV−			66

i.c. – intracerebral, s.c. – subcutaneous, i.p. – intraperitoneal, i.v. – intravenous.

In addition to CD30, most of the H-RS cell lines express CD 15 and the transferrin receptor (CD71) (see Table 2). Besides these, no characteristic pattern of marker expression can be defined either for classical H-RS cells or for the cell lines. 11 of the HD derived cell lines appear to be of B-cell origin, while 3 of the lines show a T- cell phenotype, as defined by Southern blot analysis of rearranged Ig or T- cell receptor (TCR) genes or expression of the respective rearranged genes (see Table 1). One cell line, HDMyz, has neither an Ig gene rearrangement nor a TCR rearrangement. In only 5 of the 11 B-cell derived cell lines was expression of at least one B-cell marker (such as CD 19, CD20 or CD21) and/or expression of Ig heavy and/or Ig light chain constant region genes detected. In addition, most of the T- cell lines do not express T- cell associated markers (such as CD3, CD4, CD5 or CD8). In conclusion, in most cases, immunophenotype analysis does not define the cell of origin either of H-RS cells or their cell lines.

4. CYTOKINE RELATED CHARACTERIZATION

HD shares many of the clinical and biological characteristics of an inflammatory process, including fluctuating fever, nightsweats and elevated serum levels of IL-2 receptor [20]. In affected lymph nodes the H-RS cells are surrounded mostly by T-lymphocytes [47]. This observation led to the hypothesis that T-cells are attracted by cytokines secreted from H-RS cells. In support of this observation, TARC expression was detected in 4 HD lines [64]. TARC is a chemokine that attracts TH2-cells and its expression in H-RS cells might account for the T-cell infiltration.

Cytokine expression in HD-derived cell lines is heterogenous, and most cytokines are undetectable (see Table 3). Most H-RS cells and HD cell lines do express IL-2R. Binding of IL-2 to the IL-2R leads to activation and cell proliferation. Expression of TNFα and TNFβ was observed in some of the cell lines, and might account for the symptoms. TGFβ is expressed in some cell lines, and in H-RS cells *in vivo* may result in suppression of T helper cell function.

5. GENETIC CHARACTERIZATION

Primary H-RS cells show numerical and structural chromosomal aberrations in most cases [67]. However, no H-RS cell specific chromosomal aberration has been detected [54,60,61], although cytogenetic analysis is difficult to perform due to the scarcity of the cells. Cytogenetic analysis of most of the HD derived cell lines has found grossly aberant karyotypes (see Table 4).

In addition, many of the chromosomal partners involved in translocations could not be identified using standard banding techniques. Since H-RS cells are usually derived from germinal center B cells, the frequent occurrence of chromosomal breaks within the chromosomal region 14q32 in both H-RS cells [4,47] as well as in B-cell HD derived lines may be important. The detection of chromosomal breaks affecting this region carrying the Ig heavy chain locus is also a common feature of other lymphomas (for example Burkitt's lymphoma [58] and follicular lymphoma [63]. The changes lead to oncogene deregulation; for example, c-myc in Burkitt's lymphoma [5] and bcl-2 in follicular lymphoma [62]. The characterization of translocations involving 14q32 in H-RS cells may provide new insights into the mechanism of transformation of H-RS cells.

6. FUNCTIONAL CHARACTERIZATION

In thymus aplastic T-cell deficient nude mice, HD-derived cell lines only grow after intracranial inoculation [9] (see Table 5). In contrast, most of the HD-derived cell lines grow in SCID (severe combined immunodencient) mice after subcutaneous inoculation [65]. SCID mice, due to a genetic recombinase defect, lack functional T- and B-cells. The HD-derived cell lines L540 [32], HD-MyZ [3] and L1236 [69] disseminate intralymphatically after inoculation into SCID-mice. This experimental model for the *in vivo* growth of H-RS cells has been used for the preclinical testing of new immunotherapeutic modalities, such as immunotoxins [68]. The histology of the xenografts in SCID mice resembles that of anaplastic large cell lymphoma. The typical features of HD, a few tumor cells surrounded by a large excess of reactive cells, are not present. This difference is probably due to the absence of T- cells in SCID mice, and is a major limitation of the model for HD. The transplantation of biopsy tissue into SCID mice was unsuccessful, since outgrowth of H-RS cells was never observed [33].

All HD derived cell lines but one (L591) are negative for the Epstein–Barr virus (EBV) (see Table 5). This is in contrast to the detection of EBV in H-RS cells in about half the cases of HD in industrialized countries [24].

7. PERSPECTIVES AND CONCLUSIONS

Microdissection of single H-RS cells from frozen lymph node sections and the subsequent analysis of these single cells using polymerase chain reaction (PCR) is allowing H-RS cells to be characterized genetically [36]. Analysis of rearranged immunoglubulin (Ig) genes shows that H-RS cells

are clonally derived from germinal center B-cells in most cases [35]. Also, somatic mutations have been detected, rendering potentially functional V_H gene rearrangements non-functional, thus preventing Ig gene expression in H-RS cells [35]. Since B-cells that do not express an antibody undergo apoptosis within the germinal center, it was speculated that H-RS cells are "crippled" germinal center B-cells that escape programmed cell death by a yet unidentified mechanism.

The Ig gene rearrangement has been used as a clonal marker to detect H-RS cells belonging to the same clone in other tissues obtained from the same patient during the course of the disease. Using this approach, it was demonstrated that H-RS cells clonally expand, leading to disseminated disease and to relapse of the lymphoma after clinical remission. This approach was also used to show that L1236, which grew from the peripheral blood of a patient suffering from relapse of mixed cellularity HD [69], is unequivocally derived from the H-RS cells in that patient. Sequence analysis of the rearranged Ig genes showed that Ig gene expression in L1236 cells as well as in the H-RS biopsy is prevented by a somatic mutation within the promoter region of the potentially functional V_H gene rearrangement [27].

H-RS cells, in most cases, represent clonal B-cells. Therefore, the origin of HD derived cell lines with a T-cell genotype remains to be elucidated. Some T-cell derived HD have been described [37,56], although the finding of rearranged T-cell receptor genes in H-RS cells is rare, even in those HD tissues in which the H-RS cells express several T-cell associated marker genes [37,56], Thus, the T-cell HD cell lines could also be derived from T-cells surrounding H-RS cells.

The derivation of the cell line HD-Myz, which shows a non-T, non-B cell phenotype [3], from H-RS cells is unlikely, since a myeloid derivation of H-RS cells has never been proven.

The B-cell genotype by itself does not provide unequivocal evidence for the derivation of HD derived B-cell cell lines from B-cell H-RS cells. It is essential to show clonal derivation of each cell line from the H-RS cells of the HD tissue of the patient from whom the cell line was derived.

Enrichment of H-RS cells has been achieved, providing viable purified H-RS cell populations for analysis [25], and reducing the need for cell lines. The broader application of molecular analysis of single microdissected H-RS cells will provide new insights into the pathogenesis of HD and avoid the possible disadvantages of analyzing cell populations that have been selected during *in vitro* culture.

References

1. Andreesen R, Brugger W, Löhr GW, Bross KJ. *Am J Path* 134: 187–192, 1989.
2. Alexander FE. In: Jarret RF (ed.) *Etiology of Hodgkin's Disease*, NATO ASI Series, Series A: Life Sciences, Vol. 280, Plenum Press New York, 1995.
3. Bargou RC, Mapara MY, Zugck C et al. *J Exp Med* 177:1257–1268, 1993.
4. Cabanillas F, Pathak S, Trujillo J et al. *Blood* 71: 1615–1617, 1988.
5. Dalla-Favera R, Bregni M, Erikson J et al. *Proc Natl Acad Sci USA* 79: 7824–7827, 1982.
6. DeCoteau JF, Reis MD, Griesser H et al. *Blood* 85: 2829–2838, 1995.
7. Diehl V, Burrichter H, Schaadt M et al. *Hematol Oncol* 1: 139–147, 1983.
8. Diehl V, Kirchner HH, Burrichter H et al. *Cancer Treat Rep* 66: 615–632, 1982.
9. Diehl V, Kirchner HH, Schaadt M et al. *J Cancer Res Clin Oncol* 101: 111–124, 1981.
10. Diehl V, Pfreundschuh M, Fonatsch C et al. *Cancer Surv* 4: 399–419, 1985.
11. Drexler HG. *Leukemia Lymphoma* 9: 1–25, 1993.
12. Drexler HG, Gaedicke G, Lok MS et al. *Leukemia Res* 10: 487–500, 1986.
13. Drexler HG, Gignac SM, Hoffbrand AV et al. *Recent Results Cancer Res* 117: 75–82, 1989.
14. Drexler HG, Leber BF, Norton J et al. *Leukemia* 2: 371–376, 1988.
15. Drexler HG, Dirks WG, MacLeod RAF. *Leukemia* 13: 1601–1607, 1999.
16. Dürkop H, Latza U, Hummel M et al. *Cell* 68: 421–427, 1992.
17. Falk MH, Tesch H, Stein H et al. *Int J Cancer* 40: 262–269, 1987.
18. Fonatsch C, Diehl V, Schaadt M et al. *Cancer Genet Cytogenet* 20: 39–52, 1986.
19. Foss HD, Herbst H, Oelmann E et al. *Br J Haematol* 84: 627–635, 1993.
20. Gause A, Roschansky V, Tschiersch A et al. *Ann Oncol 2* (Suppl. 2): 43–47, 1991.
21. Hansen H, Lemke H, Bredfeldt G et al. *Biol Chem Hoppe Seyler* 370: 409–416, 1989.
22. Hsu PL, Hsu SM. *Am J Pathol* 135: 735–745, 1989.
23. Hsu PL, Lin YC, Hsu SM. *Int J Hematol* 54: 315–326, 1991.
24. Hummel M, Anagnostopoulos I, Dallenbach F et al. *Br J Haematol* 82: 689–694, 1992.
25. Irsch J, Nitsch S, Hansmann ML et al. *Proc Natl Acad Sci USA* 95: 10117–1022, 1998.
26. Jones DB, Furley AJ, Gerdes J et al. In: Diehl V, Pfreundschuh M, Loeffler M (eds.), *Recent Results Cancer Res*, Springer-Verlag, Berlin 117: 62–66, 1989.
27. Jox A, Zander T, Küppers R et al. *Blood* 93: 3964–3972, 1999.
28. Jucker M, Abts H, Li W et al. *Blood* 77: 2413–2418, 1991.
29. Kamesaki H, Fukuhara S, Tatsumi E et al. *Blood* 68: 285–292, 1986.
30. Kanzaki T, Kubonishi I, Eguchi T et al. *Cancer* 69: 1034–1041, 1992.
31. Kanzler H, Hansmann ML, Kapp U et al. *Blood* 87: 3429–3436, 1996.
32. Kapp U, Dux A, Schell-Frederick E et al. *Ann Oncol* 5 (Suppl. 1):121–126, 1994.
33. Kapp U, Wolf J, Hummel M et al. *Blood* 82: 1247–1256, 1993.
34. Kapp U, Yeh WC, Patterson B et al. *J Exp Med* 189: 1939–1946, 1999.
35. Küppers R, Rajewsky K. *Annu Rev Immunol* 16: 471–493, 1998.
36. Küppers R, Rajewsky K, Zhao M et al. *Proc Natl Acad Sci USA* 91: 10962–10966, 1994.
37. Müschen M, Rajewsky K, Bräuniger A et al. *J Exp Med* 1999.
38. Naumovski L, Utz PJ, Bergstrom SK et al. *Blood* 74: 2733–2742, 1989.
39. Newcom SR, Ansari AA, Gu L. *Blood* 79: 191–197, 1992.
40. Newcom SR, Gu L. *J Clin Pathol* 48: 160–163, 1995.
41. Newcom SR, Kadin ME, Ansari AA, Diehl V. *Clin Invest* 82: 1915–1921, 1998.
42. Newcom SR, Kadin ME, Phillips C. *Int J Cell Cloning* 6: 417–431, 1988.
43. O'Connor NT, Stein H, Gatter KC et al. *Histopathology* 11: 733–740, 1987.

44. Olsson L, Behnke O, Pleibel N et al. *J Natl Cancer Inst* 73: 809–830, 1984.
45. Pfreundschuh M, Mommertz E, Meissner M et al. *Anticancer Res* 8: 217–224, 1988.
46. Poppema S, de Jong B, Atmosoerodjo J et al. *Cancer* 55: 683–690, 1985.
47. Poppema S, Kaleta J, Hepperle B. *J Natl Cancer Inst* 84: 1789–1793, 1992.
48. Poppema S, Kaleta J, Hepperle B, Visser L. *Ann Oncol* 3 (Suppl. 4): 5–8, 1992.
49. Poppema S, Visser L, de Jong B et al. In: Diehl V, Pfreundschuh M, Loeffler M (eds.), *Recent Results Cancer Res*, Springer-Verlag Berlin 117: 67–74, 1989.
50. Roberts AN, Smith KL, Dowell BL, Hubbard AK. *Cancer Res* 38: 3033–3043, 1978.
51. Schaadt M, Burrichter H, Pfreundschuh M et al. In: Diehl V, Pfreundschuh M, Loeffler M (eds.), *Recent Results Cancer Re*s, Springer-Verlag Berlin 117: 53–66, 1989.
52. Schaadt M, Fonatsch C, Kirchner H, Diehl V. *Blut* 38: 185–190, 1979.
53. Schaadt M, v Kalle C, Tesch H et al. *Cancer Rev* 10: 108–122, 1988.
54. Schouten HC, Sanger WG, Duggan M et al. *Blood* 8: 2149–2154, 1989.
55. Stein H et al. *Blood* 66: 848–858, 1985.
56. Stein H, Seitz V, Hummel M et al. *Ann Oncol* 10 (Suppl. 3): 49, 1999.
57. Smith CA, Grass HJ, Davis T et al. *Cell* 73(7): 1349–1360, 1993.
58. Taub R, Kirsch I, Morton C et al. *Proc Natl Acad Sci USA* 79: 7837–7841, 1982.
59. Tesch H, Jücker M, Falk MH et al. *Hematol Oncol* 6: 223–231, 1988.
60. Thanagavelu M, Le Beau MM. *Hemat Oncol Clin North Am* 3: 221–236, 1989.
61. Tilly H, Bastard C, Delastre T et al. *Blood* 6*:* 1298–1304, 1991.
62. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. *Science* 228(4706): 1440–1443, 1985.
63. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. *Science* 226(4678): 1097–1099, 1984.
64. Van den Berg A, Visser L, Poppema S. *Am J Pathol* 154: 1685–1691, 1999.
65. Von Kalle C, Wolf J, Becker A et al. *Int J Cancer* 52: 887–891, 1992.
66. Wagner HJ, Klintworth F, Jabs W et al. *Med Pediatr Oncol* 31: 138–143, 1988.
67. Weber-Matthiesen K, Deerberg J, Poetsch M et al. *Blood* 86: 1464–1468, 1995.
68. Winkler U, Gottstein C, Schön G et al. *Blood* 83: 466–475, 1994.
69. Wolf J, Kapp U, Bohlen H et al. *Blood* 87: 3418–3428, 1996.

Chapter 12

CD30-Positive Anaplastic Large Cell Lymphoma Cell Lines

Hermann Herbst and Hans G. Drexler
Gerhard-Domagk Institute of Pathology, University of Münster, Domagkstr 17, 48129 Münster, Germany, and DSMZ-German Collection of Microorganisms & Cell Cultures, Department of Human and Animal Cell Cultures, Braunschweig, Germany. Tel: +49-251-835-6752; Fax: +49-251-835-5460; E-mail: herbsth@uni-muenster.de.

1. INTRODUCTION

In the late 1970s, the application of the monoclonal antibody Ki-1 (CD30) to anaplastic malignancies led to the recognition of a new entity of malignant lymphomas, CD30 (Ki-1 antigen)-positive anaplastic large cell lymphoma ("Ki-1 lymphoma", ALCL) [52,53], On the basis of conventional histology alone, ALCL had previously been diagnosed as Hodgkin's sarcoma, malignant histiocytosis, malignant fibrous histiocytoma, or even as non-hematopoietic malignancies such as undifferentiated sarcoma, undifferentiated carcinoma, or amelanotic melanoma. These lymphomas were recognized as a distinct entity of high grade B- and T-cell lymphomas in the updated Kiel classification of non-Hodgkin lymphomas (NHL) [51] and are included in the Revised European-American Lymphoma (REAL) classification as a type of high grade T-/null-cell lymphoma and as a variant of diffuse large cell lymphomas of B-cell type [23]. As there is some overlap with Hodgkin's disease (HD), distinguishing ALCL with features of HD from lymphocyte depleted HD is a matter of ongoing debate.

2. CHARACTERISTICS OF ALC LYMPHOMAS

Clinically, ALCL presents most frequently in lymph nodes, often with subsequent infiltration of extranodal tissue. Among primary extranodal ALCL, cutaneous lesions are the most prevalent. ALCL may arise secondary to HD, lymphomatoid papulosis, mycosis fungoides, pleomorphic T-cell lymphoma

J.R.W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol. III, 355–370.

or T-cell lymphoma of angioimmunoblastic (AILD) type. The age distribution of primary ALCL revealed a bimodal pattern similar to HD, whereas ALCL arising simultaneously with or subsequent to other lymphomas showed a single peak in the fifth decade [52,53].

Histologically, the tumors are characterized by a preferential perifollicular involvement of lymph nodes by tumor cells often growing in coherent sheets with initial sparing of germinal centers, sinusoidal dissemination, and occasional foci of necrosis. The tumor cell morphology comprises a spectrum ranging from large pleomorphic cells with abundant, often basophilic, cytoplasm and irregularly shaped nuclei containing multiple small nucleoli or a single prominent, often rod-shaped nucleolus, to cells with more regular, rounded nuclei frequently containing a single nucleolus. The cells have a high mitotic rate and in many ALCL cases multinucleated tumor cells, often resembling Reed-Sternberg cells, may be found [52,53].

Unlike HD, where CD30 staining is a useful but not absolutely necessary diagnostic adjunct, the diagnosis of ALCL by definition requires the expression of the CD30 antigen in all of the tumor cells. Other activation antigens such as the low-affinity interleukin-2 receptor (CD25), class II histocompatibility antigens (HLA-DR), CD70 and proliferation-associated antigens such as the transferrin receptor (CD71) are usually found on ALCL tumor cells. Because activation antigens are not lineage specific markers, interest has centered on the study of cell type characteristic molecules. Similar to antigen- or mitogen-activated peripheral blood lymphocytes, which cease to express CD45 molecules, the three forms of the leukocyte common antigen (CD45, CD45RA, and CD45RO) are variably expressed on ALCL cells. Early lymphoid antigens present on precursor B- and T-cells, such as CD 10 or terminal nucleotidyl transferase (TdT), or macrophage antigens, are usually not expressed by CD30+ malignancies. ALCL of T-cell type occurs more frequently than ALCL of B-cell type, and few cases do not express B- and T-lymphoid marker molecules if a sufficient panel of antibodies is applied. Primary cutaneous ALCL are generally of T-cell type [27]. T- and null-cell ALCL have a phenotype of cytotoxic cells with expression of granzyme B, T-cell-restricted intracellular antigen (TIA)-1 and granule membrane (GMP-17) proteins as well as perform transcripts [19]. The clinical relevance of these phenotypic details has been challenged by the finding of a generally better outcome in primary ALCL of either T- or B-cell type, i.e., independent of the immunophenotype, in adults as compared to other diffuse large cell lymphomas [56].

In contrast to HD, which appears to be a neoplasm of constitutively cytokine-secreting cells, a limited body of data is available on the expression of cytokines in ALCL. Although CD25 (IL-2 receptor)-positive, these lymphomas do not express IL-2 [43], thus excluding the possibility of an

autocrine regulatory loop involving that interleukin. Similar to HD, IL-6 expression is a common finding in ALCL [36]. IL-9 expression has been found in a large proportion of HD cases and ALCL in the tumor cells, but not in other lymphoid malignancies [37]. IL-9 expression has been considered a characteristic feature of CD30-positive lymphomas with possible implications for autocrine growth control.

T-cell receptor gene rearrangements are the most frequent finding in ALCL. A minority of cases show rearranged immunoglobulin genes, and a few cases show rearrangements within both T-cell receptor and immunoglobulin loci. However, in approximately 30% of the cases, antigen receptor genes were found in germline configuration, although the cases had a tumor cell content sufficient for reliable analysis of DNA extracts, suggesting that some ALCL may represent genotypically immature lymphomas despite their display of an activated lymphoid phenotype [27].

It is not surprising that a number of ALCL cases, usually of B- or T-cell type respectively, are associated with the lymphotropic viruses, Epstein–Barr virus (EBV) and HTLV (human T-lymphotropic virus)-I, because both viruses are strong inducers of CD30 in lymphoid cells [3,25,28]. Moreover, the predominantly perifollicular distribution of EBV-infected cells in infectious mononucleosis tonsils mirrors the distribution of ALCL cells in early lesions. The expression of the latent protein (LMP)-1, an EBV gene product with transforming potential, and even of the nuclear antigen, EBNA-2, in occasional cases of B-ALCL, suggested a potential etiological role for the virus in a proportion of ALCL [25,33].

Cytogenetic analyses revealed involvement of a site at 2p23, often in a reciprocal translocation involving chromosome 5 [t(2;5)(p23;q35)], unique to ALCL associated with a T- or null-cell phenotype and genotype. The t(2;5) fuses the *ALK* (anaplastic lymphoma kinase) and the *NPM* (nucleophosmin) genes, leading to the formation of a chimaeric NPM-ALK-protein (p80) consisting of the terminal portion of NPM linked to the cytoplasmic domain of the neural receptor tyrosine kinase ALK [29,40]. It was subsequently shown that retrovirus-mediated gene transfer of *NPM-ALK* causes lymphoid malignancies in mice, indicating that the translocation indeed resulted in a dominant acting oncogene [32]. Moreover, the cloning of this novel breakpoint provided the basis for detecting the t(2;5) by reverse transcription of transcripts and subsequent polymerase chain reaction (RT-PCR) as well as of truncated *ALK* transcripts and protein by *in situ* hybridization with *ALK*-specific probes and immunohistology using polyclonal and monoclonal antibodies, respectively. The occurrence in HD of the t(2;5) and transcripts derived from the fused genes are a matter of scientific debate. On balance, if it occurs in this context, the t(2;5) is a very rare finding in HD. The same

seems to be true for lymphomatoid papulosis and primary cutaneous ALCL [26].

Recently, variants of the t(2;5) were described which involve genes other than NPM as partners for ALK, namely a t(l;2)(q25;p23), t(l;2)(q21;p23), and t(2;3)(p23,q21), all of which produce specific transcripts containing a truncated form of ALK [39,47]. The cryptic inv(2)(p23q35) abnormality was found to define another subtype of ALK-positive ALCL [59]. In t(l;2)(q25)(p23), the fusion partner was characterized as tropomyosin-3 (TPM3), which was previously found in papillary thyroid carcinomas where it forms a fusion with the TRK kinase gene [34]. Moreover, using ALK-specific antibodies, a novel subtype of diffuse large-cell lymphoma was defined which displays expression of the full-length ALK kinase, but lacks both the t(2;5) and CD30 expression [14].

3. DEFINITION OF ALCL CELL LINES

The phenotypic heterogeneity of ALCL calls for a restrictive approach to assigning a cell line to the group of *bona fide* ALCL cell lines. First, such lines should display strong expression of CD30 protein. However, this feature is shared with numerous leukemia and lymphoma cell lines, mitogen activated lymphocytes as well as EBV–, HTLV-I/II–, and HHV-8-positive cell lines, and is therefore not a sufficient criterion. Second, these lines should display the t(2;5) or an equivalent variant translocation resulting in a constitutive activation of the ALK gene. Because of the specificity of this abnormality for ALCL, a cell line may be considered an ALCL cell line even if biopsy material is not available for review. This could apply to a number of cell lines derived from tumors diagnosed in the past as malignant histiocytosis according to now obsolete classification schemes.

ALK-positive ALCL is a subset within the spectrum of these lymphomas. Therefore, cell lines corresponding to ALK-negative ALCL without genetic changes involving 2p23 presumably exist. However, in these cases the histological and phenotypic details should unequivocally meet all of the criteria for the histological diagnosis of ALCL, and the cell elements *in vitro* should closely mirror the phenotypic, genotypic and karyotypic characteristics of the tumor cells *in vivo.*

4. ALCL CELL LINES

Similar to the HD-derived cell lines, the ALCL cell lines listed in Tables 1–5 were obtained mainly from effusions rather than from cultivation of

disaggregated solid biopsy material. AMS3 was derived from a solid tissue biopsy that was heterotransplanted into nude mice and passaged several times prior to *in vitro* cultivation [50]. Many cell lines lead to tumor formation when heterotransplanted into nude or SCID mice. For many of the cell lines the currently published body of information is fragmentary. For example, UCONN-L2 and L82 were briefly mentioned in reports focusing on cloning of the t(2;5) breakpoint and on its expression in HD, respectively [40,41]. All of these cell lines share the characteristic translocation t(2;5)(p23;q35) as evidenced by karyotypic analysis and/or, in cases of a cryptic t(2;5), by analysis of NPM-ALK chimaeric gene transcripts. Moreover, expression of ALK gene products was also verified by *in situ* hybridization with *ALK*-specific RNA probes, and immunocytochemistry using ALK-specific antibodies [24,46]. The cell lines lack all of those translocations frequently found in germinal center cell, mantle cell, diffuse large B-cell, and Burkitt's lymphomas, such as t(14;18), t(11;14), t(3;14), t(3;22), t(8;2), t(8;14) and t(8;22).

5. IMMUNOPHENOTYPE

ALCL cell lines, by definition, express the CD30 antigen ("Ki-1"-antigen), a member of the TNF receptor superfamily and receptor to the CD30 ligand, CD30L. Paralleling the phenotype of Hodgkin- and Reed-Sternberg (HRS) cells and HD-derived cell lines, other markers characteristic for mitogen-activated lymphocytes ("activation markers") are regularly found on ALCL cell lines such as the IL-2 receptor (CD25, Tac antigen), the CD70 antigen ("Ki-24"-antigen, a member of the TNF family and ligand to CD27), and HLA class II-antigens (HLA-DR) (Table 2). The transferrin receptor (CD71), a widely expressed proliferation-associated marker, is also found on virtually all ALCL cell lines. CD40, another member of the TNF receptor superfamily, is expressed by Karpas 299, but may not be common to all ALCL cell lines because, and in contrast to HRS cells, less than 40% of ALCL biopsies display CD40 protein [9]. CD80 (B7 antigen), a co-regulator of T-cell activation in concert with CD86, was not expressed on three cell lines studied, despite its apparently regular expression in ALCL as previously suggested by Delabie et al. [12].

Although fragmentary in some instances, the published phenotypes of the 11 cell lines listed in Tables 1–5 reflect heterogeneous expression of T-, B-, and/or myelomonocytic markers. CD3 and T-cell receptor (TcR) transcripts or proteins are found in only a few of the lines, in some instances requiring stimulation by phorbol esters, such as SR786 [54]. There are a number of discrepancies between published phenotypic details and those evaluated at the

Table 1. ALCL cell lines: clinical characterization

Cell	Patient age/sex	Diagnosis	Previous treatment	Specimen site	Culture medium	Ig-, TcR rearrangement status*	Primary ref.
SU-DHL-1	10 M	diffuse large cell lymphoma, non-cleaved	none (at presentation)	pleural effusion	RPMI 1640 + 10% FCS	TcRβ1 G/D, TcRβ2 R/G,IgH G/G	16,17,39
Karpas 299	25 M	Ki-1+ high grade T-cell lymphoma	extensive chemotherapy	pleural effusion	RPMI 1640 + 15% FCS	TcRβ R	18
SR768	11 M	Ki-1+ large T-cell lymphoma		pleural effusion	RPMI 1640 + 15% FCS	TcRβ R, IgH G	5,54
SUP-M2	5 F	malignant histiocytosis	chemotherapy (CHOP)	cerebrospinal fluid		TcRβ1 R/R, TcRβ2 G/G, IgH G/G	39
JB6	12/M	ALCL, advanced		peripheral blood	RPMI 1640 + 15% FCS	TcRβ R, IgH G	30
DEL	51/M	malignant histiocytosis	none		RPMI 1640 + 15% FCS	TcRβ G/G, IgH R/G, IgLCκ G/G	4,20
KI-JK	Child	ALCL	none	pleural effusion	RPMI 1640 + 10% FCS	TcRβ G, IgH G	49
UCONN-L2		relapsed ALCL			McCoy's 3A + 15% FCS		40
AMS3	23 M	ALCL		"5 × 5 cm tumor in the back"	grows only in SCID mice		50
WSU-ALCL	18	primary T-ALCL		lymph node	RPMI 1640 + 10% FCS		2
L82	24 F	relapsed ALCL		pleural effusion		TcRβ R, IgH G	41

* R – rearranged, G – germline, D – deleted.

Table 2. Phenotypic characteristics of ALCL cell

Cell line	p80 (IH)	ALK (ISH)	NPM-ALK	Activation markers						Lymphoid markers	T-cell markers							
				CD30	CD25	CD70	CD71	CD80	HLA-DR	CD45	CD2	CD3	CD4	CD5	CD7	CD8	CD43	CD45R0
SU-DHL-1	+	+	+	+++	+++	++	+++	−	+++		−	−	−	+++	−	−		
Karpas 299	+	+	+	+++	+++	++	+++	−	+++	+	−	−	+	+++	−	−	++	
SR786	+		+	+++	+++		+++		+++	+	−	−	+	−	−	−		
SUP-M2	+		+	+++	+++		+++		+++		+	−	+	−	−	−		+
JB6	+	+	+	+++	+++	++	+++	−	+++		−	−	+	−	−	−		
DEL	+	+	+	+++	+++	++	+++		+++	+	−	−	−	−	−	−		−
KI-JK	+		+	+++	+++		+++		+++		−	+	−	+	+++	−		−
UCONN-L2	+		+	+++	+++		+++		+++			−		−	−			
AMS3	+		+	+++	+++		+++		+++	+		+	−			−		
WSU-ALCL	+		+	+++	−		+++		+++			+	+	+++	+++	−		
L82	+		+	+++	+++		+++		+++			+	+	+		−		

Continued on next page

Table 2. (continued)

Cell line	B-cell markers						Myelomonocytic markers							Progenitor markers		Other markers
	CD19	CD20	CD21	CD22	CD23	CD45RA	CD11b	CD11c	CD13	CD14	CD15	CD33	CD64	CD10	CD34	EMA
SU-DHL-1	–	–	–	–			–	–	–	–	–	–		–	–	+
Karpas 299	–	–	–	–	–	–	–	+	++	–	–	–	–	–	–	+
SR786	+++	+	–	–			+++		+	–	+	++		–	–	
SUP-M2	–	–	–	–				–		–	–					+
JB6	–	+/–					–		+++	–	++	–		–	–	
DEL	–	–	–	–			–	–	++	–	++	++		–	–	
KI-JK	+++	+++	–		–		+++		+++	–	+	+++		–	–	
UCONN-L2	–	++					++		–	–	–	–		–	–	
AMS3	–	–		–												
WSU-ALCL	–	–					–		–	–	+++	–		++	–	
L82																

Cell line	References
SU-DHL-1	[15,24,39]; H. Dürkop, personal communication, DSMZ database
Karpas 299	15,18,24]; H. Dürkop, personal communication, DSMZ database
SR786	[5,15,54]
SUP-M2	[35,39]
JB6	[15,24]; H. Dürkop, personal communication; DSMZ database
DEL	[4,15]; DSMZ database
KI-JK	[15,49]; DSMZ database
UCONN-L2	[15,41]; DSMZ database
AMS3	[50]
WSU-ALCL	[2,15]
L82	[41]; H. Merz, personal communication

Table 3. ALCL cell lines: cytokine, cytokine receptor and c-onc gene products

Cell line	Cytokine receptor gene expression	Cytokine gene expression	Expression of other c-onc genes	Ref.
SU-DHL-1	IL-1-R, IL-2-Rα, IL-6-R, TNFα-R, c-fms	IL-1β, IL-7, IL-10, TNFα	bcl-6	1,8
Karpas 299	IL-2-Rα, c-met	HGF	bcl-6	8,45,57
SR768	IL-2-Rα, c-met	IL-2, TGFβ, HGF, CCR4, CCR8	c-myc, H-ras	45,54,55
SUP-M2	IL-1-R, IL-2-Rα, IL-6-R, TNFα-R, c-fms			1
JB6	IL-1-R, IL-2-Rα, IL-6-R, TNFα-R			1
DEL	IL-2-Rα, c-fms		bcl-6, c-fgr, c-myb, c-myc, c-pim, K-ras	8,20
KI-JK	IL-2-Rα			1,49
UCONN-L2	c-met	HGF		45
AMS3				
WSU-ALCL			c-myc, bcl-2	2
L82				

Table 4. ALCL cell lines: genetic characterization

Cell line	Representative karyotype	Ref.
SU-DHL-1	74(67-75)<3n>XX, −Y, +1, +2, +3, +5, −7, +12, −16, −18, +19, −20, +21, +3mar, del(1)(p21), t(2;5)(p23;q35)x2, del(6)(q23)x1-2, add(8)(p12), add(9)(p21), del(10)(p14), add(12)(q24), add(14)(p12), add(16)(q24), dup(19)(q13.1qter); sdl with der(12)t(12;19(q13;q13); carries two copies of balanced t(2;5)	39; DSMZ database
Karpas 299	44, XY, −10, −22, rcp t(1;17)(p21;p11.2); rcp t(2;5)(p23.2;q35.3); rcp t(3;6)(p23;p12.2); t(13;15)(p12;q13.1); t(14;22)(p12;q11.21); mar22:der(22)22p12-q11.1	18
SR768	70-84<3n>XX, +1, +2, −4, +5, +6, +7, +8, −13, −13, +14, −18, +19, +22, +6-9mar, add(1)(q11), del(1)(p11)/der(?)t(1;?)(q11;?), der(2)t(2;5)(p23;q35)inv(2)(p23q14)x2, del(4)(q22), der(5)t(2;5)(p23;q35)x2, del(7)(q21), der(9)t(1;9)(q11;p24)x2, der(12)t(12;13)(q24.32;q11)x1–2, del(13)(q13q31), add(14)(p11)/der(?)t(14;?)(q11;?)x2, del(21)(q22)	DSMZ database
SUP-M2	47, XX, +X, −9, +der(1)t(1;?)(q44;?), del(1)(p34), t(2;5)(p23;q35)	39
JB6	49, XY, +1, der(2)t(2;5)(p23;q35)t((2;21)(q14;q11), der(5)t(2;5)(p23;q35), +8, +13, add(15)(q26), der(21)t(2;21)(q32;q11), i(22)q(10)	42
DEL	74 (74-77) <3n>XXY, +1, +3, +5, +5, +6, +7, −10, +13, −18, +20, t(5;6)(q35;p21) x2, add (10)(q24) x1–2, der(13)t(1;13)(q21;p11)t(1;13)(q21;q24)x2; add(16)(q23), add (19)(p13)	DSMZ database
KI-JK	Pseudotetraploid with modal no. of 82, cryptic t(2;5)	49
UCONN-L2		
AMS3	46, XY, t(2;5)(p23;q35), del(6)(q13q23)	50
WSU-ALCL	45, 10p−	2
L82		

Table 5. ALCL cell lines: functional characterization

Cell line	Doubling time	Viruses	Cytochemistry	Heterotransplantation into mice	Ref.
SU-DHL-1	15–18 hr	EBV– (EBER–), HBV–, HCV–, HHV-8–, HIV–, HTLV-I/II–	ACP +, ALP–, ANAE +, ANBE +, CAE–, GLC +, lysozyme–, MGP +, MPO +, PAS +, SDB–, TRAP–	tumor growth in nude mice	DSMZ database
Karpas 299	18 hr	EBV– (EBNA–, EBER–), HBV–, HCV–, HHV-8–, HTLV-I	ACP +, ANAE +, ANBE +, CAE–, MPO–, PAS +, SDB–, elastase–, muraminidase–,		18; DSMZ database
SR768	30 hr	EBV–, HBV–, HCV–, HHV-8–, HIV–, HTLV-I/II–			DSMZ database
SUP-M2	15–18 hr				
JB6	18 hr	EBV– (EBER–)		tumor growth in SCID mice	DSMZ database
DEL	18–30 hr	EBV– (EBER–), HBV–, HCV– , HHV-8–, HIV–, HTLV-I/II–	ACP+, ALP–, ANAE(+), CAE–, MPO–, oil red O+, PAS (+)	tumor growth in nude mice	DSMZ database
KI-JK	72 hr	EBV+ (LMP1, EBNA2, EBER)	ANBE +, lysozyme +	tumor growth in nude mice	49
UCONN-L2					
AMS3				*established from heterotransplanted tumor tissue*	50
WSU-ALCL					
L82					

ACP – acid phosphatase; ALP – alkaline phosphatase; ANAE – alpha-naphthylacetate esterase; ANBE – alpha-naphthylbutyrate esterase; CAE – chloroacetate esterase; MPO – myeloperoxidase; PAS – periodic acid-Schiff; GLC – β-glucuronidase; MGP – methyl green pyronine; MPO – myeloperoxidase; SDB – sudan black; TRAP – tartrate-resistant acid phosphatase.

DSMZ. In part, these discrepancies may be related to differences in culture conditions, such as differences in fetal bovine serum (FBS) supplementation, and may merely reflect inducibility of certain genes.

Rearrangements of TcR genes are observed in most cell lines and correlate with TcR gene expression in SR786 and Karpas 299. Expression of B-lineage antigens CD 19 and CD20 is restricted to three of the lines, UCONN-L2, KI-JK, and SR786; in the latter two, on a background of CD3 expression. Immunoglobulin heavy or light chain expression was not observed, even in DEL which carries a monoallelic IgH gene rearrangement [20]. Many of the cell lines display a spectrum of myelomonocytic markers, most notably CD11b (integrin αM, CR3), CD 13 (aminopeptidase M), and CD33 (gp67 sialoadhesin). CD 15 (Lewis X antigen, X hapten) expression, which is described as a distinguishing immunohistological feature of HRS cells, but not of typical ALCL, is surprisingly found in the majority of the ALCL cell lines. On balance, most lines can be assigned to a lymphoid, mainly T-cell lineage on the immunophenotypic and gene rearrangement data.

Among markers typically expressed in ALCL biopsy material is epithelial membrane antigen (EMA), an epithelial sialomucin encoded by the MUC1 gene on chromosome 1. It has been suggested that the t(2;5) might promote in lymphoid cells, by an unknown mechanism, expression of EMA and of CD30, also encoded on chromosome 1 [6].EMA is expressed on SU-DHL-l, Karpas 299 and SUP-M2. Restin (Reed-Sternberg-cell intermediate filament-associated protein) is found in HRS cells and ALCL in most instances, and was also detected in Karpas 299 cells [13].

6. CYTOKINES, CYTOKINE RECEPTORS, C-ONC GENES

In contrast to HD-derived cell lines, few studies have described the expression of cytokines and cytokine receptors (with the exception of CD25, CD30, CD40, CD70, see above) by ALCL cell lines (Table 3). Expression of interleukin (IL)-2 was demonstrated for SR768 and may result in an autocrine stimulatory loop. The same may be true for HGF (hepatocyte growth factor, scatter factor) and its receptor, c-Met, in Karpas 299, SR768, and UCONN-L2 [45]. Although the frequent expression of IL-6, IL-9 and IL-10 in HRS cells and ALCL biopsy material might suggest that expression of these cytokines may be a regular finding in ALCL cell lines, published data are limited to a report of expression IL-10 transcripts in no more than 1% of SU-DHL-1 cells [7], and to a paper describing the presence of IL-9 transcripts in an ALCL cell line designated SKA (perhaps identical to SR768) [37]. Several ALCL cell lines, among them Karpas 299, JB6 and SUP-DHL-1, were tested for CD30 ligand (CD30L) expression [21]. These lines did not

express CD30L. However, some of the cell lines listed as ALCL lines were probably EBV-positive lymphoblastoid cell lines obtained from ALCL tissue (H. Merz, personal communication). In contrast to HD-derived cell lines, it was found that CD30L produced antiproliferative effects in ALCL cell lines that could be blocked by addition of soluble CD30 [22].

Among receptors that may engage in paracrine loops is the proto-oncogene c-fms (CSF-1 receptor), displayed by SU-DHL-1 and DEL, and the chemokine receptors CCR4 and CCR8, present on Karpas 299 [57]. The cell line DEL has been studied more extensively for its expression of proto-oncogenes, and c-fgr, Ki-ras, c-myb, and c-myc have been found [20]. The significance of these findings is not clear. However, the expression of c-fgr has been claimed to point to a histiocytic origin for DEL. c-kit, encoding a membrane receptor tyrosine kinase, has been found in 11 of 16 ALCL biopsies [44] and seems to be exclusive to this lymphoma and HRS cells of HD. Corresponding data on ALCL cell lines are not yet available.

Interestingly, the zinc-finger protein Bcl-6, which has been identified in a proportion of diffuse large B-cell lymphomas by virtue of its involvement in translocations affecting chromosomal band 3q27, is often expressed by ALCL, and has been demonstrated in Karpas 299, SU-DHL-1, and DEL [8]. The biological significance of Bcl-6 expression, which is physiologically restricted to a small fraction of normal, resting CD4-positive T lymphocytes, is currently unknown. It is speculated that it may be related to the maintenance of the activated state [8]. Alterations of the PTEN tumor suppressor gene were not observed in Karpas 299, JB6, SR786, or KI-JK, indicating that abnormalities of the PTEN gene may not be relevant for ALCL [48].

7. VIRUSES

The association of ALCL with EBV has been documented by a number of studies. EBV gene expression in rare cases of T-ALCL, virtually never t(2;5)-positive, seems to be largely restricted to the EBV-encoded small nuclear RNA transcripts, EBER-1 and -2, whereas B-ALCL additionally express LMP-1. This is complemented by expression of EBNA-2 in a few cases, producing the phenotype of EBV-immortalized lymphoblastoid cell lines (type III latency). Unexpectedly, the cell line KI-JK, established from an ALCL of a child, displayed the full spectrum of latent EBV gene expression as well as NPM-ALK fusion transcripts and p80-specific immunostaining [15,49]. This finding underlines previous suggestions that EBV may be an etiologic factor relevant for at least a proportion of ALCL cases.

8. ALK-NEGATIVE ALCL CELL LINES

Few cell lines that have been considered to represent *in vitro* equivalents of ALCL have been reported, and none have been widely used. The cell lines McG-1 and McG-2 (also known as Mac-1 and Mac-2; [10]) were established from a patient with CD30-positive cutaneous T-cell lymphoma [31]. For the two cell lines, FE-PD and HKB-1, the derivation from either ALCL or HRS cells is not clear. FE-PD displays a t(1;8) and carries, with its immunoglobulin genes in germline configuration, a TcRβ rearrangement [11]. HKB-1 has been mentioned in the previous chapter on HD-derived cell lines [58]. These lines should be used with hesitation when drawing conclusions as to the distinguishing features of HD and ALCL. The cell line Michel, cited in several papers by Gruss and co-workers, is (though CD30-positive) not an ALCL cell line. Rather, it is an EBV-positive lymphoblastoid cell line (H. Merz, personal communication), and conclusions drawn from published data on the use of this cell line require particular caution.

9. CONCLUSION

ALCL cell lines have been instrumental for cloning of the genes involved in the t(2,5) and have served as controls for subsequent studies on biopsy material. Future studies may center on the definition of differences from HD-derived cell lines that may ultimately help to elucidate the molecular mechanisms leading to the diverse morphology and clinical presentation of the CD30-positive malignancies, HD and ALCL [27].

ACKNOWLEDGEMENTS

The authors are indebted to Drs. Horst Dürkop, Berlin, Germany, and Hartmut Merz, Lübeck, Germany, for communicating unpublished phenotypic data on Karpas 299, SU-DHL-1, and JB6 (H.D.) as well as on the lines L82 and Michel (H.M.).

References

1. Al Hashmi I, Cohen A, Freedman MH, Osman A et al. *Blood* 82: 132a, 1993.
2. Al-Katib A, Abubakr Y, Mohamed A, Maki A et al. *Blood* 84: 638a, 1994.
3. Anagnostopoulos I, Hummel M, Kaudewitz P, Herbst H et al. *Am J Pathol* 137: 1317–1322, 1990.

4. Barbey S, Gogusev J, Mouly H, Le Pelletier O et al. *Int J Cancer* 45: 546–553, 1990.
5. Beckwith M, Longo DL, O'Connell CD, Moratz CM et al. *J Natl Cancer Inst* 82: 501–509, 1990.
6. Benharroch D, Meguerian-Bedoyan Z, Lamant L, Amin C et al. *Blood* 91: 2076–2084, 1998.
7. Boulland ML, Meignin V, Leroy-Viard K, Copie-Bergman C et al. *Am J Pathol* 153: 1229–1237, 1998.
8. Carbone A, Gloghini A, Gaidano G, Dalla-Favera R et al. *Blood* 90: 2445–2450, 1997.
9. Carbone A, Gloghini A, Gattei V, Aldinucci D et al. *Blood* 85: 780–789, 1995.
10. Davis TH, Morton CC, Miller-Cassman R, Balk SP et al. *N Engl J Med* 326: 1115–1122, 1992.
11. Del Mistro A, Leszl A, Bertorelle R, Calabro ML et al. *Leukemia* 8: 1214–1219, 1994.
12. Delabie J, Ceuppens JL, Vandenberghe R de Boer M et al. *Blood* 82: 2845–2852, 1993.
13. Delabie J, Shipman R, Brüggen J, de Strooper B et al. *Blood* 80: 2891–2896, 1992.
14. Delsol G, Lamant L, Mariamé B, Pulford K et al. *Blood* 89: 1483–1490, 1997.
15. Dirks WG, Zaborski M, Jäger K, Challier C et al. *Leukemia* 10: 142–149, 1996.
16. Epstein AL, Kaplan HS. *Cancer* 34: 1851–1872, 1974.
17. Epstein AL, Levy R, Kim H, Henle W et al. *Cancer* 42: 2379–2391, 1978.
18. Fischer P, Nacheva E, Mason DY, Sherrington PD et al. *Blood* 72: 234–240, 1988.
19. Foss HD, Demel G, Anagnostopoulos I, Araujo I et al. *Pathobiology* 65: 83–90, 1997.
20. Gogusev J, Barbey S, Nezelof C. *Int J Cancer* 46: 106–112, 1990.
21. Grass HJ, Boiani N, Williams DE, Armitage RJ et al. *Blood* 83:2045–2056, 1994.
22. Gruss HJ, DaSilva N, Hu ZB, Uphoff CC et al. *Leukemia* 8: 2083–2094, 1994.
23. Harris NL, Jaffe E, Stein H, Banks PM et al. *Blood* 84: 1361–1392, 1994.
24. Herbst H, Anagostopoulos I, Heinze B, Dürkop H et al. *Blood* 86: 1694–1700, 1995.
25. Herbst H, Dallenbach F, Hummel M, Niedobitek G et al. *Blood* 78: 2666–2673, 1991.
26. Herbst H, Sander C, Tronnier M, Kutzner H et al. *Br J Dermatol* 137: 680–686, 1997.
27. Herbst H, Stein H, Niedobitek G. *Crit Rev Oncogenesis* 4: 191–239, 1993.
28. Herbst H, Stein H. *Leukemia Lymphoma* 9: 321–328, 1993.
29. Iwahara T, Fujimoto J, Wen D, Cupples R et al. *Oncogene* 14: 439–449, 1997.
30. Kadin ME, Cavaille-Coll MW, Morton CC. *Blood* 76: 354a, 1990.
31. Kadin ME, Sako D, Morton C, Newcom SR et al. *Lab Invest* 58: 45, 1988.
32. Kuefer MU, Look AT, Pulford K, Behm FG et al. *Blood* 90: 2901–2910, 1997.
33. Kuze T, Nakamura N, Hashimoto Y, Abe M et al. *J Pathol* 180: 236–242, 1996.
34. Lamant L, Dastugue N, Pulford K, Delsol G et al. *Blood* 93: 3088–3095, 1999.
35. Mason DY, Bastard C, Rimokh R, Dastugue N et al. *Br J Haematol* 74: 161–168, 1990.
36. Merz H, Fliedner A, Orscheschek K, Binder T et al. *Am J Pathol* 139: 1173–1180, 1991.
37. Merz H, Houssiau FA, Orscheschek K, Renauld JC et al. *Blood* 78: 1311–1317, 1991.
38. Mitev L, Christova S, Hadjiev E, Guenova M et al. *Leukemia Lymphoma* 28: 613–616, 1998.
39. Morgan R, Smith SD, Hecht BK, Christy V et al. *Blood* 73: 2155–2164, 1989.
40. Morris SW, Kirstein MN, Valentine MB, Dittmer KG et al. *Science* 263: 1281–1284, 1994.
41. Orscheschek K, Merz H, Hell J, Binder T et al. *Lancet* 345: 87–90, 1995.
42. Ott G, Katzenberger T, Siebert R, DeCoteau JF et al. *Genes Chromosomes Cancer* 22: 114–121, 1998.
43. Peuchmaur M, Emilie D, Crevon MC, Solal-Celigny P et al. *Am J Pathol* 136: 383–390, 1990.
44. Pinto A, Gloghini A, Gattei V, Aldinucci D et al. *Blood* 83: 785–792, 1994.

45. Pons E, Uphoff CC, Drexler HG. *Leukemia Res* 22: 797–804, 1998.
46. Pulford K, Lamant L, Morris SW, Butler LH et al. *Blood* 89: 1394–1404, 1997.
47. Rosenwald A, Ott G, Puford K, Katzenberger T et al. *Blood* 94: 362–364, 1999.
48. Sakai A, Thieblemont C, Wellmann A, Jaffe ES et al. *Blood* 92: 3410–3415, 1998.
49. Shimakage M, Dezawa T, Tamura S, Tabata T et al. *Intervirology* 36: 215–224, 1993.
50. Shiota M, Fujimoto J, Semba T, Satoh H et al. *Oncogene* 9: 1567–1574, 1994.
51. Stansfeld AG, Diebold J, Noel H, Kapanci Y et al. *Lancet* 1: 292–293, 1988.
52. Stein H, Herbst H, Anagnostopoulos I, Niedobitek G et al. *Ann Oncol* 2 (Suppl. 2): 33–38, 1991.
53. Stein H, Mason DY, Gerdes J, O'Connor N et al. *Blood* 66: 848–849, 1985.
54. Su IJ, Balk SP, Kadin ME. *Am J Pathol* 132: 192–198, 1988.
55. Su IJ, Kadin ME. *Am J Pathol* 135: 439–445, 1989.
56. Tilly H, Gaulard P, Lepage C, Dumontet C et al. *Blood* 90: 3727–3734, 1997.
57. van den Berg A, Visser L, Poppema S. *Am J Pathol* 154: 1685–1691, 1999.
58. Wagner HJ, Klintworth F, Jabs W, Lange K et al. *Med Pediatr Oncol* 31: 138–143, 1998.
59. Wlodarska I, de Wolf-Peeters C, Falini B, Verhoef G et al. *Blood* 92: 2688–2695, 1998.

Chapter 13

Authentication and Characterization

Roderick A.F. MacLeod and Hans G. Drexler
DSMZ – German Collection ofMicroorganisms and Cell Cultures, Mascheroder Weg 1b, 38124 Braunschweig, Germany. Tel: +49-531-2616-160; Fax: +49-531-2616-150; E-mail: rml@dsmz.de

1. BACKGROUND

Despite their many and varied origins from different stages and pathways of differentiation, human hematopoietic tumor cell lines outwardly betray little of their individual characteristics. These characteristics must be elucidated by performing a battery of assays before the derivation can be confirmed and the potential value of the cell line assessed. In addition, given the unacceptably high incidence of cross-contamination occurring among new cell lines [22,57], originators must prove authenticity.

The most versatile and informative tests for hematopoietic cell lines are immunophenotyping and cytogenetic analysis. While some characteristics of tumors are almost invariably retained by derived cell lines (such as primary chromosome rearrangements), others may change during disease progression or undergo apparent modification *in vitro* (such as immunoprofiles, which may not coincide in every detail). Thus, rather than passively adopting the diagnosis provided for the donor patient, classification of hematopoietic cell lines must take into account the results of characterization, possibly resulting in subsequent reassignment to a different lineage.

The emergence of a correlation between the morphological French-American-British (FAB) system subdividing AML [3] and the results of cytogenetic investigation encouraged the subsequent scheme widely adopted for classifying lymphoid malignancies – Morphologic, Immunologic, Cytogenetic (MIC) – to incorporate chromosomal findings. Where a particular chromosome rearrangement exhibits unusual consistency and specificity, the existence of that rearrangement (whether diagnosed cytogenetically or by PCR) is the main diagnostic criterion, as for example the presence of t(11;14)(q13;q32) in mantle cell lymphoma, or t(2;5)(p23;q35) in anaplastic large cell lymphoma (ALCL).

J.R.W. Masters and B.O. Palsson (eds.), Human Cell Culture Vol. III, 371–397.

The presence of a particular chromosome change provides strong evidence that the original tumor and its derived cell line share common ancestral origins. By the same token, the failure of a cell line to retain a primary chromosome change previously detected in the donor patient makes a common origin highly unlikely, thus permitting reactive host and neoplastic cells to be distinguished.

It is seldom sufficiently appreciated that a significant number of cell lines have been misclassified or misidentified completely due to cross-contamination by another cell line. Provision of adequate evidence of authenticity is often crucial to understanding the significance of findings obtained using cell lines. For instance, the most frequently cited human endothelial cell line, ECV-304, is spurious, having been cross-contaminated by the bladder cancer cell line, T-24 [15].

Cell lines bearing rarer recurrent chromosome translocations constitute an irreplaceable resource for cloning the genes involved and mapping rearrangements at the molecular level. Analysis of cell lines described prior to the advent of molecular cytogenetics has identified several such lines.

This chapter will deal with the detection and identification of false cell lines using both DNA profiling and cytogenetic methods, and illustrate some uses of cytogenetics in characterizing human hematopoietic tumor cell lines.

2. AUTHENTICATION

A chronic problem in cell culture, which extent is seldom fully appreciated, is the cross-contamination of one cell line by another [58]. According to a recent survey, performed using the most sensitive identification methods currently available, approximately one in six new human tumor cell lines are impostors, having been cross-contaminated by other cell lines [57]. Although the problem among human hematopoietic cell lines is of comparable magnitude, the greater availability and informativeness of karyotypes among this group may facilitate the speedier detection and removal of false examples [22]. The vast majority of cross-contaminations in our survey involved intraspecies contamination of one human cell line by another. Another survey concluded that about a third of cell lines in circulation were false [39].

The number of human leukemia-lymphoma cell lines is estimated at more than one thousand [19], indicating the high level of sensitivity to be demanded of any system adopted for detecting and identifying cross-contamination by recipients of cell lines. Unfortunately, the number of cell lines which may be confidently identified from published references is limited. Rapid advances in molecular genetics have hitherto hampered the general adoption of standardized DNA fingerprinting methods for cell line authentication, and in-

formative published DNA fingerprints are still conspicuously rare. Although detailed karyotypes are increasingly provided for many cell lines, most predate the advent of sensitive molecular methods such as fluorescence *in situ* hybridization (FISH) needed for interpreting all but the most straightforward structural chromosome rearrangements. As a result, karyotypes of a significant number of the older cell lines may be incomplete or even misleadingly inaccurate.

The paucity of reports documenting the provenance or authenticity of cell lines suggests a lack of general awareness of the extent of cross-contamination among contributors and editors alike. Confirming this view, and despite the rapid expansion in the numbers and varieties of available cell lines, many of the same contaminants are involved as when the problem was first encountered some twenty years ago. The vast majority of cross-contamination occurs intraspecies, with a distinct tendency for the contaminating cell lines to mimic the supposed attributes of their targets, While deliberate fraud cannot be excluded, our experience indicates that carelessness prevails over deceit as the main cause of the problem.

2.1. Distortions Attributable to Cross-Contaminated Cell Lines

U-937, a misidentified cell line masquerading as a representative *in vitro* model of macrophage-monocyte differentiation, was widely distributed before cross-contamination by the chronic myeloid leukemia (CML) cell line K-562 was discovered [77]. Misidentified samples of U-937 and many other cell lines have been used in countless published studies, but in contrast to other types of scientific fraud, reports are rarely retracted or publicly qualified in the light of the use of the false cell line.

A second type of error occurs when misidentification involves mischaracterization. This error is most problematic when the disease has few *in vitro* models, and so the reporting of inconsistent data is less apparent. For example, one study of Hodgkin's disease (HD) was in fact carried out using a T-cell acute lymphoblastic leukemia (ALL) cell line [30] and another contamination involved the well-known acute megakaryocyte leukemia (AMegL) cell line, DAMI [32], now known to have been cross-contaminated at initiation [56] by the classic erythroleukemia cell line, HEL [59]. Unfortunately, at the time, DAMI was one of few AMegL cell lines freely available, having been deposited by its originators with the ATCC, resulting in its rapid and widespread distribution. DAMI continues to be used despite its fictitious claim to represent AMegL, registering more than 100 retrievable citations on Evaluated Medline, which is an underestimate of the actual usage. Given their close developmental proximity, it is likely that many findings performed using erythrocytic cells are indeed applicable to megakaryocytes: hence, it is

doubly unfortunate that, had its true origin been known at the time, the validity of many experiments inadvertently performed using the DAMI-subclone of HEL would have remained untainted.

Misclassification may also occur in the absence of cross-contamination. Perhaps the most widely used and best-known hematopoietic cell line is HL-60, described as originating from a case of acute promyelocytic leukemia (AML-M3) [10,29] – a misclassification which persists despite having been revised on morphological grounds to AML-M2 by the original investigators [11]. This reassignment gains crucial support from the results of cytogenetic investigations confirming the absence of t(15;17)(q22;q21), a rearrangement confined to AML-M3 and now known to occur in practically all cases with this disease subtype [5,49].

A further type of distortion occurs with the unwitting use of misidentified subclones. For example, the observation of induction of *RALDH2* expression by *TAL1* in T-cell leukemia was based on inducibility in "both" CCRF-CEM and MKB-1 cell lines [74] the latter being, unknown to these authors, a subclone of the former (Table 1). This type of distortion may lead to ill-founded conclusions. For example, a novel leukemia subtype was identified by the occurrence of molecularly identical chromosome translocations in three supposedly independent acute leukemia cell lines NALM-6, PBEI and LR10.6 [94], subsequently shown to be genetically identical by both karyotyping and DNA profiling [22,57]. Thus, both PBEI and LR10.6 are cross-contaminants of NALM-6, the first of the three to be established and, thereafter, widely distributed.

It is a cause for concern that false cell lines should remain undetected for so long despite yielding inappropriate data. Is is likely that negative results go unreported or, if reported, simply ignored. Those least likely to learn of doubts concerning particular cell lines, such as beginners or associates, are also those least empowered to act as "whistleblowers", and there appears to be a conspiracy of silence over this form of passive scientific fraud. Thus, it is often left to cell repositories like the ATCC or DSMZ, which perform multiparameter cell line authentication, to pronounce on matters concerning the true identity of cell lines with any degree of objectivity [54].

Restrictions on distribution are also a barrier to authenticating cell lines. By sidestepping checks by other users or cell repositories, restricted cell lines may evade authentication altogether. For example, the singular and restricted cell line AG-F [30], was purportedly established from an HD patient after previous treatment for high grade neuroblastoma. Uniquely among hematopoietic cell lines, it was reported to retain the MYC-N amplification seen in the tumor of origin, despite displaying a typical T-cell immunoprofile. The well-documented karyogram accompanying the original description of AG-F reveals a tetraploid karyotype which is an almost exact two-fold iteration

Table 1. False or misidentified cell lines

False cell lines		Actual cell lines	
Name	Classification	Name	Classification
(a) *Cross contaminations occurring in originators' laboratories*:			
207*	BCP-ALL	REH	BCP-ALL
AG-F	Hodgkin's disease	CCRF-CEM	T-ALL
DAMI	AML-M7	HEL	AML-M6
ECV-304	endothelial	T-24	bladder carcinoma
F2-4B6, F2-4E5	thymic epithelial	SK-HEP-1	liver carcinoma
FQ, Rb, SpR	Hodgkin's disease	monkey	?
HS-SULTAN	multiple myeloma	Jiyoye	Burkitt's lymphoma
J-111	monocytic leukemia	HeLa	cervix carcinoma
JOSK-I	AML-M4	U-937	histiocytic lymphoma
JOSK-K	AML-M5	U-937	histiocytic lymphoma
JOSK-M	CML-BC	U-937	histiocytic lymphoma
JOSK-S	AML-M5	U-937	histiocytic lymphoma
LR10.6	BCP-ALL	NALM-6	BCP-ALL
MKB-1	T-ALL	CCRF-CEM	T-ALL
MOLT-15	T-ALL	CTV-1	AML-M5
OCI-AML-12	AML	OCI-AML-1	AML-M4
P1-4D6, P1-4E5	thymic epithelial	SK-HEP-1	liver carcinoma
SPI-801/802	T-ALL	K-562	CML-BC
(b) *Cross-contaminations occurring subsequently*:			
207§	BCP-ALL	CCRF-CEM	T-ALL
CO	Hodgkin's disease	CCRF-CEM	T-ALL
KE-37§	T-ALL	CCRF-CEM	T-ALL
KM-3	BCP-ALL	REH	BCP-ALL
MB-02§	AML-M7	HU-3	AML-M7
RC-2A	AML-M4	CCRF-CEM	T-ALL

The table lists some false cell lines of hematologic interest and the authentic prototypes from which they are derived. Part (a) lists spurious cell lines, i.e. cross-contaminations known to have occurred in originators' laboratories and except where indicated (*) may be taken to affect most or all stocks in circulation. Part (b) lists subsequent misidentifications *probably* occurring in recipient laboratories, although only for those labelled (§) can the existence of authentic stocks be confirmed. In most intraspecies contaminations, identities were confirmed by both DNA profiling and cytogenetics. Modified from Drexler et al. [22] and MacLeod et al. [57].

of the major diploid clone present in the "classic" T-cell line CCRF-CEM established some thirty years earlier and widely distributed [57]. CCRF-CEM and AG-F are the only two cell lines reported to display t(8;9)(p11;p24), pointing again to cross-contamination.

In the absence of a central register of cell line descriptors (DNA profiles and karyotypes), which might enable originators of cell lines and those reviewing manuscripts to check for untoward matches indicative of cross-contamination, false cell lines will continue to be described. There also seems to be an information barrier concerning the existence of false cell lines, perhaps because publication of cross-contamination incidents occurs haphazardly and in specialist journals [58]. For this reason, the DSMZ will, in future, list false cell lines on its website (www.dsmz.de). Secondly, even widespread publicity may be insufficient in itself. Despite repeated alerts to the risks of HeLa contamination and the availability of a variety of methods for its detection, our data show it remains the most prolific contaminant, at levels comparable to those reported 15 years ago. Thirdly, most reports describing new cell lines lack DNA profiling to document identity with the biopsy material, and detailed karyotypes are provided in few cases.

2.2. Prevention of Cross-Contamination

Some obvious precautions may be adopted to reduce the risks of receiving false cell lines. Cell lines are usually obtained from three different sources: friends and colleagues, cell repositories, and the orginators themselves. While the originators might be thought to offer the most impeccable provenance, this is the very group least likely to suspect cross-contamination. Furthermore, cell lines obtained from originators are in about a third of all cases contaminated with mycoplasma [90]. Cell lines obtained from friends or colleagues compound the problems caused by originators by adding to the risks of misidentification and cross-contamination at each remove. For cell repositories, the obvious consequences of late discovery means that the risks of cross- contamination among stocks distributed to investigators must be kept to a minimum. This awareness has led such institutions to undertake systematic identity testing programs among cell lines [35].

The risks of cross-contamination may be enhanced among unpublished cell lines. Those expecting to acquire for the first time new or untested cell lines from the originators can, and should, check if their genetic identity with biopsy material has been confirmed. Failing that, a well-documented karyotype, with accompanying karyogram, should be made available. Reports claiming unprecedented success in establishing cell lines where others have repeatedly failed should raise the suspicion of cross-contamination. For example, the reported establishment of a large series of thymic epithelial cell

lines by "spontaneous immortalization" [26] have now all been shown to be identical cross-contaminants [57]. Reports describing the serial establishment of several cell lines from the same type of tumor should be treated with similar caution. In the same study, we found that multiple instances of cross-contamination appeared to be clustered within certain institutions, more than half arising within six laboratories. Untoward cytogenetic similarities among cell lines of shared provenance is a further ground for suspicion as, with few exceptions, notably t(8;14)(q24;q32) in cell lines derived from Burkitt's lymphoma and other B-cell neoplasms, or t(9;22) in CML cell lines [18,20,21], recurrent chromosome changes usually occur too rarely in cell lines to be a conspicuous feature. Reports describing consistent chromosome changes should be treated with scepticism unless cross-contamination has been excluded by DNA fingerprinting. The similar rearrangements described in the four monocytic leukemia cell lines, JOSK-I/K/M/S, [72] were due to multiple cross-contamination by U-937 rather than the operation of consistent primary chromosome change. Instructively, the only sizeable panel of cell lines whose establishment was verified by DNA profiling passing through our hands [82] was also the only example totally free from cross-contamination when retested by us.

2.3. Detection of Cross-Contamination

A further problem contributing to the difficulty of detecting intraspecies cross-contamination is that, until recently, the necessary methods for its accurate detection were unavailable. The first breakthrough came with the discovery of stably inherited polymorphic variant satellite DNA regions which led eventually to the development of forensic DNA profiling [42]. Specific probes for polymorphic single loci were used for cell line identification by Masters et al. [60]. DNA profiling has three advantages. Firstly, it may be used by originators of new cell lines to confirm the identity of a new cell line with the primary culture or the biopsy material. Secondly, it may be used to confirm that subsequent passages remain free from cross-contamination by the simple expedient of comparing their DNA profiles with those of standards prepared fom early passage material. Thirdly, it is now being used at the DSMZ to detect false cell lines by comparing DNA profiles of candidate cell lines during accession with those already held. The effectiveness of such positive vetting relies on the construction of a searchable databank compiled from DNA profiles stored on disk after digitization which is being made freely available to assist other investigators wishing to check the identity of their cell lines [16].

2.4. DNA Profiling

A number of routine DNA profiling methods available for cell line authentication rely on either Southern blotting or electrophoresed PCR products, which detect one or more polymorphic loci to reveal "signature" profiles. Traditional multilocus profiling by Southern analysis detecting restriction fragment length polymorphisms (RFLP) potentially offers the highest levels of discrimination but is relatively slow, requires more DNA, and is less reproducible and more resource-consuming than PCR fingerprinting. In addition, the multilocus profiles so generated are ill-suited to the automation essential for archival comparison, and important information conveyed by band intensity levels may be lost on digitization, and undue weight automatically attached to invariant high molecular weight bands. Single-locus profiling, on the other hand, is flexible, as the desired sensitivity level may be tailored to the needs of the user by judicious choice of allele number and, in addition, the band profiles are ideally suited to digitization.

Before choosing a particular profiling system, two problems peculiar to tumor cell culture must be considered. First, loss of heterozygosity (LOH), taken to indicate the presence of tumor suppressor genes in the region affected, is known to occur widely in many different types of tumor and derived cell lines and, if chromosomally generated (for example by deletion or sister chromatid exchange), is likely to affect neighboring loci. A polymorphic locus favored in identity testing, D17S5 (*YNZ22*), is located on the short-arm region of chromosome 17 and may be lost together with *TP53*. Enhanced LOH simultaneously reduces the numbers of alleles available for comparison, encouraging "false positive" type errors. In addition, some cell lines may be prone to LOH, as we and others have observed among different samples of U-937 cells [57,84]. Curiously, the LOH we observed in U-937 occurred in samples obtained directly from its originator and affected all four polymorphic loci tested: D1S80 at 1p35-36, *ApoB* at 2p23-24, D2S44 at 2q21, and D17S5 at 17p13.3, whereas indirectly sourced material (from Japan) had retained heterozygosity at both D1S80 and D2S44.

A second problem concerns the instability experienced by microsatellite repeat loci in some tumor cells. It has been observed recently that microsatellite instability and frameshift mutations in *BAX* and transforming growth factor-β RII genes, though relatively uncommon in leukemias, may arise within hematopoietic cell lines at establishment or during subsequent culture [66]. We have observed significant variation in DNA profiles of $(GTG)_5$ microsatellite loci between subclones of some cell lines, confirming that instability may complicate identification based on microsatellite loci. In contrast to LOH, microsatellite instability tends to cause "false negative" errors by disguising similarities between subclones. In the cross-contamination in-

volving subclones of NALM-6 referred to above, microsatellite instability is thought to have hidden the common identities of the cell lines involved (P. Marynen, personal communication).

Taking these and other difficulties into account, for routine DNA profiling of cell lines, we have adopted a system measuring RFLP affecting minisatellite variable number tandem repeat (VNTR) loci by multiplexed PCR amplification [16] based on the work of King et al. [45]. In this system the number of polymorphic loci chosen for testing is tailored to the required degree of sensitivity. The four VNTR loci used for routine DNA profiling at the DSMZ are described in Table 2. Positive matches can arise between candidate cell lines undergoing authentication and those previously validated, usually suggesting cross-contamination of the former by the latter. "False positives" are excluded by multilocus fingerprinting using the $(GTG)_5$ microsatellite probe and by comparison with the results of karyotyping which is carried out in parallel, principally for reasons of authentication.

In our experience, multiparameter authentication of human cell lines combining cytogenetics with DNA profiling is not only prudent but, because the two methods are complementary, may be the only way to detect and identify the culprit cell line [31]. The principal objection to using cytogenetic methods for authentication, its lower cost-effectivness when compared to DNA profiling, disappears if it is being used for characterization.

2.5. Cytogenetic Authentication

The human haploid karyotype has about 300 bands even in mediocre chromosome preparations. Allowing for an observer error of plus/minus one band, this resolves to about 100 microscopically distinct zones. Thus, a conservative estimate of the number of different two break chromosome rearrangements involving different chromosomes (i.e. most chromosome translocations) that can be detected is in the order of 100 × 100 (i.e. 10,000). Therefore, the chance of identical non-primary translocations arising by chance in different cell lines is in the order of 0 .01 % (1 in 10,000). In addition to being invaluable for identifying cell lines and cross-contamination, it is also possible to identify secondary translocations present in subclones, as seen with CCRF-CEM (Table 1). This permits the subclones to be distinguished cytogenetically and is an advantage over DNA fingerprinting and profiling.

The classic CML cell line, K-562, uniquely carries two marker chromosomes in which *BCR-ABL* fusion effected by t(9;22)(q34;q11) is amplified in tandem [18,21]. Their occurrence, therefore, in another pair of cell lines, SPI-801 and SPI-802 [22], supposedly derived from T-ALL, is spurious and actually due to cross-contamination by K-562 [31]. Unsurprisingly therefore,

Table 2. VNTR loci used for authentication at DSMZ

Probe [ref.]	VNTR type	Alias	Chromosome location	Repeat-length (bp)	Product-length (bp)	Heterozygosity (%)
Apo-B [6]	minisatellite	–	2p23-p24	15	522–909	80
D1S80 [75a]	minisatellite	*MCT118*	1p35-p36	16	400–940	79
D17S5 [95]	minisatellite	*YNZ22*	17p13.3	70	170–1080	78
D2S44 [70]	minisatellite	*YNH24*	2q21	31	600–>5000	94
$(GTG)_5$ [79a]	microsatellite	–	subtelomeric	3	10–>15000	>99.9

The table lists salient attributes of four single-locus minisatellite probes used in multiplexed PCR detection and microsatellite $(GTG)_5$ multilocus probe used to detect polymorphic variation. Table modified from Dirks et al. [16].

excluding autologous cell lines derived from the same patient and those with near-normal karyotypes, no two tumor cell lines in the DSMZ collection are karyotypically identical.

The main hindrances to using cytogenetics for identification are both technical and interpretational. Unlike DNA extraction and purification, for which standard methods apply irrespective of cell type, those intending to obtain usable chromosome preparations from cell lines must be prepared to try a variety of methods (for example, the use of different hypotonic treatments). Only after obtaining adequate chromosome preparations may the problems of analysis be addressed. Cell lines show many different types of alteration: primary changes affecting specific oncogene rearrangements, secondary changes whose molecular consequences may be unknown but which are believed to promote tumor progression, random changes (for example due to DNA instability), and those possibly associated with adaptation to growth in culture. The most complex karyotypes among lymphomas and leukemias are those derived from HD or AML-M7, respectively, in which full analysis may be impractical even when augmented by FISH.

Under the common notation used for transcribing pictorial karyograms into written format, the International System for Chromosome Nomenclature (ISCN), chromosome rearrangements (markers) unidentifiable by banding analysis are binned together as "mar", irrespective of size, shape or form, reducing their informativeness for subsequent detection of untoward karyotypic similarities (indicative of cross-contamination). Although the compositions of marker chromosomes are theoretically solvable with the help of FISH, in the absence of additional clues, trial and error may require an impractical number of repeated attempts before the correct informative combination of chromosome painting probes is arrived at.

However, the latest technical developments spawned by FISH obviate at a stroke the necessity for such laborious and frustrating procedures. These methods generally involve hybridizing chromosomes with sets of whole chromosome (painting) probes combinatorially labelled so that each homolog (or part thereof) is identifiable via a unique spectral signature. This requires the use of sensitive cameras and optical devices for distinguishing the labelling spectra as well as specialized computer software. Spectral karyotyping (SKY) utilizes interferometry and Fourier analysis of a spectroscopic image to assess the color composition of individual light pixels [80]. Multiplex FISH (M-FISH) involves merging images for each of five or more fluorochromes collected separately through the appropriate passband filter sets [83]. In both systems, material originating from the different homologs are pseudocolored to highlight their differences for subsequent manipulation and documentation. Among more complex karyotypes, weeks or even months of work and the materials needed for scores of experi-

ments are therefore replaced by a single procedure. The relative advantages and disadvantages of both systems have been discussed elsewhere [52]. Neither system dispenses with the need for conventional banding analysis, however, as the chromosome painting probes used are not informative regarding the intrachromosomal locations of the rearrangements detected, and although a multicolor FISH system with band-specific probes (exploiting cross-hybridization with prosimian chromosome painting probes) has been recently developed [68,69], it is probably unsuitable for tumor cell karyotyping as breakpoints juxtaposed by translocation may share common syntenic origins and spectral signatures.

A second problem concerns so-called "chromosome instability". Although vanishingly few of the necessary longitudinal studies using cloned cell lines have been reported, the charge of instability is widely voiced regarding cell lines. The existence in a tumor cell line of a complex series of chromosome rearrangements, while often taken as evidence of "instability", is, of course, no proof that the rearrangements in question actually arose *in vitro.* In fact, comparison of three highly complex cell lines (HDLM-1/2/3) established independently from a patient with HD has revealed a high degree of similarity indicating relative stability *in vitro* [57a]. It is therefore probable that many instances of supposed chromosome instability may be due to the differential expansion of clones which had acquired their distinctive features *in vivo.* After comparing several subclones of the classic T-ALL cell line, CCRF-CEM [28] whose identities were all confirmed by DNA fingerprinting, we observed minor distinctions between the karyotypes in all examples except in one case (MKB-1) where the differences were extensive. MKB-1 is near-tetraploid and was reported to display a complex series of changes: 89, XX, −X, −X, −2, −4, t(5;6)(q13;q21–23), add(7)(q35), del(7)(q22), −9, inv del(9)(ql?3-pl?3::ql?3-qter)[sic], t(10;14)(q24;q11.2), del(12)(q21), del(13)(q12), dup(13)(q22qter), −16, −20, +21, der(21)t(21;21)(p11;q11), +22, del(22)(q11) by its originators (renotated from Matsuo et al. [61]). On the other hand, CCRF-CEM and its remaining subclones masquerading as 207, AG-F, KE-37, RC-2A, though distinct, display relatively simple, closely related karyotypes including a common change, t(8;9)(p11;p24) which is absent from MKB-1 (Table 1). This discrepancy prompted our examination of early-passage stocks of CCRF-CEM which revealed the presence of two distinct subclones, with and without the t(8;9), which had expanded differentially to give rise to the two families of subclones. Interestingly, Molenaar et al. [66] have shown that MKB-1, unlike the parent CCRF-CEM, exhibits microsatellite instability, suggesting a possible mechanism underlying its apparent chromosome instability. It remains to be ascertained whether this is a general mechanism leading to the acquisition of additional chromosome changes *in vitro.*

Chromosome instability may not be a general feature of hematopoietic cell lines, but some types of cell line, not necessarily those established the longest, do have both aneuploid and structurally rearranged karyotypes. Our experiences suggest that in the different lineages of hematopoietic tumor cell lines, control of numerical and structural rearrangement has been relinquished differentially. Of all types of cell line, those derived from mature B-cells retain the most stringent control of both ploidy and chromosome structure, followed by T-cell lines which, though often tetraploid at later passage, display relatively few structural rearrangements, while myeloid cell lines tend to lose control of both – the most extreme karyotypic deviants being those assigned to the erythoid-megakaryocytic pathway. Recent studies have shown that numerical chromosome change, as well as structural changes targeted at specific oncogenes, may be a key step in carcinogenesis [8,50,51].

The third main difficulty is that because of their complexity and unavoidable ambiguity in some cases, ISCN karyotypes resist digitization, preventing the karyotypic identification of false cell lines by computer. The problems inherent in the computerized "understanding" of written hematopoietic tumor karyotypes have been summarized by Anthony Moorman who has written some programs ([67]; A. Moorman, personal comm.).

The singular virtue of cytogenetics as applied to authentication is that karyotypes, unlike DNA profiles, are increasingly published for new cell lines by their originators and sometimes updated for older cell lines. Recently published guidelines for describing newly established cell lines [23] stress the importance of adequate cytogenetic documentation. By comparison of the detailed and accurate karyotype prepared by its originators for the false DAMI cell line [32] we were able to confirm that samples of DAMI held by the DSMZ and ATCC truly represented the material described by its originators, thus proving that the similarity of DAMI to the classic HEL cell line [59] shown by DNA profiling was due to cross-contamination by its originators [56]. In this way, comparative cytogenetics was able to fill the gap occasioned by the loss of stored early passage ampoules of DAMI which its originators claimed was caused by the untimely breakdown of a storage freezer.

2.6. False Cell Lines

In our experience, the most likely contaminants to invade and take over primary cell cultures are well known, long-established "classic" cell lines [57]. It is scarcely coincidental that the "classic" T-cell line, CCRF-CEM, has been instrumental in spawning so many impostors (Table 1). In addition to the above-mentioned karyotypically and genetically distinct MKB-1, a further authenticated subclone is available which grows adherently, almost unprecedented among hematopoietic cell lines. In addition to seniority

and ubiquity, CCRF-CEM displays the unusual, though dubious, virtue of thriving at clonal cell densities, heightening the risk of transmission by, for example, aerosols. Other prolific cross-contaminants affecting cell lines of interest in hematology (Table 1) include U-937 and the SK-HEP-1 liver cancer cell line, which we have shown to be the source of a large series of supposed thymic epithelial cell lines [57].

In the absence of previously published data, information relevant to authentication is also afforded by characterization (see below). Table 3 lists hematopoietic cell lines with recurrent primary chromosome changes and shows how, within the limits of incompleteness, some recurrent translocations are sufficiently rarely represented to be of value in identification.

2.7. Interspecies Cross-Contamination

Among the earliest well-known instances of cross-contamination affecting hematopoietic cell lines is the curious case of several purported HD cell lines, FQ, Rb, and SpR, which were subsequently instead shown to be of simian origin [34]. According to our survey, although the vast majority of cross-contamination affecting human tumor cell lines occurs intraspecies, there is some evidence that interspecies contamination may come to predominate downstream when a greater variety of cell lines than those present within the parent laboratory may be encountered [39]. Equally, interspecies contamination should be easier to detect than that occurring intraspecies. A simple and relatively inexpensive method for its detection by isoenzyme analysis is discussed by Steube et al. [85]. As with most electrophoretic methods, the cost effectiveness of isoenzyme analysis disappears unless batch testing is performed. For those wishing to detect individual instances therefore, cytogenetics is the obvious alternative, though some familiarity is required with the chromosome banding patterns of human and the more common animal cell lines, including rodent, ungulate and primate. Murine cell lines are statistically the most likely animal contaminant: mouse chromosomes distinguish themselves from human chromosomes at a glance by virtue of their morphology: telocentric (or Robertsonian fusions thereof) with unmistakeably dense G-band positive paracentromeric heterochromatin, apparent after G-banding [81].

3. CYTOGENETIC CHARACTERIZATION

The notion that tumor cells tend to carry chromosome changes long predates the advent of molecular cytogenetics. Over a century ago, von Hansemann proposed that aberrant mitoses might be typical of cancer cells [92]. The

Table 3. Hematopoietic malignant cell lines with recurrent chromosome and gene changes

Recurrent alteration	Genes fused	Cell lines
(a) BCP (ALL-derived)		
t(1;19)(q23;p13)	*PBX1/E2A*	697*, ALL-2, KMO-90, KOPN63, LC1;19, LILA-1, LK63, MHH-CALL-3*, PER-278, SUP-B27
t(4;11)(q21;q23)	*AF4/MLL*	AN4;11, B1, KOCL-45, KOCL-58, KOCL-69, RS4;11, SEM
t(8;14)(q24;q32)	*MYC/IGH*	380* (3-way translocation with 18q21), KHM-2B, MHH-PREB-1*
t(9;22)(q34;q11)	*ABL/M-BCR*	ALL-1, NALM-27/28
t(9;22)(q34;q11)	*ABL/m-BCR*	ALL/MIK, KOPN-30bi/57bi, MHH-TALL1, NALM-20, PALL-1/-2, SUP-B13/15, TOM-1
t(11;19)(q23;p13)	*MLL/ENL*	BS, KOCL-33, KOCL-44, KOCL-50, KOCL-51, KOPN-1, KOPN-8
t(12;21)(p13;q21)	*TEL/AML1*	REH *(3-way translocation with 4q32 and 16q24), SUP-B26, UOC-B4
t(17;19)(q22;p13)	*HLF/E2A*	HAL-01, UOC-B1
(b) BCP (CML-derived)		
t(9;22)(q34;q11)	*ABL/M-BCR*	BV-173*, NALM-1*
(c) B-cell (ALL- or lymphoma-derived)		
t(3;4)(q27;p11–13)	*BCL6/LAZ3*	VAL
t(8;14)(q24;q32)	*MYC/IGH*	BALL2, BALM-6/7/8,MC-116*, MN-60*, OCI-LY18, TANOUE*
t(14;18)(q32;q21)	*IGH/BCL2*	FL-18 (mcr), FL-218 (mbr), FL-318 (mbr), KARPAS-422* (mbr), SU-DHL6 (mbr), WSU-NHL* (mbr)
t(8;14;18)(q24;q32;q21)	*MYC/IGH/BCL2*	DOHH2* (mbr), ROS-50 (mcr), SU-DUL5 (mcr), VAL (mcr)
t(8;22)(q24;q11)	*MYC/IGL*	BALM-16
t(11;14)(q13;q32)	*BCL1/IGH*	GRANTA-519*, JVM-2*, NCEB-1

Continued on next page

Table 3. (continued)

Recurrent alteration	Genes fused	Cell lines
(d) B-cell (Burkitt's lymphoma-derived)		
t(8;14)(q24;q32)	*MYC/IGH*	BL-41*, BL-70*, CA-46*, DAUDI*, DG-75*, EB-1*, RAJI*, WIEN-133
t(8;22)(q24;q11)	*MYC/IGL*	BL-2
(e) T-cell (ALL- or lymphoma-derived)		
t(1;7)(p34;q34)	*LCK/TCRB*	CCRF-HSB-2, CTV-1*, SUP-T12
t(1;14)(p32;q11)	*TAL1/TCRA/D*	DU.528
t(7;9)(q34;q34)	*TCRB/TAL2*	SUP-T1*
t(9;22)(q34;q11)	*ABL/M-BCR*	CML-T1*
t(11;14)(p13;q11)	*TTG2 /TCRA/D*	KOPT-K1, LALW-2, TALL-104
t(11;14)(p15;q11)	*TAL1/TCRA/D*	RPMI-8402*
inv(14)(q11q32) or t(14;14)(q11;q32)	*TCRA/IGH*	HT-1, SUP-T1*, TALL-106
(f) myelomonocytic (AML- or CML-derived)		
t(4;11)(q21;q23)	*AF4/MLL*	KOCL-48, MV4;11*
inv(3)(q21q26) or t(3;3)(q21;q26)	*EVI1/?*	MUTZ-3*, UCSD/AML1
t(6;11)(q27;q23)	*AF-6/MLL*	CTS , ML-1/2*
t(9;11)(p21;q23)	*AF9/MLL*	IMS-M1 , MOLM-13/14 (cryptic insertion), MONO-MAC-1/6*, THP-1*
t(9;22)(q34;q11)	*ABL/M-BCR*	EM-2*/3* (myelocytic); JK-1*, K-562* (erythrocytic); LAMA-84*/87* (erythro-megakaryocytic); MEG-01* (megakaryocytic)

Continued on next page

Table 3. (continued)

Recurrent alteration	Genes fused	Cell lines
(f) myelomonocytic (AML- or CML-derived) (continued)		
t(9;22)(q34;q11)	*ABL/m-BCR*	AR230 (CML-derived), KOPM30, MR-87 – (both AML-derived)
t(10;11)(p13;q14)	*AF10/CALM*	U-937*
t(15;17)(q22;q21)	*PML/RARA*	NB-4*
inv(16)(p13q22)	*MYH11/CBFB*	ME-1
(g) ALCL-derived		
t(2;5)(p22;q35)	*ALK/NPM*	DEL* (masked translocation), KARPAS-299*, SU-DHL-1*

The table lists recurrent chromosome changes and gene fusions in hematopoietic cell lines classified according to cell type. (*) available from DSMZ. Abbreviations: m/M-BCR, minor/major breakpoint cluster region – t(9;22); mbr, major breakpoint region; mcr, minor (breakpoint) cluster region – t(14;18). Table modified from Drexler et al. [18,20].

introduction of chromosome banding in the 1970s soon revealed the identities of consistent chromosome changes identified in the previous decade in CML and the precise localization of the chromosome breakpoints involved. With hindsight, it is clear that those who looked for consistent chromosome changes among hematopoietic malignancies were fortunate in their choice of disease as, along with tumors of the nervous system, these display specific changes with the greatest frequency and consistency [36]. It seems that one class of consistent chromosome rearrangement, the balanced translocation, whereby material is reciprocally exchanged between chromosomes from different homologous pairs with highly conserved breakpoints on each of the two chromosomes involved, is typical of hematopoietic neoplasms [64]. Investigation of these translocations (including the functionally equivalent inversions) has illuminated the central role played by genie alteration in cancer. Recurrent translocations effect the fusion of specific pairs of oncogenes, one of which is usually a transcriptional activator. These translocations exhibit remarkable specificity for hematopoietic cells blocked at different stages of differentiation [53], explaining the special power of cytogenetics in characterizing this class of cells. A list of cell lines known to carry recurrent translocations leading to known gene fusions is presented in Table 3.

Two types of fusion may be distinguished:

(a) juxtapositional, whereby proteins (typically transcription factors) are activated by being brought under the influence of the promoter regions of constitutively active genes. Examples are the immunoglobulin heavy chain genes – at 2p12 (*IGK*), 14q32 (*IGH*), or 22q11 (*IGL*) – in B-cell leukemia-lymphoma, and the T-cell receptor genes – at 7q35 (*TCRB*) or 14q11 (*TCRA/D*) – in T-cell leukemia-lymphoma.

(b) chimeric, whereby some of the exons from both participant genes are transcribed into a single mRNA and translated into a single novel protein. The latter are found across a wide variety of hematopoietic, and a few solid, tumors and derived cell lines. Examples are REH with t(12;21)(p12;q21) causing *TEL-AML1* fusion in BCP-ALL [89]; MONOMAC-6 with t(9;11)(p21;q23) causing *MLL-AF9* fusion in AML FAB-MS [55]; and KARPAS-299 with t(2;5)(p23;q35) causing *NPM-ALK* fusion in ALCL [14].

The simultaneous demonstration of identical recurrent chromosome rearrangements in both the biopsy and in a derived cell line is overwhelming evidence of a shared clonal relationship. The availability of hematopoietic malignant cell lines bearing recurrent translocations has played an important role in advancing these studies. In some cases, cell lines known to carry the chromosomal rearrangement in question were used for cloning the translocation breakpoints. Examples are K-562 for *BCR-ABL* in t(9;22)(q34;q11)

[37], NB-4 for *PML* in t(15;17)(q22;q12-21) [24], and KASUMI-1 for *ETO* in t(8;21)(q22;q22) [65].

It is not possible to discuss how cytogenetic characterization has contributed to the characterization of all types of cell line listed in Table 3. However, consideration of one of the largest cytogenetic groups, cell lines with t(9;22), a typical cytogenetic feature of two distinct neoplasms, CML and ALL, is instructive and illustrates how the characterization of hematopoietic cell lines is related to chromosome and related gene changes [21]. In addition, the way in which cytogenetics may be used to characterize cell lines derived from tumors in which no recurrent change is known is discussed with reference to Hodgkin's disease. Finally, some cell lines which have been incorrectly or incompletely characterized cytogenetically are referred to briefly.

3.1. t(9;22)(q34;q11) Cell Lines as Models

An example of a cytogenetic change occurring in more than one distinct category of hematopoietic neoplasm is the t(9;22) which, together with t(8;14)(q24;q32) in B-cell leukemia- lymphoma, is the translocation studied in most detail at the molecular level. The der(22) partner of this reciprocal translocation was the first somatic chromosome change specifically associated with any tumor [71] and designated the "Philadelphia chromosome" (Ph). However, it was not until the advent of chromosome banding that the rearrangement was seen to be a reciprocal translocation [79], marking a milestone in cancer cytogenetics.

t(9;22) is primarily associated with CML and occurs in more than 95% of all cases, including a minority where the translocation may be cryptic (due to a genomic insertion) or masked by the involvement of one or more additional chromosomes [43]. However, the t(9;22) rearrangement is also the single most frequent acquired cytogenetic alteration in ALL [4] and has been also recorded in rare cases of AML (Table 3). In addition, two B-lymphoblastoid cell lines with t(9;22) have been described: SD-1 from blood taken from a patient at diagnosis of ALL [13], and PhB1 from a patient with CML in blast crisis [47]. No other leukemic translocation targets such a wide variety of cells, implying the occurrence of t(9;22) at primitive stages during hematopoietic differentiation, a conjecture supported by the observation that transgenic mice with *BCR-ABL* may develop either myeloid, or B/T-cell lymphoid leukemias [75]. This lineage diversity is also an indication that *BCR-ABL* fusion is in itself insufficient to induce full neoplastic transformation with differentiation arrest.

Following the results of molecular investigation, we now know that it is possible to distinguish three different types of t(9;22) based on the breakpoint cluster region (BCR) location of the breakpoints in chromosome 22 band

q11: M(ajor)-BCR, m(inor)-BCR and *μ-BCR* are translated into proteins of 210, 190 and 230 kDa, respectively [62]. By means of RT-PCR, these various possibilities may be readily distinguished, while cytogenetic probes are commercially available to identify the M/m-*BCR* variants. Following such studies, it has become clear that *BCR-ABL* variants are unequally partitioned between CML and ALL and derived cell lines. Almost all CML, about half of all ALL and some AML patients carry M-*BCR.* A few CML, about half of all ALL and remaining AML patients carry m-*BCR,* while *μ-BCR* has only been recorded so far in a subtype of CML, termed "chronic neutrophilic leukemia". As far as cell lines are concerned, more than 40 have been established from CML and more than 20 from ALL, while only three have been established from AML [21]. CML-derived cell lines have been shown to reflect the distribution of the different *BCR* breakpoints *in vivo*, almost all carrying the M-*BCR* breakpoint, the exceptions including AR230 with μ-BCR [93]. In contrast only 2/17 molecularly characterized ALL-derived cell lines carry M-*BCR*, less than a quarter of the number expected. These are ALL-1 [48] and the autologous pair NALM-27/28 [1]. Of the Ph positive B-LCL, SD1 carries m-*BCR* and PhB1 M-*BCR.* It should be noted that in the best known *BCR- ABL* positive cell line of all, K-562, the original Ph is no longer recognizable, having undergone secondary rearrangement, resulting in an approximate thirty-fold coamplification of the fusion gene via tandem duplication. This rearrangement is unprecedented in both patients and cell lines. SPI-801/802, which also carries this change, is now known to be merely a subclone of K-562 (Table 1). The rearrangement has been recorded in several independent samples of K-562 [18,78,96], implying its origin *in vivo* or at early passage.

A less obvious feature of t(9;22) cell lines concerns the numbers of Ph-fusion genes present and their relationship to unrearranged alleles of *ABL* and to ploidy levels. The question is more than of theoretical interest. For example, where cell lines are being employed as positive controls for cytogenetic or PCR detection of *BCR-ABL*, it is important to know whether, or how many, rearranged and unrearranged copies of each allele are present, particularly for cytogenetic detection among interphase cells. In addition, it has been sugggested that *ABL* and *BCR-ABL* may act antagonistically [33], reminiscent of *TEL-AML1* fusion in BCP-ALL accomplished via the recurrent t(12;21)(p12;q12). The latter rearrangement is normally accompanied by deletion of the remaining allele of TEL [76], and this antagonistic pairing has been also observed in a t(12;21) BCP-ALL cell line, REH [89]. Similarly, loss of the unrearranged allele of ABL has been noted in some *BCR-ABL* cell lines (R.A.F. MacLeod, unpublished). Among *BCR-ABL* cell lines (excluding the B-LCL which tend to undergo tetraploidization at late passage irrespective of their origins), all 20 examples established from patients with ALL

remain diploid, while 14/40 cell lines established from CML patients are triploid or tetraploid [21] refuting the assertion that *BCR-ABL* promotes genetic instability per se. In primary CML, the t(9,22) is present at disease onset. Nevertheless, none of the forty-odd available CML- derived cell lines was established prior to blast crisis, revealing a second type of representational disparity when compared to the primary disease.

3.2. Hodgkin's Disease

Hodgkin's disease is the most enigmatic of all hematopoietic neoplasms on account of unresolved questions regarding the origin of the Hodgkin/Reed-Sternberg (H-RS) cell and its relationship to other hematopoietic lineages. It might be expected that continuous HD cell lines could play a valuable role, and the relatively small number of HD cell lines are, indeed, the subjects of much inquiry [17]. Yet their relevance is controversial. In addition to the cross-contaminations discussed above, several so-called HD cell lines have been misclassified. Establishment of continuous cell lines from tumor material taken from HD patients is difficult, and many of the cell lines resulting are lymphoblasts immortalized by EBV. Unfortunately, several of the cell lines described as having originated from HD are merely B-LCL, including HS-445 and RPMI-6666. Nevertheless, these continue to be cited as HD in some publications.

The major problem in characterizing HD cell lines is that, unlike all other major common hematopoietic neoplasms, no consistent chromosome translocation has been identified. This is attributed to the difficulty of isolating scarce H-RS cells from reactive normal tissue. In the absence of any recurrent primary translocation to identify the malignant clone and allow cloning of a putative oncogene target, cytogenetics has added little to the positive identification of the putative HD precursor. While immunological studies have implicated a lymphocyte precursor, molecular biological studies at the level of single cells have suggested that a substantial fraction of cases of classical HD may represent clonal expansion of B-cells in which immunoglobulin expression is disabled by mutation during the germinal center reaction. A minority of cases are derived from T-cells, in contrast to the HD cell lines where both T- and B-cell phenotypes are equally represented. A second problem is the sheer karyotypic complexity of H-RS cells, in which subtle rearrangements might be unnoticed and for which the technical advances necessary for their analysis, such as M-FISH and SKY (discussed above), are only now becoming available. Nevertheless, with every patient series failing to reveal the existence of a common translocation, it is becoming increasingly likely that some other kind of primary tumorigenic change may underly HD,

such as the tumor suppressor gene deletions commonly found in epithelial solid tumors.

A number of cell lines have been established from HD and made available to other investigators, such as HDLM-1/2/3, HD-MyZ, KM-H2, L-480 [20]. Given the possibility that host cells become immortalized by EBV often present, candidate cell lines should be characterized thoroughly prior to being accepted as suitable *in vitro* models for HD. As cytogenetics is uninformative in HD, evidence linking the cell lines to putative H-RS precursors has mainly rested on immunophenotyping. In particular, to be classed as HD, cell lines should constitutively express CD30 (Ki-1) at high levels without evidence of monocytic differentiation. On this criterion alone, the affinity with H-RS cells of the Ki-1-negative HD-MyZ cell line, which has a monocytic phenotype, is questionable, whereas those of HDLM- 1/2/3, KM-H2 and L-428 are reinforced. All HD cell lines exhibit extremely high levels of cytogenetic rearrangement. According to our initial findings, this appears to follow a non-random pattern resembling that reported among HD patients [2,63], inviting the conjecture, supported by twin studies, that HD may be a type of chromosome instability disease, as recently proposed [25]. We have observed "jumping translocations" involving certain types of DNA repeat in HD cell lines [57a]. HDLM-1/2/3, KM-H2, and L-428 all display several landmark bands recurrent in HD [57a]. On the other hand, HD-frequent breakpoints were no more likely than HD-infrequent breakpoints to be rearranged in other types of hematopoietic cell lines, whose breakage patterns HD-MyZ resembled. Consistency between the results of immunophenotyping and cytogenetics serves to confirm the validity of HDLM-1/2/3, KM-H2 and L-428, and suggests that when full and accurate karyotyping of HD becomes feasible by the routine application of SKY and M-FISH, sufficiently strong non-random patterns of breakpoints may emerge to justify their molecular cloning, perhaps with the help of these very cell lines.

3.3. Cell Lines with Revised Karyotypes

Several cell lines, more particularly those described prior to the routine use of image analysis and molecular cytogenetic methods, have been subsequently shown to carry recurrent primary chromosome translocations [20,55]. Some of these are listed in Table 4. In most cases the rearrangements are quite subtle, for example t(9;11) as in MONO-MAC-1, MONO-MAC-6, and THP-1, which is notoriously difficult to spot in suboptimal preparations. t(12;21), as present in REH, involving the reciprocal exchange of visually identical G-banding regions, is undetectable without FISH. An additional factor which hampers detection of subtler changes in myeloid leukemias is their ka-

Table 4. Cell lines with revised karyotypes

Cell line	Type	Ref.	Original karyotype	Revised karyotype (structural changes only)	Key change
697	BCP-ALL	27	−46, XY, t(7;19)(q11;q13)	−46, XY, t(1;19)(q23;p13), del(6)(q21)	t(1;19)
CTV-1	T-ALL	9	−47, X, −X, −11, t(12;11), +3mar	−47, X, t(1;7)(p34.2;q34), i(6)(q10), del(10)(p13), t(12;16)(q24;q11)	t(1;7)
DOHH-2	B-NHL	46	−48, XY, +7, del(12)(q24), t(14;18)(q23;q21)	−47, XY, der(8)t(8;18)(q24;q21), der(14)t(8;14)(q24;q32), der(18)t(14;18)(q32;q21)	t(8;14;18)
MHH-TALL-1	BCP-ALL	Tomeczkowski, unpublished	−46, XY, BCR–ABL negative	−48, XY, t(1;9;22)(q32;q34;q11)inv(9)(p11q13), del(7)(p15), del(19)(p13)	t(9;22)
MONO-MAC-1	AML-M5	97	−41 −43, XY, +3, −12, −17, +mar	−49(43–52)XY, dup(3)(q21q27), t(9;11)(p13;q23), t(10;12, 17)(q24;q13;q11), del(13)(q13q21), t(16;21)(q13;q22.2)	t(9;11)
MONO-MAC-6	AML-M5	97	−41 −43, XY, +3, −12, −17, +mar	−84 −90, XX/XXX, t(9;11)(p22;q23)x2, add(10)(p11) x2, add(12)(q?21), del(13)(q13q14)der(13)t(13;14)(p11;q;12)x2	t(9;11)
ML-1/2	AML-M4	73	−91, XX, del(1)(p23), add(1)(p22), del(6)(q23), der(11)t(11;13)(q23;q32), der(13)t(11;13)(q23;q32), add(14)(q13), del(17)(q23), der(16)t(1;16)(p13;p35)	−92(84–94)XX, der(1)t(1;?)(p21;?)x2, del(6)(q23)x2, der(6)t(6;11)(q27;q23)x2, der(11)t(6;11)(q27;q23), add(11)(p11;q23)x2, add(11)(q11–13), dup(13)(q32–>qter)x2, der(18)t(15;?;18)(q21;?;q11)x2	t(6;11)
THP-1	AML-M5	88	−46, XY. Normal karyotype	−94, XY/XXYadd(1)(p11), del(1)(q42.2), i(2q), del(6)(p21), i(7)(p10), der(9)t(9;11)(p22;q23)i(9)(p10)x2, der(11)t(9;11)(p22;q23)x2, add(12)(q24), der(13)t(8;13)(p11;p12)x2, add(?18)(q21)	t(9;11)

Continued on next page

Table 4. (continued)

Cell line	Type	Ref.	Original karyotype	Revised karyotype (structural changes only)	Key change
U-937	histiocyt. NHL	86	−58, XXY, t(1q;14q), t(1p;13q), add(2)(q?), der(3)t(1;3)(q21;q27), der(5)t(1;5)(p13;q31), t(6p;12q)	−63, XX/XXY, der(5)t(1;5)(p32;q3?), t(1;12)(q21;p13), der(2;3)(q10;p10), add(9)(p22), t(10;11)(p12;q14), i(11)(q10), i(12)(p10), add(16)(q24), del(17)(p13), add(19)(p13), add(21)(p1)	t(10;11)
REH	BCP-ALL	91	−45, XX, −2B, +1C	−46, X, del(3)(p22), t(4;12;21;16)(q32;p13;q22;q24.3), inv(12)(p13q22), t(5;12)(q31–32;p12), der(16)t(16;21)(q24.3;q22)	t(12;21)

The table lists cell lines carrying primary key changes overlooked or misinterpreted in original descriptions. Key changes are primary recurrent translocations effecting gene fusions (see Table 3). For brevity, revised karyotypes show structural rearrangements only.

ryotypic complexity, combined with the variety and number of different translocations effecting gene fusions within this group.

4. CONCLUSION

Amongst the various ways of detecting and identifying cross-contamination, cytogenetics displays unique versatility, complementing both DNA profiling (intraspecies) and isoenzyme analysis (interspecies). It combines with immunophenotyping to enable the characterization of hematopoietic cell lines to an unrivalled degree.

Abbreviations

ALCL – anaplastic large cell lymphoma;
ALL – acute lymphoblastic leukemia;
AMegL–acute megakaryocytic leukemia;
ATCC – American Type Culture Collection;
AML – acute myeloid leukemia;
B-LCL – B-lymphoblastoid cell line;
BCP – B-cell precursor;
BCR – breakpoint cluster region;
CML – chronic myeloid leukemia;
DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen;
EBV – Epstein-Barr virus;
FISH – fluorescence in *situ* hybridization;
HD – Hodgkin's disease;
H-RS – Hodgkin's/Reed-Sternberg;
ISCN – International System for Human Chromosome Nomenclature;
LOH – loss of heterozygosity;
NHL – non-Hodgkin's lymphoma;
PCR – polymerase chain reaction;
RFLP – restriction fragment length polymorphism;
RT – reverse transcriptase;
SKY – spectral karyotyping;
VNTR – variable number tandem repeats.

References

1. Ariyasu T et al. *Human Cell* 11: 43, 1998.
2. Atkin NB. *Cytogenet Cell Genet* 80: 23, 1998.
3. Bennett JM et al. *Br J Haematol* 33:451, 1976.
4. Berger R et al. *Cancer Genet Cytogenet* 44: 143, 1990.
5. Berger R et al. *Genes Chrom Cancer* 3: 332, 1991.
6. Boerwinkle E et al. *Proc Natl Acad Sci USA* 86: 212, 1989.
7. Budowle B et al. *Am J Hum Genet* 48: 137, 1991.
8. Cahill DP et al. *Nature* 392: 300, 1998.

9. Chen PM et al. *Gann* 75: 660, 1984.
10. Collins SJ et al. *Nature* 270: 347, 1977.
11. Dalton WTet al. *Blood* 71: 242, 1988.
12. Delabie J et al. *Br J Haematol* 94: 198, 1996.
13. Dhut S et al. *Leukemia* 5: 49, 1991.
14. Dirks WG et al. *Leukemia* 10, 142, 1996.
15. Dirks WG et al. *In Vitro Cell Dev Biol Animal* 35: 558, 1999.
16. Dirks WG et al. *Cell Mol Biol* 45: 841, 1999.
17. Drexler HG. *Leukemia Lymphoma* 9: 1, 1993.
18. Drexler HG et al. *Leukemia* 9: 480, 1995.
19. Drexler HG et al. *Human Cell* 11: 51, 1998.
20. Drexler HG et al. *DSMZ Catalogue of Human and Animal Cell Lines*, 7th edn., Braunschweig, 1999.
21. Drexler HG et al. *Leukemia Res* 23: 207, 1999.
22. Drexler HG et al. *Leukemia* 13: 1601, 1999.
23. Drexler HG, Matsuo Y. *Leukemia* 14: 777, 2000.
24. Du Thé H et al. *Nature* 347: 558, 1990.
25. Falzetti D et al. *Haematologica* 84: 298, 1999.
26. Fernandez E et al. *Blood* 83: 3245, 1994.
27. Findley HW et al. *Blood* 60: 1305, 1982.
28. Foley GE et al. *Cancer* 18: 522, 1965.
29. Gallagher R et al. *Blood* 54: 713, 1979.
30. Gazitt Y et al. *Leukemia* 7: 2034, 1993.
31. Gignac SM et al. *Leukemia Lymphoma* 10: 359, 1993.
32. Greenberg SM et al. *Blood* 72: 1968, 1988.
33. Guo XY et al. *Leukemia Lymphoma* 30: 225, 1998.
34. Harris NL et al. *Nature* 289: 228, 1981.
35. Hay RJ. et al. *J Cell Biochem* 24: 107, 1996.
36. Heim S, Mitelman F. *Cancer Cytogenetics*, Wiley, 1995.
37. Heisterkamp N et al. *Nature* 306: 293, 1983.
38. Hirata J et al. *Leukemia* 4: 365, 1990.
39. Hukku B et al. *Adv Exp Med Biol* 172: 13, 1984.
40. Hummel M et *al. N Engl J Med* 333: 901, 1986.
41. ISCN. In: Mitelman F (ed.), *An International System for Human Cytogenetic Nomenclature*, S Karger, Basel, 1995.
42. Jeffreys AJ et al. *Nature* 314: 67, 1985.
43. Kantarjian HM et al. *Blood* 82: 691, 1993.
44. Kanzler H et al. *J Exp Med* 184: 1495, 1986.
45. King BL et al. *Am J Pathol* 144: 486, 1994.
46. Kluin-Nelemann JM et al. *Leukemia* 5: 221, 1991.
47. Knuutila S et al. *Acta Hematol* 90: 190, 1994.
48. Lange B et al. *Blood* 70: 192, 1987.
49. Larson RA et al. *Am J Med Genet* 76: 827, 1984.
50. Lengauer C et al. *Nature* 386: 623, 1997.
51. Li R et al. *Proc Natl Acad Sci USA* 94: 14506, 1997.
52. Lichter P. *Trends in Genetics* 13: 475, 1997.
53. Look AT. *Science* 278, 1997.
54. MacLeod RAF et al. *In vitro Cell Dev Biol* 28A: 591, 1992.
55. MacLeod RAF et al. *Blood* 82: 3221, 1993.

56. MacLeod RAF et al. *Leukemia* 11: 2032, 1997.
57. MacLeod RAF et al. *Int J Cancer* 83: 555, 1999.
57a. MacLeod RAF et al. *Leukemia* 14: 1803, 2000.
58. Markovic O, Markovic N. *In vitro Cell Dev Biol* 34A: 1, 1998.
59. Martin P, Papayannopoulou T. *Science* 216: 1233, 1982.
60. Masters JRW et al. *Br. J. Cancer* 57: 284, 1988.
61. Matsuo Y et al. *Human Cell* 2: 423, 1989.
62. Melo JV. *Blood* 88: 2375, 1996.
63. Mitelman F. *Catalog of Chromosome Aberrations in Cancer,* 5th edn., Wiley-Liss, 1994.
64. Mitelman F et al. *Nat Genet* 15: 417, 1997.
65. Miyoshi H et al. *EMBO J* 12: 2715, 1993.
66. Molenaar JJ et al. *Blood* 92: 230, 1998.
67. Moorman A et al. *Cytogenet Cell Genet* 77: 35, 1997.
68. Muller S et al. *Human Genet* 101: 149, 1997.
69. Muller S et al. *Cytogenet Cell Genet* 78: 260, 1998.
70. Nakamura Y et al. *Science* 235: 1616, 1987.
71. Nowell PC, Hungerford DA. *Science* 132: 1497, 1960.
72. Ohta M et al. *Cancer Res* 46: 3067, 1986.
73. Ohyashiki K et al. *Cancer Genet Cytogenet* 37: 103, 1989.
74. Ono Y et al. *Mol Cell Biol* 18: 6939, 1998.
75. Pasternak G et al. *J. Cancer Res Clin Oncol* 24: 643, 1998.
75a. Pepinski W et al. *Rocz Akad Med Bialynst* 41: 316, 1996.
76. Raynaud S et al. *Blood* 87: 2891, 1996.
77. Reid YA et al. *J Leuk Biol* 57: 804 (letter), 1985.
78. Rodley P et al. *Genes Chrom Cancer* 19: 36, 1995.
79. Rowley JD. *Nature* 243: 290, 1973.
79a. Schäfer R et al. *Nucleic Acids Res* 16: 5196, 1988.
80. Schröck E et al. *Science* 273: 494, 1996.
81. Seabright M. *Lancet* ii: 971, 1971.
82. Shimada Y et al. *Cancer* 69: 277, 1992.
83. Speicher MR et al. *Nat Genet* 12: 368, 1996.
84. Stacey GN et al. *Cytotechnology* 8: 13, 1992.
85. Steube KG et al. *In vitro Cell Dev Biol* 31 A: 115, 1995.
86. Sundström K et al. *Int J Cancer* 13: 808, 1974.
87. Thacker J et al. *Somat Cell Mol Genet* 14:519, 1988.
88. Tsuchiya S et al. *Int J Cancer* 26: 171, 1980.
89. Uphoff CC et al. *Leukemia* 11: 441, 1997.
90. Uphoff CC, Drexler HG. In: Spiers RE (ed.), *Encyclopedia of Cell Technology,* Wiley, in press, 2000.
91. Venuat AM et al. *Cancer Genet Cytogenet* 3: 327, 1981.
92. Von Hanseman D. *Virchows Arch A Pathol Anat* 119: 299, 1890.
93. Wada H et al. *Cancer Res* 55: 3192, 1995.
94. Wlodarska I et al. *Blood* 89: 1716, 1997.
95. Wolff RK et al. *Genomics* 3: 347, 1988.
96. Wu SQ et al. *Leukemia* 9: 858, 1995.
97. Ziegler-Heitbrock HW et al. *Int J Cancer* 41: 456, 1988.
98. Zischler H et al. *Human Genet* 82: 227, 1989.

Human Cell Culture

1. J.R.W. Masters and B. Palsson (eds.): *Human Cell Culture, Vol. I.* 1998 ISBN 0-7923-5143-6
2. J.R.W. Masters and B. Palsson (eds.): *Human Cell Culture, Part 2. Vol. II.* Cancer Cell Lines. 1999 ISBN 0-7923-5878-3
3. B.O. Palsson and J.R. Masters (eds.): *Human Cell Culture, Part 3.* Cancer Continuous Cell Lines: Leukaemias and Lymphomas. 2001 ISBN 0-7923-6225-X
4. M.R. Koller, B.O. Palsson and J. R. W. Masters (eds.): *Human Cell Culture, Volume IV.* Primary Hematopoietic Cells. 1999 ISBN 0-7923-5821 -X
5. M.R. Koller, B.O. Palsson and J.R.W. Masters (eds.): *Human Cell Culture, Part 5.* Primary Mesenchymal Cells. 2001 ISBN 0-7923-6761 -8

KLUWER ACADEMIC PUBLISHERS – DORDRECHT / BOSTON / LONDON