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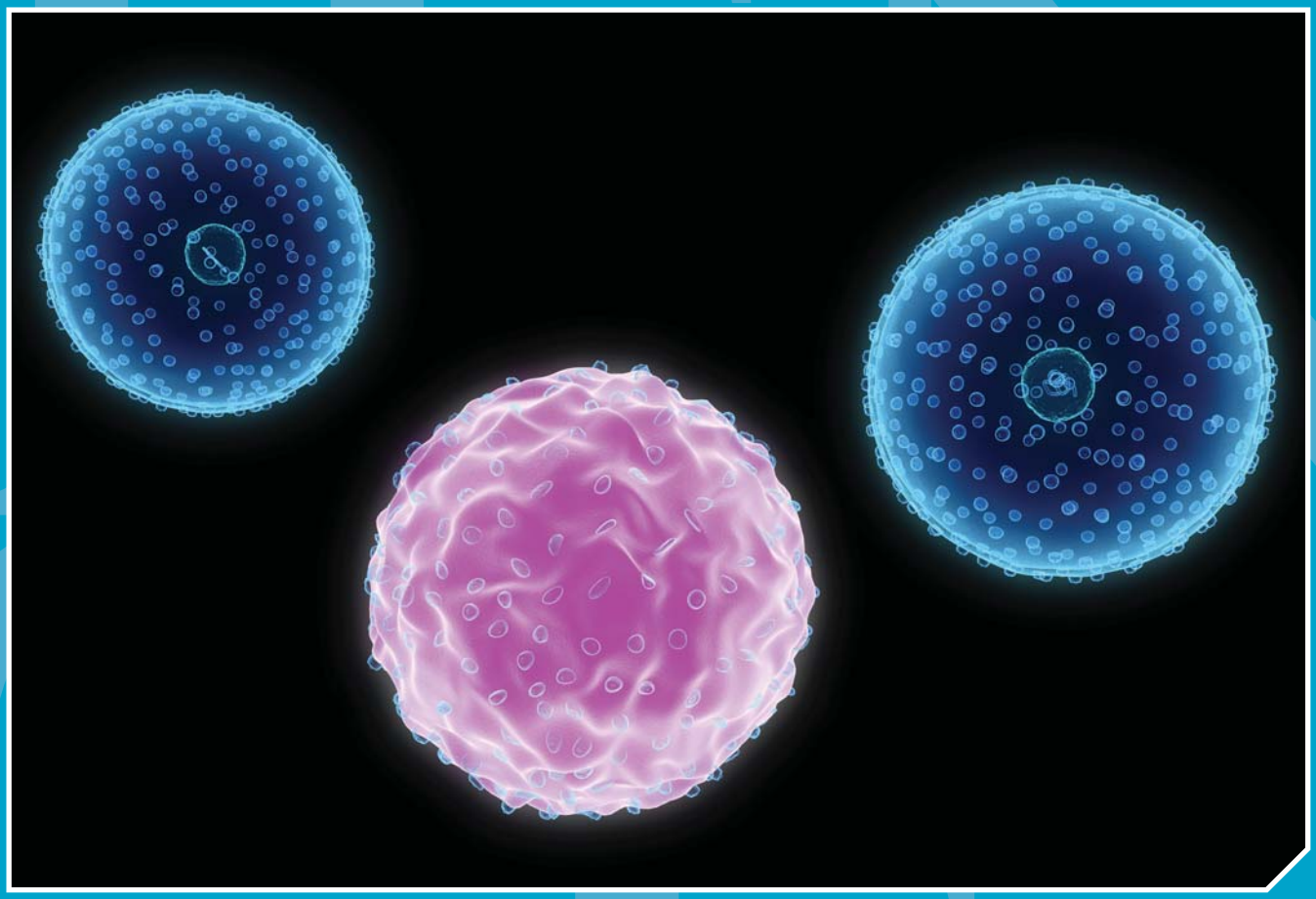
Lymphohematopoietic Cancers Induced by Chemicals and Other Agents: Overview and Implications for Risk Assessment



National Center for Environmental Assessment
Office of Research and Development

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National Center for Environmental Assessment
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Washington, DC

DISCLAIMER

ABSTRACT

The objective of this report is to provide an overview of the types and mechanisms underlying the lymphohematopoietic cancers induced by chemical agents and radiation in humans, with a primary emphasis on acute myeloid leukemia and some of the known agents that induce this type of cancer. Following a brief discussion of hematopoiesis and leukemogenesis, an overview of the major classes of leukemia-inducing agents—radiation, chemotherapeutic alkylating agents, and topoisomerase II inhibitors—is presented along with information on plausible mechanisms by which these leukemias occur. The last section focuses on how mechanistic information on human leukemia-inducing agents can be used to better inform risk assessment decisions. It is evident that there are different types of leukemia-inducing agents that may act through different mechanisms. Even though most have been concluded by IARC to act through mutagenic or genotoxic mechanisms, leukemia-inducing agents may have different potencies and associated risks, which appear to be significantly influenced by the specific mechanisms involved in leukemogenesis. Identifying the specific types of cancer-causing agents with their associated mechanisms and using this information to inform key steps in the risk assessment process remains an ongoing challenge.

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LIST OF ABBREVIATIONS

ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
ANLL	acute nonlymphocytic leukemia
CLL	chronic lymphocytic leukemia
CML	chronic myeloid leukemia
ENU	*N*-nitroso-*N*-ethylurea
EPA	U.S. Environmental Protection Agency
FAB	French-American-British
IARC	International Agency for Research on Cancer
MALT	mucosa-associated lymphoid tissues
MDS	myelodysplastic syndromes
MM	multiple myeloma
NCI	National Cancer Institute
NHL	non-Hodgkin lymphoma
NI	no information
NK	natural killer
NQO1	NADPH quinone oxidoreductase 1
RAR*a*	retinoic acid receptor *alpha*
SCA	structural chromosomal aberrations
t-AML	therapy-related acute myeloid leukemia
TCDD	Tetrachlorodibenzo-*p*-dioxin
UNSCEAR	United Nations Scientific Committee on the Effects of Atomic Radiation
WHO	World Health Organization

PREFACE

This report represents the update and expansion of an earlier U.S. Environmental Protection Agency (EPA) document entitled “Chemical and Radiation Leukemogenesis in Humans and Rodents and the Value of Rodent Models for Assessing Risks of Lymphohematopoietic Cancers” (EPA/600/R-97/090, May 1997). This report provides an overview of chemically induced leukemias and lymphomas with a primary emphasis on acute myeloid leukemia (AML). It is intended to provide insights into how mechanistic information on AML-inducing agents may be used in risk assessment.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

PROJECT MANAGER AND CONTRIBUTORS

Nagalakshmi Keshava, PhD
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Babasaheb Sonawane, PhD
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

AUTHOR

David A. Eastmond, PhD
University of California, Riverside
Riverside, CA

INTERNAL REVIEWERS

Glinda Cooper, PhD
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Maureen Gwinn, PhD
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

George Woodall, PhD
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

Chao Chen, PhD
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Jennifer Jinot, PhD
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Brian Pachkowski, PhD
Oak Ridge Institute for Science and Education Postdoctoral Fellow
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Cheryl Scott, MS
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Suryanarayana Vulimiri, BVSc, MVSc, PhD, DABT
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

EXTERNAL PEER REVIEWERS

James E. Klaunig, PhD
School of Public Health
Indiana University
Bloomington, IN

David Ross, PhD
School of Pharmacy
University of Colorado Denver
Aurora, CO

Martha S. Sandy, MPH, PhD
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
Oakland, CA

Martyn T. Smith, PhD
School of Public Health
University of California
Berkeley, CA

Roel Vermeulen, PhD
Utrecht University
Utrecht, The Netherlands

EXECUTIVE SUMMARY

Lymphohematopoietic neoplasias represent a heterogeneous group of clonal hematopoietic and lymphoid cell disorders and are one of the most common types of cancer induced by environmental and therapeutic agents. Leukemia is a lymphohematopoietic cancer originating in the bone marrow that affects cells of either the myeloid lineages (myeloid leukemia) or the lymphoid lineage (lymphoid or lymphoblastic leukemia). These can be further categorized as acute or chronic depending upon the rate of clonal expansion or stage of differentiation of the cancer cell. The objective of this report is to provide an overview of the types and mechanisms underlying the lymphohematopoietic cancers induced by chemical agents and radiation in humans, with a primary emphasis on AML and agents that induce this type of cancer. Following a brief discussion of hematopoiesis and leukemogenesis, a review of the major classes of leukemia-inducing agents—radiation, chemotherapeutic alkylating agents, and topoisomerase II inhibitors—is presented along with information on the mechanisms by which these leukemias occur. The last section focuses on how mechanistic information on human leukemia-inducing agents can be used to better inform risk assessment decisions. A brief overview of the major points in the report is presented below.

It is widely recognized that lymphohematopoietic neoplasms originate through multistep processes involving a series of genetic and epigenetic alterations that transform a normal hematopoietic or lymphoid cell into a malignant tumor. The various lymphohematopoietic cancers are believed to originate in specific types of pluripotent or lineage-restricted cells at different stages in hematopoiesis and immune cell development. Current evidence indicates that the acute and chronic myeloid leukemias (CMLs) as well as precursor lymphoid neoplasms, including the acute lymphoblastic leukemias (ALLs)—B lymphoblastic leukemia/lymphoma and T lymphoblastic leukemia/lymphoma, originate in hematopoietic stem or progenitor cells; whereas, other lymphomas and chronic lymphocytic leukemia (CLL) have their origins in mature lymphoid cells. An understanding of the origin of these cancers as well as morphologic, cytochemical and immunophenotypic features of the neoplastic cells provide valuable information for grouping various lymphohematopoietic cancers and for identifying the cell types targeted by carcinogenic agents.

Lymphohematopoietic neoplasia represents one of the most common cancers induced by chemical, physical, and infectious agents. To date, the International Agency for Research on Cancer has identified over 100 agents as human carcinogens. Of these, approximately 25% have been shown to induce either leukemias or lymphomas in humans. Many of these identified human carcinogens are antineoplastic drugs, but a variety of other agents including industrial chemicals, various forms of radiation, immunosuppressive drugs, and infectious agents have also been shown to induce lymphohematopoietic cancers in humans. In evaluating the different types of induced leukemias and lymphomas, a number of general patterns become apparent. Leukemias are the primary type of cancer induced by chemical agents. Most of these are acute nonlymphocytic leukemias (synonymous with AML) with relatively short median latency periods that are formed through the induction of mutations affecting critical cancer-related genes. Radiation, which is also thought to act through a mutagenic mechanism, is frequently associated with AML as well as CML and ALL.

Exposure to a variety of infectious agents is causally related to the formation of lymphoid neoplasms. The induced lymphomas identified to date appear to be primarily associated with chronic infection with either viruses or *Helicobacter* bacteria, agents that are immunomodulating, and/or specifically target lymphoid cells. Two chemical agents, cyclosporine and azathioprine, which are associated with the development of non-Hodgkin lymphoma (NHL) are also strongly immunosuppressive, and this immunosuppression is believed to play a critical role in the development of the associated lymphomas.

Among the induced acute myeloid leukemias, different subtypes with particular characteristics have shown to be induced by different classes of agents. For example, the alkylating agent class of chemotherapeutic agents typically induces acute myeloblastic leukemias that are often preceded by a myelodysplastic phase and are characterized by loss of all or part of chromosomes 5 or 7. These leukemias generally develop with a median latency period of 5−7 years from the beginning of treatment. In contrast, the leukemias induced by the epipodophyllotoxin class of topoisomerase II inhibitors develop much sooner with a median latency period of 2−3 years. These leukemias are typically characterized as monocytic or myelomonocytic subtypes and exhibit reciprocal translocations involving a specific region on the long arm of chromosome 11 (11q23).

The last section of the report discusses how mechanistic information on human leukemia-inducing agents can be used to better assess the risks from exposure to environmental chemicals. A range of topics is considered including brief discussions on the usefulness of short-term and chronic animal bioassays; the combining of various tumor types for analysis; the impact of latency periods on the detection of leukemias; and the modifying influences of metabolism, pharmacokinetics, DNA-adduct type and repair, as well as individual and age-related susceptibility factors in assessing the risks associated with leukemia-inducing agents.

In conclusion, it is evident that there are different types of leukemia-inducing agents that act through different mechanisms. In its evaluations, IARC has concluded that most leukemia-inducing agents act through mutagenic and/or genotoxic mechanisms, with different potencies and associated risks, which can be significantly influenced by the specific mechanisms involved in leukemogenesis. Identifying the specific types of cancer-causing agents with their associated mechanisms and using this information to inform key steps in the risk assessment process remains one of the ongoing challenges for researchers and risk assessors. For the alkylating agent class of carcinogens, an approach such as that described by Vogel and colleagues supplemented with more recent genomic, proteomic, and biomarker information would appear to present a reasonable and scientifically valid step towards this objective.

1. INTRODUCTION TO LYMPHOHEMATOPOIETIC CANCERS

Lymphohematopoietic neoplasia can be described as an uncontrolled proliferation or expansion of hematopoietic and lymphoid cells that are unable to differentiate normally to form mature blood cells (Sawyers et al., 1991). These neoplasms represent clonal expansions of hematopoietic cells, almost always within either the myeloid or lymphoid lineage (Nowell, 1991; WHO, 2008). Infrequently, some leukemias exhibit both myeloid and lymphoid characteristics and are known as biphenotypic leukemias (Russell, 1997). The myeloid clones are designated as chronic or acute leukemias, depending upon the rate of clonal expansion and the stage of differentiation that dominates the leukemic clone. Lymphoid neoplasms typically manifest themselves in the blood as chronic or acute lymphoblastic leukemias (ALLs) or remain confined to lymphoid proliferative sites such as the lymph nodes or spleen and are designated as lymphomas (Nowell, 1991). Acute leukemias tend to have a rapid onset with a predominance of immature cells whereas chronic leukemias have a more insidious onset and progress over a period of months or years to a blast or acute leukemic phase.

Using this basic classification, leukemias can be described as one of four major types—ALL, acute myeloid leukemia (AML), chronic lymphoblastic leukemia (CLL), and chronic myeloid leukemia (CML). Similarly, lymphomas are broadly classified as Hodgkin or non-Hodgkin lymphomas (NHLs) depending upon the appearance of a specific cancer cell type, the Reed-Sternberg cell, which is found in Hodgkin lymphomas (ACS, 2009). Within these larger groupings, there are numerous subtypes involving specific cells that have unique characteristics, origins, and increasingly recognized clinical significance. These subtypes are generally classified according to morphologic, cytogenetic, immunophenotypic, and more recently, molecular characteristics according to the French-American-British (FAB) or World Health Organization (WHO) classification systems (Head and Pui, 1999; WHO, 2001, 2008; Haferlach et al., 2005). For convenience, a simplified classification scheme for the leukemias and lymphomas based largely on the FAB classification system (shown in Table 1 [Sullivan, 1993]) will be the basis for most descriptions used in this document. A more thorough and complicated classification of the primary types and subtypes of lymphohematopoietic diseases based on the most recent WHO classification (WHO, 2008; Vardiman et al., 2009) is shown in Tables 2 and 3 for myeloid and lymphoid neoplasms, respectively. It should be noted that in the

Table 1. Simplified classification of the major lymphohematopoietic neoplastic diseases in humans based largely on the French-American-British (FAB) classification

I.	**Neoplasms of multipotent stem cell origin**	
	Chronic myelogenous leukemia	CML
II.	**Neoplasms possibly originating in the multipotent stem cell**	
	Myelodysplastic syndromes	MDS
	Refractory anemia	RA
	Refractory anemia with ring sideroblasts	RARS
	Refractory anemia with excess blasts	RAEB
	Chronic myelomonocytic leukemia	CMML
	Refractory anemia with excess blasts in transformation	RAEB
	Chronic myeloproliferative disorders	
III.	**Neoplasms originating in stem cells or myeloid-committed precursors**	
	Acute myeloid leukemia or acute nonlymphocytic leukemia	AML/ANLL
	Acute myeloblastic leukemia with minimal differentiation	M0
	Acute myeloblastic leukemia without maturation	M1
	Acute myeloblastic leukemia with maturation	M2
	Acute promyelocytic leukemia	M3
	Acute myelomonocytic leukemia	M4
	Acute monocytic leukemia	M5
	Acute erythroleukemia	M6
	Acute megakaryoblastic leukemia	M7
	Malignant histiocytosis	
IV.	**Neoplasms of lymphoid-committed precursors**	
	Immature phenotype: Acute lymphoblastic leukemia	ALL, L1,L2
	B-cell lineage	b-ALL
	T-cell lineage	t-ALL
	Intermediate or mature phenotype: non-Hodgkin lymphoma	NHL
	Nodal/splenic phase	
	Leukemic phase	
	B-cell lineage	
	non-Burkitt’s	
	Burkitt’s	L3
	T-cell lineage	
	Lymphoblastic lymphoma	

Table 1. Simplified classification of the major lymphohematopoietic neoplastic diseases in humans based largely on the French-American-British (FAB) classification (continued)

Adult T-cell leukemia/lymphoma	
Mature lymphocytic phenotype	
Prolymphocytic leukemia	
Chronic lymphocytic leukemia	CLL
B-cell lineage	
T-cell lineage	
Hairy cell leukemia	
Plasmacytoid phenotype: Marrow-phase predominant	
Macroglobulinemia	
Heavy chain diseases	
Myeloma	
Hodgkin lymphoma	

Adapted from Sullivan (1993).

Table 2. WHO classification of myeloid and related neoplasms

Myeloproliferative neoplasms (MPNs)
Chronic myelogenous leukemia, *BCR-ABL1*-positive
Chronic neutrophilic leukemia
Polycythemia vera
Primary myelofibrosis
Essential thrombocythemia
Chronic eosinophilic leukemia, not otherwise specified
Mastocytosis
Cutaneous mastocytosis
Systemic mastocytosis
Mast cell leukemia
Mast cell sarcoma
Extracutaneous mastocytoma
Myeloproliferative neoplasms, unclassifiable
Myeloid and lymphoid neoplasms associated with eosinophilia and abnormalities of *PDGFRA, PDGFRB,* or *FGFR1*
Myeloid and lymphoid neoplasms with *PDGFRA* rearrangement
Myeloid neoplasms with *PDGFRB* rearrangement
Myeloid and lymphoid neoplasms with *FGFR1* abnormalities
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
Chronic myelomonocytic leukemia
Atypical chronic myeloid leukemia, *BCR-ABL1*-negative
Juvenile myelomonocytic leukemia
Myelodysplastic/myeloproliferative neoplasm, unclassifiable
Refractory anemia with ring sideroblasts and thrombocytosis (provisional entry)
Myelodysplastic syndrome (MDS)
Refractory cytopenia with unilineage dysplasia
Refractory anemia
Refractory neutropenia
Refractory thrombocytopenia
Refractory anemia with ring sideroblasts
Refractory cytopenia with multilineage dysplasia
Refractory anemia with excess blasts

Table 2. WHO classification of myeloid and related neoplasms (continued)

Myelodysplastic syndrome with isolated del(5q)
Myelodysplastic syndrome, unclassifiable
Childhood myelodysplastic syndrome
Refractory cytopenia of childhood (provisional entry)
Acute myeloid leukemia and related neoplasms
Acute myeloid leukemia with recurrent genetic abnormalities
AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
Acute promyelocytic leukemia with t(15;17)(q22;q12); *PML-RARA*
AML with t(9;11)(p22;q23); *MLLT3-MLL*
AML with t(6;9)(p23;q34); *DEK-NUP214*
AML with inv(3)(q21;q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
AML with mutated NPM1 (provisional entry)
AML with mutated CEBPA (provisional entry)
Acute myeloid leukemia with myelodysplasia-related changes
Therapy-related myeloid neoplasms
Acute myeloid leukemia, not otherwise specified
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Acute erythroid leukemia
Pure erythroid leukemia
Erythroleukemia, erythroid/myeloid
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome

Table 2. WHO classification of myeloid and related neoplasms (continued)

Transient abnormal myelopoiesis
Myeloid leukemia associated with Down syndrome
Blastic plasmacytoid dendritic cell neoplasm
Acute leukemias of ambiguous lineage
Acute undifferentiated leukemia
Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); *BCR-ABL1*
Mixed phenotype acute leukemia with t(v;11q23); *MLL* rearranged
Mixed phenotype acute leukemia, B-myeloid, NOS
Mixed phenotype acute leukemia, T-myeloid, NOS
Natural killer (*NK*) *cell lymphoblastic leukemia/lymphoma (provisional entry)*

Source: WHO (2008).

Table 3. WHO classification of lymphoid neoplasms

Precursor lymphoid neoplasms
B lymphoblastic leukemia/lymphoma
B lymphoblastic leukemia/lymphoma, NOS
B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2);*BCR-ABL 1*
B lymphoblastic leukemia/lymphoma with t(v;11q23);*MLL* rearranged
B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22) *TEL-AML1 (ETV6-RUNX1)*
B lymphoblastic leukemia/lymphoma with hyperdiploidy
B lymphoblastic leukemia/lymphoma with hypodiploidy
B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32) *IL3-IGH*
B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*
T lymphoblastic leukemia/lymphoma
Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic B-cell lymphoma/leukemia, unclassifiable (provisional entry)
Splenic diffuse red pulp small B-cell lymphoma (provisional entry)
Hairy cell leukemia—variant (provisional entry)
Lymphoplasmacytic lymphoma
Waldenstrom macroglobulinemia
Heavy chain diseases
Alpha-heavy chain disease
Gamma-heavy chain disease
Mu heavy chain disease
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT-lymphoma)

Table 3. WHO classification of lymphoid neoplasms (continued)

Nodal marginal zone B-cell lymphoma
Pediatric nodal marginal zone lymphoma (provisional entry)
Follicular lymphoma
Pediatric follicular lymphoma (provisional entry)
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma
Diffuse large B-cell lymphoma (DLBCL), NOS
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg type
EBV positive DLBCL of the elderly (provisional entry)
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK-positive large B-cell lymphoma
Plasmablastic lymphoma
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
Primary effusion lymphoma
Burkitt lymphoma/leukemia
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkett lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma
Mature T-cell and NK-cell neoplasms
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cells (provisional entry)
Aggressive NK-cell leukemia

Table 3. WHO classification of lymphoid neoplasms (continued)

Systemic EBV-positive T-cell lymphoproliferative disease of childhood
Hydroa vacciniforme-like lymphoma
Adult T-cell leukemia/lymphoma
Extranodal NK/T-cell lymphoma, nasal type
Enteropathy-type T-cell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30-positive anaplastic large cell lymphoma
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous gamma-delta T-cell lymphoma
Primary cutaneous CD8 positive aggressive epidermotropiccytotoxic T-cell lymphoma (provisional entry)
Primary cutaneous CD4 positive small/medium T-cell lymphoma
Peripheral T-cell lymphoma, NOS (provisional entry)
Angioimmunoblastic T-cell lymphoma
Anaplastic large cell lymphoma, ALK positive
Anaplastic large cell lymphoma, ALK positive (provisional entry)
Hodgkin lymphoma
Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma

Table 3. WHO classification of lymphoid neoplasms (continued)

Histiocytic and dendritic-cell neoplasms
Histiocytic sarcoma
Langerhans cell histiocytosis
Langerhans cell sarcoma
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma
Fibroblastic reticular cell tumor
Indeterminate dendritic cell tumor
Disseminated juvenile xanthogranuloma
Post-transplant lymphoproliferative disorders (PTLDs)
Early lesions
Plasmacytic hyperplasia
Infectious mononucleosis-like PTLD
Polymorphic PTLD
Monomorphic PTLD (B- and T/NK-cell types)
Classical Hodgkin lymphoma type PTLD

Source: WHO (2008).

more recent WHO classifications (International Agency for Research on Cancer [IARC], 2008c), lymphocytic leukemias and lymphomas are no longer considered to be different diseases and should be evaluated accordingly. However, for simplicity and due to the difficulties involved in reclassifying neoplasms based on studies that were conducted many years ago, the classifications used in the original studies or in the IARC monographs are used in this document. It should be recognized, however, that considerable heterogeneity exists among NHL subtypes as classified according to older classification systems (Morton et al., 2007). In addition, the older schemes do not consider temporal changes in classifying distinct variants of NHL. For example, lymphomas of mucosa-associated lymphoid tissues (MALT) and mantle cell lymphoma as classified by WHO (2008), were previously considered as either pseudolymphomas or benign lymphoid disorders. Similarly, some forms of T-cell lymphomas and aplastic large-cell lymphomas were earlier classified as Hodgkin disease (Banks, 1992).

Among the leukemias, the two major diagnostic categories, ALL and AML, can be further classified based upon cellular features. ALL is subdivided by FAB morphology (L1, L2, and L3) and by immunophenotype (B-cell, early pre-B, pre-B, and T-cell) (Bhatia et al., 1999). AML is classified primarily by morphological characteristics into eight different FAB subgroups (M0–M7) based upon the myeloid lineage and degree of maturation involved. Similarly, the myelodysplastic syndromes (MDSs), a series of blood disorders characterized by maturation defects resulting in ineffective hematopoiesis, have also been classified by the FAB and WHO systems (see Tables 1 and 2, respectively). These are commonly considered to be preleukemic because a variable, but significant, proportion (1 to 33%) of the various disorders progress to frank leukemia (Wright, 1995; WHO, 2001, 2008; Hasle et al., 2003).

The objective of this report is to provide an overview of the types of lymphohematopoietic neoplasia induced by chemical agents and radiation in humans, and to summarize current information on the mechanisms of chemical leukemogenesis. Much of the discussion focuses on AML due to the limited and evolving knowledge of chemically induced lymphoid leukemias and lymphomas that is only now being integrated into ongoing epidemiological and clinical studies. An overview of the major classes of leukemia-inducing agents—radiation, the alkylating agents, and topoisomerase II inhibitors is presented followed by a short discussion of factors influencing chemical leukemogenesis. Lastly, the report ends with a

discussion of how mechanistic information on human leukemia-inducing agents can be used to better assess the risks from exposure to environmental chemicals.

1.1. OVERALL INCIDENCE AND TRENDS

Lymphohematopoietic neoplasms are an uncommon, yet significant, cause of cancer-related deaths. In 2009, it was estimated that leukemia would be diagnosed in 44,790 people in the United States (ACS, 2009). Slightly over half of these will be chronic leukemias (20,540) with the remainder being acute leukemias (18,570) and others that have not been clearly identified (5,680). Because of the limitations of current therapies, leukemia represents the 5th leading cause of cancer deaths among males in the United States and the 7th leading cause among females (ACS, 2009). Furthermore, it was estimated that 74,490 new cases of lymphoma would be diagnosed in the United States in 2009 (ACS, 2009). Of these, 89% (65,980 cases) were estimated to have been NHL and 11% Hodgkin lymphoma (8,510 cases). NHL represents the 9th leading cause of cancer-related deaths in males and the 6th in females. It should be noted that more recent cancer incidence and mortality data can be obtained from the National Cancer Institute (NCI)'s Surveillance Epidemiology and End Results Web site (http://seer.cancer.gov/statistics/).

While leukemia occurs much more commonly in adults than in children, childhood leukemia still accounts for approximately 30% of all childhood cancers in the United States and is a leading cause of disease-related death among children (Smith et al., 2005; ACS, 2006). The incidence of leukemia in children (36 per million) is similar to that seen in young to middle-aged adults (ages 20−44) but roughly one-tenth of that of adults aged 45 years and older, where the annual incidences increase with age from 144 to 545 per million (Xie et al., 2003; ACS, 2006). In adults, roughly 85% of the acute leukemias are myeloid in origin with the remainder being lymphoid (Greaves, 1999). In children, the opposite occurs with 80% of the leukemias originating from lymphoid cells. Moreover, the incidence trends for adult and pediatric leukemias differ substantially. While the overall trend for adult leukemia has generally declined with time, the incidence of childhood leukemias in the United States appeared to increase during the early 1980s with rates increasing from 3.3 cases per 100,000 in 1975 to 4.6 cases per 100,000 in 1985 (NCI, 2008). In the subsequent years, the rates have shown no consistent upward or downward trend. Over the past 30 years, increases in childhood lymphoid leukemia and

lymphoma have also been reported for Europe and in other developed countries (Hrusak et al., 2002; Steliarova-Foucher et al., 2004). Fortunately, there has been significant progress in treating childhood leukemias so that the 5-year survival rate for the affected children is now approximately 80% (ACS, 2006). The survival rate for adult leukemias varies by type with 5-year survival rates of 22% for AML patients, 66% for ALL patients, and 76% for CLL patients (ACS, 2009).

2. HEMATOPOIESIS

Hematopoiesis is the process by which the cells of the blood are formed. The development of the hematopoietic system begins in several mesodermal lineages in the mammalian conceptus with cells migrating from the primitive streak to three blood-forming tissues: the yolk sac, the para-aortic splanchnopleura/aorta-gonadal-mesonephros region, and the chorio-allantoic placenta (for review, see Dzierzak and Speck, 2008). Hematopoietic stem cells capable of conferring complete long-term and multilineage repopulation of hematopoiesis in irradiated adult recipient mice appear in the aorta-gonadal-mesonephros and other tissues during the middle of embryogenesis (Embryonic Day 10.5 in the mouse). These cells proceed to colonize the liver, then the thymus, spleen and bone marrow (by Embryonic Day 15 in the mouse) where hematopoiesis primarily occurs after birth.

The formation of blood cells originates with the hematopoietic stem cell. As described by Wilson and Trumpp (2008), stem cells are functionally defined as cells that can both self-renew (maintain their numbers at a constant level) and give rise to all mature cells in the tissue in which they reside. The formation of blood cells is supported by a small population of pluripotent stem cells that exhibit the capacity to self-renew and are capable of extensive proliferation. The balance between stem cell self-renewal and differentiation is believed to be controlled by interactions between the stem cells and the adjacent niche-forming stromal cells or soluble factors produced by the stromal cells (Wilson and Trumpp, 2008). The pluripotent stem cells can also reconstitute all hematopoietic lineages and are capable of long-term reconstitution of the hematopoietic system of recipient animals. The primitive pluripotent stem cells are estimated to comprise 1 in 100,000 bone marrow cells and give rise to multipotent and committed progenitor cells, which represent approximately 2−5 per 1,000 marrow cells (Mihich and Metcalf, 1995). Each of these progenitor cells can generate 100,000 or more maturing progeny. The process of proliferation and differentiation is regulated by more than 25 growth factors, cytokines, and other regulators that may act directly upon one or more of the major lineages of blood cells or interact to influence cell growth (Mihich and Metcalf, 1995). A diagram illustrating the relationships between the major cell types involved in hematopoiesis is shown in Figure 1 (adapted from Bryder et al., 2006).

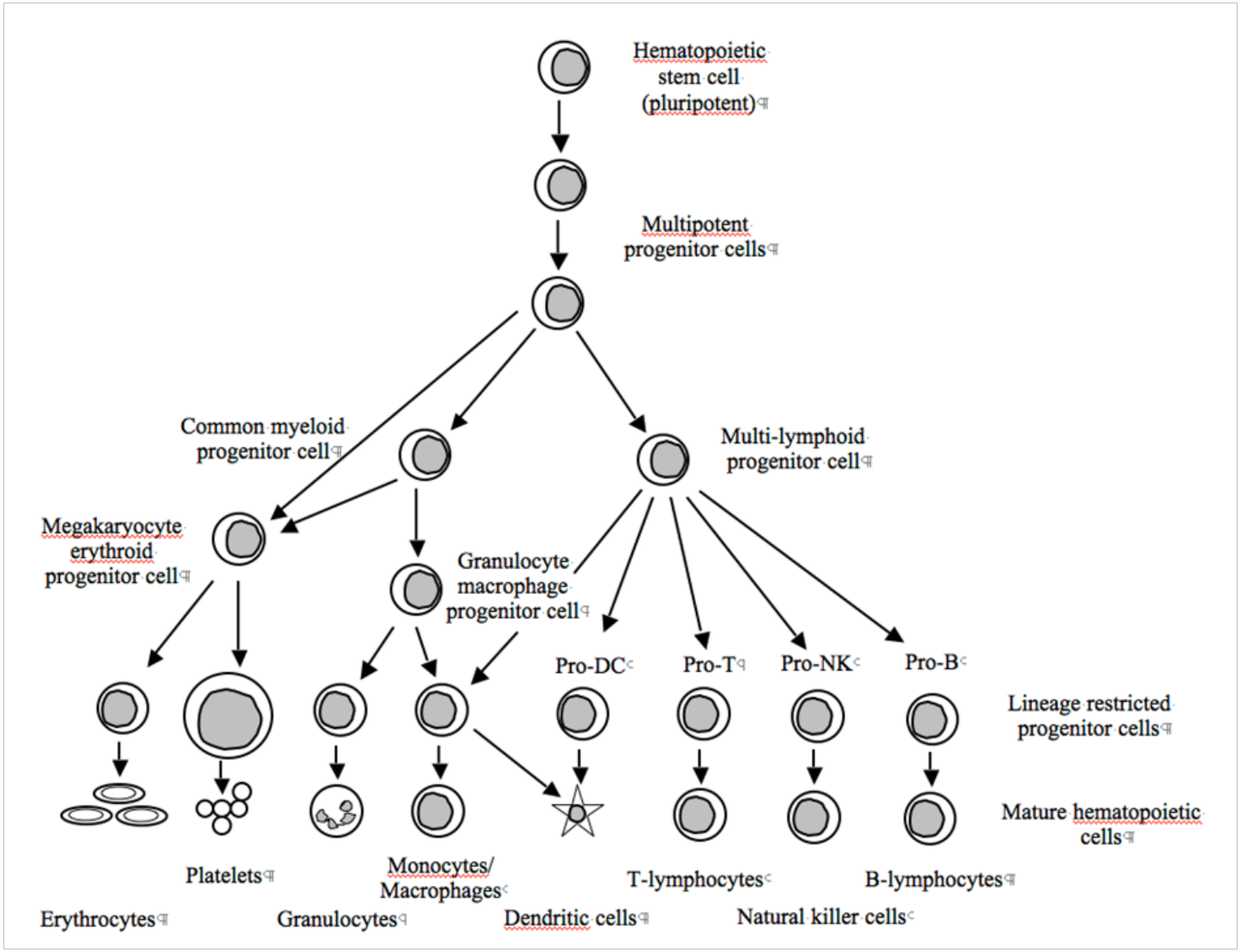

Figure 1. Simplified model of hematopoiesis showing lineages of major types of hematopoietic cells.

With the exception of lymphocytes where maturation also continues in the thymus, spleen and peripheral tissues, the formation of blood cells in normal human adults occurs almost exclusively in the bone marrow. All mature circulating blood cells have a finite life with the majority of cells being terminally differentiated and unable to replicate (Bagby, 1994). In order to maintain steady-state levels, the formation of cells in the marrow must equal the rate of cellular senescence and elimination. As a result, the hematopoietic system has a tremendous proliferative capacity with estimates of cell turnover ranging from 130 to 500 billion cells per day in a 70-kg man (Jandl, 1996; Bryder et al., 2006). In addition, the hematopoietic system in the central bone marrow helps respond to a variety of environmental stresses and infection by increasing the blood cell counts of specific lineages when needed (Bagby, 1994; Jaiswal and Weissman, 2009). For example, upon exposure to a hypoxic environment, erythrocyte production will increase without a change in the production of neutrophils. Similar lineage-specific responses are required following exposure to myelotoxic agents. To maintain steady-state blood cell levels and to respond to environmental pressures, hematopoiesis, of necessity, must be a highly regulated process. Historically, the responses to infection and environmental stresses were believed to occur exclusively within the bone marrow. However, as described below, it has recently been shown that an immune response to infection can occur at extramedullary sites due to the homing, proliferation, and differentiation of hematopoietic stem and progenitor cells at these ecotopic sites within the body (Jaiswal and Weissman, 2009; Schulz et al., 2009).

As indicated above, the hematopoietic stem cells normally reside within the bone marrow, a tissue with specialized vasculature and shielding that provides an excellent environment for the development of blood cells (Papayannopoulou and Scadden, 2008). In addition to their ability to self-renew, primitive pluripotent hematopoietic stem cells give rise to a number of multipotent progenitor cells, which, in turn, give rise to oligopotent progenitor cells (see Figure 1 and Bryder et al., 2006). Among these, the multilymphoid progenitors give rise to mature B lymphocytes, T lymphocytes, and natural killer (NK) cells whereas the common myeloid progenitors give rise to granulocyte-macrophage progenitors (which differentiate into monocytes/macrophages and granulocytes), and megakaryocyte/erythrocyte progenitors (which differentiate into megakaryocytes/platelets and erythrocytes). Both the myeloid and lymphoid progenitor cells have also been shown to form monocytes and dendritic cells, which play a role

in regulating the immune system (Doulatov et al., 2010; Dorshkind, 2010). In addition to these major types of blood cells, a number of the cell types, for example, the granulocytes can further differentiate into more specialized blood cells such as neutrophils, eosinophils, and basophils. As a result, the number of unique cell types derived from the hematopoietic stem cell is quite large, and, as indicated above, the total number of individual cells formed per day numbers in the hundreds of billions.

Hematopoietic stem and progenitor cells are commonly identified based on the presence or absence of lineage markers or antigen expression on the cell surface or in the cytoplasm. As presented by Bryder and associates, the most primitive hematopoietic stem cell identified to date in humans exhibits the cell surface markers, $Lin^-CD90^+CD38^-CD34^+$ meaning that it is negative for the lineage differentiation surface antigen Lin, namely $B^-G^-M^-T^-$ (B220 for B cells, Gr-1 for granulocytes, Mac-1 for myelomonocytic cells, and CD4 and CD8 for T cells), negative for the surface antigen CD38 and positive for the surface antigens CD90 and CD34 (Bryder et al., 2006; Li and Li, 2006). As described by Iwasaki and Akashi (2007) and Bryder et al. (2006), the lineage markers and differentiation pathways differ in notable ways between the mouse and the human.

While hematopoietic stem and progenitor cells normally reside in specialized niches in the bone marrow, they can be mobilized into the peripheral blood either at low levels spontaneously or in large numbers as a result of cytokine or chemical treatment (Levesque et al., 2007; Schulz et al., 2009). These mobilized cells remain in circulation for only short periods of time (minutes to hours) before homing to another peripheral tissue or, for a small to very small percentage of cells under normal conditions, returning to the bone marrow (Wright et al., 2001; Abkowitz et al., 2003; McKinney-Freeman and Goodell, 2004). As a result, there appears to be a constitutive recirculation of hematopoietic stem and progenitor cells between the bone marrow, extramedullary tissues, and the lymphoid compartments (Schulz et al., 2009). Consequently, it is theoretically possible for DNA damage or other types of potentially leukemogenic alterations to affect hematopoietic stem cells while they circulate in the blood and extramedullary spaces. Upon exposure to mobilizing agents such as specific chemokines, toxicants, antibodies, or growth factors, or under conditions of stress or myelosuppression, large numbers of these stem and progenitor cells can be released into the peripheral blood (Levesque et al., 2007). Clinically, these mobilized cells can be harvested for use in bone marrow transplantation. The natural role

of mobilization is not fully known, but there is evidence that the circulating hematopoietic stem and progenitor cells may help replenish tissue-residing myeloid cells such as specific monocytes, macrophages, and dendritic cells or help in rapidly responding to tissue injury and infection (Jaiswal and Weissman, 2009; Schulz et al., 2009).

3. ORIGINS OF LYMPHOHEMATOPOIETIC NEOPLASIA

As with other cancers, leukemogenesis and lymphomagenesis are multistep processes involving a series of genetic and epigenetic alterations involved in the transformation of a normal cell into a malignant cell. The various lymphohematopoietic cancers are believed to originate in specific types of pluripotent or lineage-restricted cells at different stages in hematopoiesis (see Figure 2; Greaves, 1999). As illustrated in the figure, leukemias and lymphomas can originate within many types of hematopoietic or hematopoietic-forming cells. With a few rare exceptions, most leukemias and lymphomas originate at the hematopoietic stem cell stage or at later progenitor or lineage-restricted stages. For example, most adult leukemias of myeloid origin (AML, MDS, and CML) as well as adult ALL are believed to originate at the pluripotent stem or progenitor cell stage whereas childhood leukemias are believed to originate during a subsequent stage of differentiation at either the lineage-restricted lymphoid or myeloid stem cell stage. For most types of adult AML, the key leukemic transformations appear to occur in hematopoietic stem cells (Warner et al., 2004). However, for acute promyelocytic leukemia, the key transformative event may occur at the committed myeloid progenitor stage (Passegue et al., 2003; Warner et al., 2004). For a few others, such as those possessing the MLL-ENL fusion gene, the transformative event can occur either at the pluripotent or the committed stem cell stage (Cozzio et al., 2003). In contrast, many lymphomas (NHL, Hodgkin lymphoma, Burkitt lymphoma) and all myelomas, as well as, several rare leukemias/lymphomas (adult T-cell leukemia, prolymphocytic leukemia, hairy cell leukemia) and one common leukemia (CLL) are believed to originate in mature lymphoid cells (Greaves, 1999; Harris et al., 2001). An understanding of the origin of these cancers can be useful for grouping various lymphohematopoietic cancers and can provide insight into the cell types targeted by carcinogenic agents.

Consistent with the multistep nature of leukemogenesis, over 300 different genetic alterations and mutations have been identified (Kelly and Gilliland, 2002). Indeed, a recent examination of alterations affecting the MLL gene, a cancer-related gene located at band 11q23, in pediatric and adult leukemias, identified a total of 87 different rearrangements, primarily translocations, involving this one gene (Meyer et al., 2006). While most of the detected alterations are rare, certain translocations and genes are more prevalent and are typically

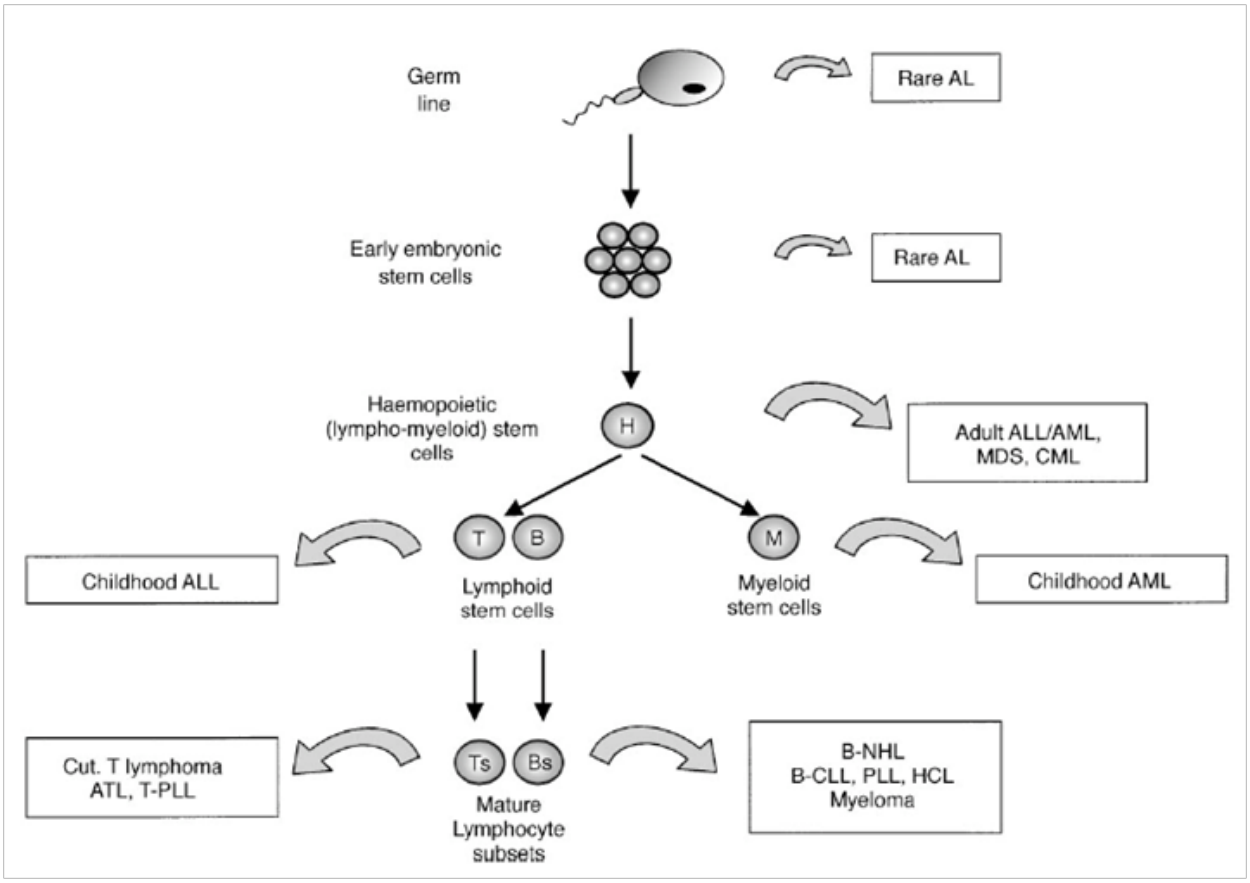

Figure 2. Hierarchical stem cell origins of leukemia and related cancers. The arrows denote the likely level of clonal selection for the majority of the leukemia subtypes listed.

Abbreviations: AL = acute leukemia; Cut. T lymphoma = cutaneous T-cell lymphoma; ATL = adult T-cell leukemia; T-PLL = T-cell prolymphocytic leukemia; B-cell non-Hodgkin lymphoma; PLL = prolymphocytic leukemia; HCL = hairy cell leukemia. For other abbreviations, see Table 1.

Source: Greaves (1999). Reprinted by permission from Elsevier.

associated with specific leukemic subtypes (Bhatia et al., 1999; Greaves and Wiemels, 2003). Many of these genetic alterations can only be detected at the molecular level. However, others have been detected using cytogenetic, molecular cytogenetic, and genomic approaches. Nonrandom chromosomal alterations are detected in the neoplastic cells of a majority of patients with leukemias or lymphomas, and the identification of genes involved in these alterations has provided valuable insights into leukemogenesis and lymphomagenesis in humans (Chen and Sandberg, 2002; Mrozek et al., 2004; Pedersen-Bjergaard et al., 2006; Qian et al., 2009). It is also likely that in addition to genetic alterations, other processes such as epigenetic modifications (altered DNA methylation, dysregulation of miRNA, etc.) play an important role in leukemogenesis (Pedersen-Bjergaard et al., 2006; Nervi et al., 2008; Liu et al., 2011). Because much more is known about the genetic alterations in carcinogenesis and their recognized importance, the overview presented below focuses primarily on genetic changes in the development of myeloid leukemia.

Common clonal cytogenetic changes seen in leukemias include alterations in chromosome number such as loss or gain of one or more chromosomes (e.g., monosomy 7 and Trisomy 8, respectively), balanced and unbalanced chromosome translocations, deletions, or inversions involving specific chromosomal regions as well as complex arrangements involving combinations of the above. In some cases, these changes are a reflection of the genetic instability that is common in many types of cancers. However in many cases, there is evidence that these have originated from a single ancestral cell (i.e., clonal), and are highly specific involving genes that are directly involved in the cancer process. For example, the translocation between chromosomes 15 and 17, (t[15;17][q22;q12−21]) that is characteristic of the promyelocytic form of AML (FAB M3), results in a fusion gene involving the PML gene on chromosome 15 and the retinoic acid receptor *alpha* (RARα) gene on chromosome 17. This hybrid gene blocks differentiation of the developing myeloid cells at the promyelocytic stage (Downing, 1999), a characteristic feature of the disease. As seen in Table 4, significant differences have been seen in the karyotypes of patients who have developed leukemia following therapy and those who have no history of exposure to chemotherapy or other leukemogenic agents. The former are called t-AML and the latter, de novo leukemias. In addition to these microscopically visible chromosomal alterations, mutations at the molecular level affecting specific cancer-related genes such as RAS, FTL3, GATA1, and TP53 have also been seen in

Table 4. Cytogenetic comparisons of de novo leukemias and t-AMLa

Cytogenetic features	AML	
	de novo ***n*** **= 3,649 (%)**	**t-AML** ***n*** **= 581 (%)**
Number of anomalies		
1 anomaly	2,186 (60)	242 (42)
2 anomalies	641 (18)	104 (18)
≥3 anomalies	822 (23)[d]	235 (40)
Ploidy level		
hypodiploid	813 (22)	224 (39)
pseudodiploid	1,843 (51)	242 (42)
hyperdiploid	974 (27)[d]	108 (19)
tri-/tetraploid	13 (0.4)	7 (1.2)
unknown ploidy	6 (0.2)	0
Unbalanced anomalies	2,734 (75)[d]	491 (85)
3p−	33 (0.9)[d]	17 (2.9)
−5	152 (4.2)[d]	73 (13)
5q−	249 (6.8)[d]	77 (13)
−7	340 (9.3)[d]	167 (29)
−7 (sole)	114 (3.1)[d]	51 (8.8)
7q−	147 (4.0)[d]	36 (6.2)
der(1;7)	8 (0.2)[d]	12 (2.1)
loss of 5 and/or 7	717 (20)[d]	284 (49)
+8	614 (17)	84 (14)
+8 (sole)	269 (7.4)[d]	19 (3.3)
11q−	82 (2.2)	17 (2.9)
der(12p)	153 (4.2)[d]	37 (6.4)
13q−	32 (0.9)	7 (1.2)
−17	172 (4.7)[d]	53 (9.1)
der(17p)	104 (2.9)[d]	36 (6.2)
−18	129 (3.5)[d]	40 (6.9)
20q−	45 (1.2)	13 (2.2)
−21	92 (2.5)[d]	41 (7.1)
Balanced anomalies	1,713 (47)[d]	215 (37)
t(1;3)(p36;q21)	2 (0.1)[d]	3 (0.5)

Table 4. Cytogenetic comparisons of de novo leukemias and t-AML[a] (continued)

Cytogenetic features	AML	
	de novo ***n* = 3,649 (%)**	**t-AML** ***n* = 581 (%)**
inv(3)(q21q26)[b]	26 (0.7)	1 (0.2)
t(6;11)(q27;q23)	7 (0.2)	2 (0.3)
t(6;9)(p23;q34)	18 (0.5)	1 (0.2)
t(8;16)(p11;p13)	10 (0.3)	2 (0.3)
t(8;21)(q22;q22)	335 (9.2)[d]	11 (1.9)
t(9;11)(p22;q23)	64 (1.8)[d]	35 (6.0)
t(9;22)(q34;q11)	52 (1.4)[d]	0
t(11;19)(q23;p13)	16 (0.4)[d]	14 (2.4)
t(11q23)	144 (3.9)	72 (12)
t(15;17)(q22;q12)	388 (11)[d]	16 (2.8)
inv(16)(p13q22)[c]	144 (3.9)[d]	4 (0.7)
t(21q22)	375 (10)[d]	20 (3.4)

[a]Includes 'unselected' cases from the Mitelman Database of Chromosome Aberrations in Cancer.
[b]Includes also cases with t(3;3)(q21;q26).
[c]Includes also cases with t(16;16)(p13;q22).
[d]Differs significantly ($p \leq 0.025$) from the t-AML group.

Source: Mauritzson et al. (2002). Reprinted by permission from the Nature Publishing Group.

t-AML and de novo leukemias at varying frequencies (see Table 5). For example, mutations in genes of the receptor tyrosine kinase/RAS-BRAF signal transduction pathway are reported to be present in over 50% of de novo AML (Christiansen et al., 2005). These occur through many mechanisms including base pair substitutions, frame shifts, internal tandem duplications, gene fusions, and splicing errors (for examples in AML1, see [Roumier et al., 2003]). In addition, epigenetic alterations such as changes in the methylation patterns in leukemia-related genes or their promoter regions have also been seen in many types of leukemia, including t-AML (Zheng et al., 2004; Pedersen-Bjergaard et al., 2006; Nervi et al., 2008).

In recent years, researchers have begun to identify patterns among the myriad of translocations, gene arrangements, and point mutations involved in myeloid leukemias and have

Table 5. Frequency of molecular mutations in de novo AML and t-MDS/t-AML[a]

Mutated gene	AML de novo (%)	t-MDS/t-AML (%)
FLT3 (ITD[b]*)*	35	0
FLT3 (TKD[c]*)*	9	<1
NRAS	10−15	10
KITD816	~5	NA[d]
MLL (ITD[b]*)*	3	2−3
RUNX1	10−15	15−30
TP53	10	25−30
PTPN11	~2	3
NPM1	35−50	4−5
CEBPA	6−15	Rare
JAK2V617F	2−5	2−5

[a]From Qian et al. (2009). Reprinted with permission from Elsevier.
[b]ITD = internal tandem duplication.
[c]TKD = tyrosine kinase-domain.

created a model for leukemogenesis (Deguchi and Gilliland, 2002; Kelly and Gilliland, 2002). According to the model proposed by (Kelly and Gilliland, 2002),

> AML is the consequence of collaboration between at least two broad classes of mutations. Class I mutations, exemplified by constitutively activated tyrosine kinases or their downstream effectors, such as BCR/ABL, TEL/PDGFβR, N-RAS, or K-RAS mutants, or constitutively activated FLT3, confer a proliferative or survival advantage to hematopoietic cells. When expressed alone, these mutant genes confer a CML-like disease characterized by leukocytosis with normal maturation and function of cells. Class II mutations result in loss of function of transcription factors that are important for normal hematopoietic differentiation and include the AML1/ETO, CBFβ/SMMHC, PML/RARα, and NUP98/HOXA9 fusions as well as point mutations in hematopoietic transcription factors such as AML1 and C/EBPα. These mutations would also be predicted to impair subsequent apoptosis in cells that do not undergo terminal differentiation. When expressed alone, these mutations may confer a phenotype like most MDS. Regardless of the timing or order of acquisition of mutations, individuals who accrue both Class I and Class II mutations have a clinical phenotype of AML characterized by a proliferative and/or survival advantage to cells and by impaired hematopoietic differentiation.

A list of the gene mutations seen in patients with t-MDS or t-AML separated into these two mutation classes is shown in Table 6.

More recently, additional interrelationships between new and previously identified genetic alterations and leukemogenic agents, and types of leukemia have been reported (Christiansen et al., 2001, 2004; Harada et al., 2003; Zheng et al., 2004; Klymenko et al., 2005; Rege-Cambrin et al., 2005; Wiemels et al., 2005). As illustrated by Pedersen-Bjergaard et al., 2008, the interactions between these various genes can become quite complicated, and the outcome of these interactions is not fully understood. It should also be noted that some genes such as TP53 have cellular functions that are quite different from those involved in the Class I and II mutation pathways and may, therefore, represent a third class of mutated genes that contribute to malignant transformation in t-AML and t-MDS. These results suggest that multiple types of genetic changes are likely to be necessary for the conversion of a normal hematopoietic stem or progenitor cell into a fully transformed leukemic cell (Pedersen-Bjergaard et al., 2008).

Table 6. Gene mutations observed in the Copenhagen series of 140 patients with t-MDS (*n* = 89) or t-AML (*n* = 51)[a]

-	t-MDS	t-AML
Class I mutations		
Tyrosine kinases		
FLT3 ITD+ point mutations	1	10
KIT point mutations	0	2
FMS point mutations	0	0
JAK2 point mutations	2	0
Genes in the RAS/BRAF pathway		
KRAS or NRAS point mutations	7	7[b]
BRAF point mutations	0	3[b]
PTPN11 point mutations	2	2
Class II mutations		
Transcription factors		
AML1/CBFB chimerically rearranged	3	7
AML1 point mutations	20	2
MLL chimerically rearranged	0	11
MLL ITD	1	1
RARA chimerically rearranged	0	2
EVI1 chimerically rearranged	3	1
CEBPA point mutations	0	0
NPM1 point mutations	3	7
Tumor suppressor gene		
TP53 point mutations	25	9
Total 131 mutations observed	67	64

[a]From Pedersen-Bjergaard et al. (2008). Reprinted with permission from the Nature Publishing Group.

[b]One patient had a mutation of KRAS together with a mutation of BRAF possibly in different subclones.

4. LEUKEMIA- AND LYMPHOMA-INDUCING AGENTS

While most leukemias occur in individuals with no obvious exposure to radiation or chemical carcinogens (de novo leukemias), a significant number, known as secondary leukemias develop in individuals who have previously been exposed to radiation, industrial chemicals, or chemotherapeutic agents. Approximately 40 years ago, the IARC began evaluating chemicals (and later other types of agents) for their ability to induce cancer in humans. Since 1971, IARC has evaluated more than 900 agents (including exposure circumstances) and identified more than 100 as being carcinogenic to humans (classified as Group 1: *Carcinogenic to Humans* [IARC, 2010]). Another 66 have been classified in Group 2A, which indicates agents that are *Probably Carcinogenic to Humans*. Among the 100+ agents in Group 1, approximately 25% have been established as causing a lymphohematopoietic cancer (see Table 7). These include eight therapeutic drugs or mixtures, five industrial chemicals or contaminants, two immunosuppressive drugs, six forms of radiation, two occupational or lifestyle exposures, and six infectious agents. Thus, leukemias and lymphomas represent one of the most common types of cancer induced by a wide variety of carcinogens. In addition, three other Group 1 carcinogens (see Table 7), which were classified based on the induction of cancer at one or more other sites, are also thought to be likely to induce either a leukemia or lymphoma. Of the 66 Group 2A agents, there is evidence based either on human studies, animal bioassays, or structural similarities that at least 10 of these chemicals are also likely to cause leukemia in humans. Table 7 lists the Group 1 and 2A agents that have been established, or are likely to cause leukemia or lymphoma, as well some of their characteristics. The same list of agents with key information related to the likely mechanisms responsible for the lymphohematopoietic cancers is shown in Table 8.

In examining the information in Tables 7 and 8, a number of general patterns become apparent. Leukemias are the primary type of cancer induced by the chemical agents, and most of these are acute nonlymphocytic leukemias (ANLLs, synonymous with AML). These were almost always induced by agents that are either directly DNA-reactive or that can be metabolized (or otherwise converted) into DNA-reactive species. Many of the leukemogens are bifunctional alkylating agents; chemicals that form adducts on DNA bases involved in base-pairing such as the 0^6 of guanine, or induce chromosomal damage through the inhibition of topoisomerase II.

Table 7. Characteristics of selected known and probable human leukemia- and lymphoma-inducing agents

Agents carcinogenic to humans	CAS	1° Lymphohematopoietic cancer	2° limited evidence	Myelotoxicity	Chromosomal aberrations[a]	Source(s)
DNA-reactive						
1,3-Butadiene		Lympho-hematopoietic cancers		–[b]	–	IARC, 2008a, 2009; Baan et al., 2009
1,4-Butanediol dimethanesulfonate (Busulfan, Myleran)	55-98-1	ANLL		+	SCA	Grosse et al., 2009; IARC, 1987b
Chlorambucil	305-03-3	ANLL		+	SCA	Grosse et al., 2009; IARC, 1987b
(1-(2-)Chlorethyl)-3-(4-methylcyclohexyl) nitrosurea (Methyl-CCNU, Semustine)	13909-09-6	ANLL		+	NI[c]	Grosse et al., 2009
Cyclophosphamide	50-18-0	ANLL		+	SCA	Grosse et al., 2009; IARC, 1987b
Ethylene Oxide	75-21-8		NHL, MM, CLL	–	SCA	IARC, 2009, 2008b; Baan et al., 2009
Formaldehyde	50-00-0	Myeloid leukemias		+/–	+/–	IARC, 2009, 2006
Melphalan	148-82-3	ANLL		+	SCA	Grosse et al., 2009; IARC, 1987b
MOPP therapy		ANLL		+	SCA	Grosse et al., 2009; IARC, 1987b
Treosulfan	299-75-2	ANLL		+	NI	Grosse et al., 2009; IARC, 1987b
Thio-TEPA (tris(1-aziridinyl)-phosphine)	52-24-4	Leukemia		+	SCA	Grosse et al., 2009; IARC, 1987b
Topoisomerase II-inhibitor						
Etoposide	33419-42-0	ANLL		+	MN	Grosse et al., 2009; IARC, 2000a
Immunosuppressive agents						
Cyclosporine	79217-60-0	NHL		–	SCA	Grosse et al., 2009; IARC, 1990c
Azathioprine	446-86-6	NHL		+	SCA	Grosse et al., 2009; IARC, 1987b

Table 7. Characteristics of selected known and probable human leukemia- and lymphoma-inducing agents (continued)

Agents carcinogenic to humans	CAS	1° Lymphohemato-poetic cancer	2° limited evidence	Myelotoxicity	Chromosomal aberrations[a]	Source(s)
Other						
Benzene	71-43-2	ANLL	NHL, ALL, CLL, MM	+	SCA	IARC, 2009; Baan et al., 2009
2,3,7,8-TCDD	1746-01-6		NHL	–	–	IARC, 2009
X- and *Gamma*-radiation		ANLL, CML, ALL		+	SCA	IARC, 2000b
Neutron radiation			Leukemia	NI	SCA	IARC, 2000b
Thorium-232 and its decay products		ANLL, CML, ALL		NI	SCA	El Ghissassi et al., 2009; IARC, 2001
Phosphorus-32, as phosphate		ANLL		+	SCA	IARC, 2001
Fission products including strontium-90		Leukemia (non-CLL)		NI	NI	El Ghissassi et al., 2009; Krestinina et al., 2010
Tobacco smoking and tobacco smoke		ANLL		NI	SCA	IARC, 2004
Tobacco smoking (parental exposure)			Childhood Leukemia (ALL)	NI	NI	Secretan et al., 2009
Rubber manufacturing occupation		Leukemia, Lymphoma		NI	SCA	IARC, 2009
Painting occupation (maternal exposure)			Childhood leukemia	NI	NI	IARC, 2009
Infectious agents						
Epstein-Barr virus		Burkitt's lymphoma, NHL NK/T-cell lymphoma, Hodgkin lymphoma		–	NI	Bouvard et al., 2009; IARC, 1997
Human immunodeficiency virus Type 1		NHL, Hodgkin lymphoma		+	NI	Bouvard et al., 2009; IARC, 1996a
Human T-cell lymphotrophic virus Type 1		Adult T-cell leukemia and lymphoma		–	NI	Bouvard et al., 2009; IARC, 1996b
Hepatitis C virus		NHL		–	NI	Bouvard et al., 2009
Helicobacter pylori		low-grade B-cell MALT		–	NI	Bouvard et al., 2009

Table 7. Characteristics of selected known and probable human leukemia- and lymphoma-inducing agents (continued)

Agents carcinogenic to humans	CAS	1° Lymphohemato-poetic cancer	2° limited evidence	Myelotoxicity	Chromosomal aberrations[a]	Source(s)
Kaposi's sarcoma herpes virus		1°effusion lymphoma		–	NI	Bouvard et al., 2009
Selected agents probably carcinogenic to humans (IARC Group 2A)						
DNA-reactive						
Bischloroethyl nitrosourea (BCNU; carmustine)	154-93-8		ANLL	+	NI[d]	IARC, 1987b
1-(2-)Chlorethyl-3-cyclohexyl-1-nitrosurea (CCNU; lomustine)	13010-47-4		ANLL	+	NI	IARC, 1987b
N-Ethyl-*N*-nitrosourea	759-73-9		ANLL?	NI	NI[d]	IARC, 1987b
Cisplatin	15663-27-1		leukemia	+	NI[d]	IARC, 1987b
Nitrogen Mustard (Mechlorethamine)	51-75-2		ANLL	+	SCA	IARC, 1987b
Procarbazine	671-16-9		ANLL	+	NI[d]	IARC, 1987b
Chlorozotocin	54749-90-5		ANLL	+	NI	IARC, 1990b
Topoisomerase II-inhibitor						
Adriamycin	25316-40-9		ANLL	+	SCA	IARC, 1987b
Teniposide	29767-20-2		ANLL	+	NI[d]	IARC, 2000c
Other						
Azacytidine	320-67-2		Leukemia	+	NI[d]	IARC, 1990a
Chloramphenicol	56-75-7		ANLL	+	NI	IARC, 2000c

[a]SCA = structural chromosome aberrations; MN = micronuclei; MM = multiple myeloma; TCDD = Tetrachlorodibenzo-*p*-dioxin; NI = no information.
[b]Myelotoxicity is either not seen or infrequently seen.
[c]No information located for humans.
[d]Increases seen in the blood or bone marrow of experimental animals.

Table 8. Likely mechanisms involved in the carcinogenesis of selected known and probable human leukemia- and lymphoma-inducing agents

Agent	Cancer	Class	Activation	1° Mechanism	Other	1° Source
Agents carcinogenic to humans (IARC Group 1 or similar)						
1,3-Butadiene	Various	Industrial chemical	Bioactivated to mono- and bifunctional alkylating agents	Mutation resulting from DNA binding and/or chromosomal alterations	Metabolized into diepoxybutane	IARC, 2008a, 2009; Baan et al., 2009
Busulfan (Myleran; 1,4-Butanediol dimethanesulfonate)	ANLL	Therapeutic agent	Direct-acting bifunctional alkylating agent	Mutation resulting from DNA binding and/or chromosomal alterations		Grosse et al., 2009
Chlorambucil	ANLL	Therapeutic agent	Direct-acting bifunctional alkylating agent	Mutation resulting from DNA binding and/or chromosomal alterations		Grosse et al., 2009
Semustine (Methyl-CCNU; 1-(2-Chlorethyl)-3-(4-methylcyclohexyl)1-nitrosurea)	ANLL	Therapeutic agent	Degrades to direct-acting alkylating and carbamoylating agents	Mutation resulting from DNA binding and/or chromosomal alterations		Grosse et al., 2009
Cyclophosphamide	ANLL	Therapeutic agent	Bioactivated to bifunctional alkylating agent and acrolein	Mutation resulting from DNA binding and/or chromosomal alterations		Grosse et al., 2009
Ethylene oxide	Various	Industrial chemical	Direct-acting alkylating agent	Mutation resulting from DNA binding and/or chromosomal alterations		IARC, 2008b, 2009; Baan et al., 2009
Formaldehyde	ANLL	Industrial chemical	Direct-acting, forms DNA-protein crosslinks	Unknown, possibly mutation resulting from DNA binding and/or chromosomal alterations		IARC, 2006, 2009; Baan et al., 2009
Melphalan	ANLL	Therapeutic agent	Direct-acting bifunctional alkylating agent	Mutation resulting from DNA binding and/or chromosomal alterations		Grosse et al., 2009

Table 8. Likely mechanisms involved in the carcinogenesis of selected known and probable human leukemia- and lymphoma-inducing agents (continued)

Agent	Cancer	Class	Activation	1° Mechanism	Other	1° Source
MOPP therapy	ANLL	Combination of therapeutic agents	A direct-acting bifunctional and an indirect monofunctional alkylating agent, a microtubule inhibitor, and a glucocortocoid	Mutation resulting from DNA binding and/or chromosomal alterations		Grosse et al., 2009
Treosulfan	ANLL	Therapeutic agent	Converts to a mono- and bifunctional alkylating agent	Mutation resulting from DNA binding and/or chromosomal alterations	Converts into diepoxybutane, an (intrastrand?) crosslinking agent	Hartley et al., 1999; Grosse et al., 2009
Thio-TEPA (tris(1-aziridinyl)-phosphine)	Leukemia	Therapeutic agent	Direct-acting trifunctional alkylating agent. Also, metabolized to monofunctional alkylating agent aziridine	Mutation resulting from DNA binding and/or chromosomal alterations		Maanen et al., 2000; Grosse et al., 2009
Etoposide	ANLL	Therapeutic agent	Topoisomerase II-poison	Mutation resulting from chromosomal breakage and translocations	Results in modified transcription factor	Grosse et al., 2009
Cyclosporine	NHL	Therapeutic agent	Inhibition of transcription factors that regulate inducible cytokine expression	Immunosuppression		Grosse et al., 2009
Azathioprine	NHL	Therapeutic agent	Metabolized into nucleotide analog	Immunosuppression	Also genotoxic	Grosse et al., 2009

Table 8. Likely mechanisms involved in the carcinogenesis of selected known and probable human leukemia- and lymphoma-inducing agents (continued)

Agent	Cancer	Class	Activation	1° Mechanism	Other	1° Source
Benzene	ANLL	Industrial chemical and environmental agent	Metabolized into reactive protein and DNA-binding species	Unknown, likely mutation resulting from either DNA binding, topoisomerase II-inhibition, and/or oxidative damage	Likely multiple metabolites and modes of action involved	Baan et al., 2009; IARC, 2009
2,3,7,8-TCDD	NHL[a]	Environmental contaminant	Receptor-mediated effects modifying cellular replication and apoptosis	Unknown, likely immunosuppression	Also can lead to DNA damage through oxidative stress	Baan et al., 2009; IARC, 2009; Holsapple et al., 1996
X- and *Gamma*-radiation	ANLL CML ALL	Therapeutic, energy and military uses	Direct and indirect DNA damage	Mutation resulting from DNA damage and/or chromosomal alterations		IARC, 2000b; El Ghissassi et al., 2009
Alpha and beta particle emitters	ANLL (also CML and ALL for Th-32)	Therapeutic, energy and military uses	Direct and indirect DNA damage	Mutation resulting from DNA damage and/or chromosomal alterations		IARC, 2001; El Ghissassi et al., 2009
Tobacco smoking and tobacco smoke	ANLL	Lifestyle use	Direct and indirect DNA damage	Unknown, likely mutation resulting from DNA binding and/or chromosomal alterations		IARC, 2004; Secretan et al., 2009
Tobacco smoking (parental)	Childhood ALL	Parental use	Direct and indirect DNA damage	Unknown, assumed mutation resulting from DNA binding and/or chromosomal alterations occurring in germ cells or in utero	Epigenetic changes could also contribute	IARC, 2004; Secretan et al., 2009

Table 8. Likely mechanisms involved in the carcinogenesis of selected known and probable human leukemia- and lymphoma-inducing agents (continued)

Agent	Cancer	Class	Activation	1° Mechanism	Other	1° Source
Rubber manufacturing occupation	Leukemia Lymphoma	Occupational exposure	Unknown but DNA-reactive chemicals are used	Unknown, assumed to be mutation resulting from DNA binding and/or chromosomal alterations and/or immunosuppression		IARC, 2009
Painting occupation	Childhood leukemia[a]	Occupational exposure	Unknown but DNA-reactive chemicals are used	Unknown, assumed mutation resulting from DNA binding and/or chromosomal alterations occurring in germ cells or in utero	Other mechanisms are also likely	IARC, 2009
Epstein-Barr virus	Burkitt's lymphoma NHL NK/T-cell lymphoma Hodgkin lymphoma	Infectious agent	Viral infection and expression of viral proteins leading to lymphocyte transformation	Alteration in normal B-lymphocyte function leading to cell proliferation, inhibition of apoptosis, genomic instability, and cell migration		Hjalgrim and Engels, 2008; Bouvard et al., 2009
Human immunodeficiency virus Type 1	NHL	Infectious agent	Viral infection and expression of viral proteins leading to loss of CD 4+ T lymphocytes	Immunosuppression (as an indirect effect)		Hjalgrim and Engels, 2008; Bouvard et al., 2009
Human T-cell lymphotrophic virus Type 1	Adult T-cell leukemia and lymphoma	Infectious agent	Viral infection and expression leading to lymphocyte transformation	Immortalization and transformation of T cells		Hjalgrim and Engels, 2008; Bouvard et al., 2009

Table 8. Likely mechanisms involved in the carcinogenesis of selected known and probable human leukemia- and lymphoma-inducing agents (continued)

Agent	Cancer	Class	Activation	1° Mechanism	Other	1° Source
Hepatitis C virus	NHL	Infectious agent	Viral infection and expression of viral proteins leading to chronic immune stimulation	Chronic immune stimulation		Hjalgrim and Engels, 2008; Bouvard et al., 2009
Helicobacter pylori	Low-grade B-cell MALT	Infectious agent	Inflammation leading to cellular alterations	Oxidative stress, altered cellular turnover and gene expression, methylation, and mutation	Chronic immune stimulation	Hjalgrim and Engels, 2008; Bouvard et al., 2009
Kaposi's sarcoma herpes virus	1° Effusion lymphoma	Infectious agent	Viral infection and expression of viral proteins	Cell proliferation, inhibition of apoptosis, genomic instability, cell migration		Bouvard et al., 2009
Selected agents probably carcinogenic to humans (IARC Group 2A)						
Bischloroethyl nitrosourea (BCNU; carmustine)	ANLL	Therapeutic agent	Direct-acting bifunctional alkylating agent	Mutation resulting from DNA binding and/or chromosomal alterations		IARC, 1987b; Vogel et al., 1998
(1-(2-)Chlorethyl)-3-cyclohexyl-1-nitrosurea (CCNU; lomustine)	ANLL	Therapeutic agent	Direct-acting bifunctional alkylating agent	Mutation resulting from DNA binding and/or chromosomal alterations		IARC, 1987b; Vogel et al., 1998
N-Ethyl-*N*-nitrosourea	ANLL?	Experimental reagent	Direct-acting alkylating agent	Mutation resulting from DNA binding and/or chromosomal alterations		IARC, 1987b; Vogel et al., 1998
Cisplatin	Leukemia	Therapeutic agent	Direct-acting bifunctional DNA binding agent	Mutation resulting from DNA binding and/or chromosomal alterations		IARC, 1987b; Vogel et al., 1998

Table 8. Likely mechanisms involved in the carcinogenesis of selected known and probable human leukemia- and lymphoma-inducing agents (continued)

Agent	Cancer	Class	Activation	1° Mechanism	Other	1° Source
Nitrogen Mustard (Mechlorethamine)	Leukemia	Therapeutic agent	Direct-acting bifunctional alkylating agent	Mutation resulting from DNA binding and/or chromosomal alterations		IARC, 1987b; Vogel et al., 1998
Procarbazine	ANLL	Therapeutic agent	Bioactivated to a monofunctional alkylating agent	Mutation resulting from DNA binding and/or chromosomal alterations		IARC, 1987b; Vogel et al., 1998
Chlorozotocin	ANLL	Therapeutic agent	Direct-acting bifunctional alkylating agent	Mutation resulting from DNA binding and/or chromosomal alterations		IARC, 1987b; Vogel et al., 1998
Adriamycin	ANLL	Therapeutic agent	Topoisomerase II-poison and redox-cycling agent	Mutation resulting from chromosomal breakage and translocations		IARC, 1987b
Teniposide	ANLL	Therapeutic agent	Topoisomerase II-poison	Mutation resulting from chromosomal breakage and translocations		IARC, 2000c
Azacytidine	Leukemia	Therapeutic agent	DNA-methyltransferase inhibitor through metabolism and incorporation into DNA	Alters DNA methylation and gene expression. Is also genotoxic		NTP, 2005; Stresemann and Lyko, 2008
Chloramphenicol	ANLL	Therapeutic agent	Binds to ribosomal subunit blocking protein synthesis in mitochondria. Metabolite may also induce DNA damage	Unknown, presumed to be mutation resulting from DNA damage and/or chromosomal alterations		NTP, 2005

[a]Limited evidence.

The primary mode of action for these agents is through the induction of mutations, either gene mutations or chromosomal mutations (IARC, 2012). Consistent with their proposed mutagenic mechanisms, most of the leukemia-inducing agents have been reported to induce structural chromosome aberrations in the peripheral blood of exposed humans. Myelotoxicity is also commonly seen in humans (and animals) exposed to these leukemogenic agents. Exposure to radiation in various forms was frequently associated with ALL and CML, in addition to ANLL. These are also likely due to the effects of ionizing radiation on DNA, either directly or indirectly, and are believed to occur through mutagenic and epigenetic mechanisms (IARC, 2012).

In contrast to these DNA-damaging agents, exposure to a variety of infectious agents is causally related to the formation of lymphoid neoplasms. The induced lymphomas appear to be primarily associated with chronic infection with either viruses or *Helicobacter* bacteria that are immunomodulating and/or specifically target lymphoid cells. These agents are believed to act as either direct carcinogens acting on the cellular DNA, indirect carcinogens acting through chronic inflammation, or through immune-suppression (IARC, 2012). The two chemical agents, cyclosporine and azathioprine, which also induce NHL, are also strongly immunosuppressive. In addition, Tetrachlorodibenzo-*p*-dioxin (TCDD), which may induce NHL, has been shown in animal studies to be immunosuppressive (Holsapple et al., 1996), so this could be the mechanism underlying its lymphoma-inducing effects. Although not reviewed by IARC and, hence, not presented in Tables 7 and 8, autoimmune disorders and other diseases associated with immune stimulation and inflammation are increasingly recognized from large consortia studies as risk factors for lymphoma (Vajdic et al., 2009, Ekstrom Smedby et al., 2008; Cocco et al., 2008; Nieters et al., 2006).

Similar to the Group 1 agents, the Group 2A leukemogens are primarily associated with the induction of ANLL. For chemicals for which information is available, these agents are also myelotoxic and clastogenic and are believed to most likely induce their leukemogenic effects through a mutagenic mechanism (see Table 8).

In summary, based on the IARC evaluations, the vast majority of the leukemia-inducing agents are believed to act through a mutagenic or genotoxic mechanism(s) whereas the lymphoma-inducing agents most likely act through immunomodulation and related effects. An overview of the major classes of leukemia-inducing agents is presented below.

5. OVERVIEW OF THE MAJOR CLASSES OF LEUKEMIA-INDUCING AGENTS

5.1. IONIZING RADIATION

As a result of its widespread medical, military, and energy-related uses, large numbers of people have been exposed to ionizing radiation, and its adverse effects have been extensively documented (IARC, 2000b, 2001; UNSCEAR, 2000a, b; Ron, 2003; NRC, 2006). Exposure to ionizing radiation has been shown to result in numerous types of cancer including several types of leukemia as well as adverse effects that are likely to be involved in leukemogenesis including myelotoxicity, immune suppression, and genetic damage leading to chromosomal and gene mutations. The earliest association between radiation and cancer was seen for leukemia, and it has been repeatedly seen in numerous population studies including those exposed through medical, military, and environmental exposures (IARC, 2000b, 2001; UNSCEAR, 2000b; Ron, 2003; NRC, 2006). It should be noted that there are a variety of types of radiation and radioactive materials. Most of the studies that have been conducted have investigated the effects of ionizing radiation, which for simplicity; will be considered as one individual agent in this section. Assessing the risks associated with radiation exposure is challenging and is influenced by the type of radiation, the tissue dose, the proportion of the body exposed, the extent of cell killing, and the DNA-repair capacity of the individual or tissue (Curtis et al., 1994). Host factors such as age, sex, genetic composition, and health of the exposed individuals can also influence the risk of radiation-induced leukemia.

All age groups are affected, but children, in particular, have been shown to be at an elevated risk for radiation-induced leukemia as seen in the Life Span Study of the atomic bomb survivors in Hiroshima and Nagasaki. Among the survivors who were exposed to both gamma, and to a lesser extent, neutron radiation, the highest leukemia risks were seen in children (Preston et al., 1994, 2004). Significant dose-related increases were seen for ALL, AML, and CML, with the excess risks for ALL and CML being approximately 100% higher in the exposed males than in the females. When the leukemias were reclassified using the FAB classification scheme, all AML subtypes were present with a predominance of myeloblastic leukemias with and without maturation (M1 and M2) (Matsuo et al., 1988). Among the ALL cases, most cases exhibited the L2 subtype, but a significant portion also showed the L1 subtype. Latency periods were shorter for those under 15 years of age with a leukemia peak at 5−7 years post exposure as

compared to later peaks for those exposed at later ages (Kamada and Tanaka, 1983). The leukemia risk also decreased more rapidly in children as compared to those in older age groups. Overall, the risks to children were highest during the period from 1950 to 1965 and returned to near background levels thereafter (Preston et al., 1994).

Similarly, the use of radiation for medical purposes has been associated with increases in leukemia. Increased risk of leukemia has been reported following the use of radiation for diagnostic tests, for the treatment of benign diseases, and following radiotherapy for cancer (Ron, 1998, 2003). For hematopoietic and lymphoid tissues that are diffuse, a significant portion of the tissue must be irradiated to increase the incidence of neoplasia (Storer et al., 1982). The leukemia risks from medical irradiation appear to have diminished in recent years due to a use of lower and more restricted doses as well as changes in therapeutic strategy in which high doses are applied within limited fields. High radiation doses (above 3−4 Gray) result in extensive killing of marrow-containing progenitor cells and have been associated with a reduced risk of developing leukemia (Boice et al., 1987; NRC, 1990; Curtis et al., 1994).

5.2. CHEMOTHERAPEUTIC AGENTS

Following the initiation of intensive chemotherapy with genotoxic agents, increases in therapy-related leukemias began to appear (Seiber and Adamson, 1975; Kantarjian and Keating, 1987; Levine and Bloomfield, 1992; Leone et al., 1999). These leukemias, also known as treatment-related or secondary leukemias, consisted primarily of AML, although a small increase in therapy-related ALL has also been seen (Hunger et al., 1992; Andersen et al., 2001). With increased periods of follow-up, increases in other types of solid tumors have also been observed (Tucker et al., 1988; Loescher et al., 1989; Boffetta and Kaldor, 1994; van Leeuwen et al., 1994; Vega-Stromberg, 2003; Travis et al., 2005; Hodgson et al., 2007).

Over time, as new agents and therapeutic strategies have been employed; additional agents have been recognized as inducing leukemia in humans. Indeed, as seen in Table 7, chemotherapeutic drugs comprise the largest group of agents generally recognized as human leukemia-inducing agents. Currently, therapy-related leukemias constitute 10 to 20% of the leukemia cases seen at major medical institutions (Deschler and Lubbert, 2006; Pedersen-Bjergaard et al., 2007).

The identification of specific agents involved in leukemogenesis and the interpretation of many of the studies can be challenging due to the use of multiple therapeutic agents, varying dosing regimens, concurrent use of radiotherapy, and variable periods of patient follow-up. Over time, two main classes of leukemogenic therapeutic drugs have been identified—alkylating agents and topoisomerase II inhibitors (Pedersen-Bjergaard and Philip, 1991; Pedersen-Bjergaard and Rowley, 1994; Pedersen-Bjergaard et al., 2006). An overview of the AMLs (including myelodysplastic syndromes) induced by these two classes of chemotherapeutic drugs is briefly discussed in the following sections.

5.2.1. Alkylating Agent-Related Leukemias

A large number of studies have demonstrated that patients treated with alkylating agent-based chemotherapy are at an increased risk of MDS and AML (Levine and Bloomfield, 1992; Pedersen-Bjergaard and Rowley, 1994; Smith et al., 1994). These risks have been seen for both children and adults and are strongly related to the cumulative dose of the alkylating agent (Pedersen-Bjergaard and Philip, 1987; Tucker et al., 1987; Hunger et al., 1992; Pedersen-Bjergaard et al., 2000; Pyatt et al., 2005). The administered agents also exhibit varying degrees of hematopoietic toxicity, immunotoxicity, and genotoxicity, which can also be affected by the agent, dose, and route of administration (Gale, 1988; Ferguson and Pearson, 1996; Sanderson and Shield, 1996). The incidence of therapy-related MDS and AML (t-MDS/t-AML) in adults treated with antineoplastic drugs has been reported to range from 1 to >20% (Felix, 1998; Leone et al., 2001). The increases in this type of therapy-related leukemia generally appear 1 to 2 years after treatment and may remain elevated for 8 or more years following the completion of chemotherapy (Pedersen-Bjergaard and Philip, 1987; Pedersen-Bjergaard and Philip, 1991; Davies, 2000; Schonfeld et al., 2006).

In adults, leukemias induced by alkylating agents exhibit characteristics that generally allow them to be distinguished from de novo leukemias and those induced by topoisomerase II inhibitors (Pedersen-Bjergaard and Rowley, 1994; Eastmond et al., 2005). These leukemias are typically myeloblastic with or without maturation (FAB M1 and M2 subtypes) and are characterized by trilineage dysplasia and clonal unbalanced chromosome aberrations, most commonly involving loss of the entire chromosome or part of the long arms of chromosomes 5and 7 (−7, 7q−, −5, 5q−). These t-AML have a modal latency of 4−7 years, and

the onset of the actual leukemia is often preceded by myelodysplasia. It should be noted that AML induced by ionizing radiation tends to exhibit similar features (MDS, clonal unbalanced chromosomal aberrations, etc.) although the range of the FAB subtypes induced by radiation tends to be broader (Matsuo et al., 1988; Philip and Pedersen-Bjergaard, 1988; Gundestrup et al., 2000).

Using structure-activity relationships as well as other predictive approaches with in vivo rodent and *Drosophila* data, Vogel et al. (1998) were able to identify three major categories of DNA-reactive chemical and therapeutic agents. As described by the authors, Category 1 consisted of mono-functional alkylating agents such as ethylene oxide and methyl methane sulfonate, which primarily react at the N7 and N3 moieties of purines in DNA. Efficient DNA repair was the major protective mechanism against the relatively weak genotoxic effects of these agents, which might not be detectable in repair-competent cells. High doses were generally needed for the induction of tumors in rodents. Strong target site specificity for the adverse effects was seen, and this appeared to be related to DNA repair capacity. Category 2 agents such as procarbazine and *N*-nitroso-*N*-ethylurea (ENU), induce both *O*-alkyl adducts and *N*-alkyl adducts in DNA. In general, the induced *O*-alkyl adducts appeared to be slowly repaired, or not repaired, which made these agents potent carcinogens and germ cell mutagens. The inefficient repair of the *O*-alkyl-pyrimidines, in particular, was responsible for their potent mutagenic activity. Category 3 agents such as melphalan and busulfan induced structural aberrations through their ability to cross-link DNA, and this was the major factor contributing to their high genotoxic potency, which appeared to be related to the number of DNA crosslinks per target dose unit that were induced. The genotoxic effects for the Category 3 agents occurred at, or near, toxic levels. For all three categories of genotoxic agents, strong correlations were observed between their carcinogenic potency, acute toxicity, and germ-cell specificity.

5.2.2. Topoisomerase II Inhibitor-Related Leukemias

In the late 1980s, a new type of therapy-related leukemia was recognized that exhibited unusual features that differed from those previously seen following treatment with alkylating agents and radiation (Pui et al., 1989; Pui and Relling, 2000). The affected patients had been treated with etoposide or teniposide, two of a newly developed epipodophyllotoxin class of chemotherapeutic agents (Pedersen-Bjergaard and Philip, 1991). In the ensuing years, these

epipodophyllotoxins have become widely used, especially for treating childhood cancers, and a large number of studies have been published establishing an association between treatment with these drugs and the subsequent development of leukemia (Haupt et al., 1993; Smith et al., 1994; Pedersen-Bjergaard et al., 1995; Pui and Relling, 2000; Leone et al., 2001; Hijiya et al., 2009).

The leukemia risk following epipodophyllotoxin therapy was reported to be very high in early studies, with cumulative incidences approaching 19% in some treatment groups (Pui et al., 1989). Interestingly, in some cases, the risk of a treatment-related cancer appeared to be more closely related to the treatment regimen than to total dose (Pui et al., 1991; Pui and Relling, 2000). Patients receiving epipodophyllotoxins on a weekly or twice-weekly basis had much higher cumulative risks (12.4%) than those receiving the drugs on a biweekly schedule (1.6%). Similar results have been seen in more recent studies (Smith et al., 1999) although others have seen a correlation between cumulative dose and epipodophyllotoxin-induced t-AML (Neglia et al., 2001; Le Deley et al., 2003). With implementation of newer treatment protocols, the risk of t-AML has been reduced substantially and is now within the range of that seen with alkylating chemotherapeutic agents (Smith et al., 1993; Pui and Relling, 2000). There is also evidence that the combined treatment of topoisomerase II inhibitors with alkylating agents (or cisplatin) confers a greater risk of t-AML than seen with either type of chemotherapeutic agent alone (Sandoval et al., 1993; Smith et al., 1994; Blatt, 1995). In most reported cases, the incidence of t-AML induced by these drugs is estimated to range from 2 to 12% (Felix, 1998; Leone et al., 2001).

The epipodophyllotoxins exhibit moderate myelosuppression, identifiable chromosomal damage, and their leukemogenic effects by inhibiting topoisomerase II through a process that involves stabilization of the DNA-enzyme complex (Smith et al., 1994; Ferguson and Pearson, 1996). Topoisomerase II is a nuclear enzyme involved in a wide variety of cellular functions, including DNA replication, transcription, and chromosome segregation (Anderson and Berger, 1994; Nitiss, 2009). Leukemias induced following treatment with epipodophyllotoxin-type topoisomerase inhibitors generally appear from 10 months to 8 years following the initiation of chemotherapy, with a median latency of 2 to 3 years (Smith et al., 1994; Leone et al., 2001). The induced AMLs are primarily of the monocytic or myelomonocytic subtypes (M4 and M5) and are rarely preceded by a myelodysplastic phase, a pattern that differs significantly from

alkylating agent or radiation-induced leukemias (Pedersen-Bjergaard and Rowley, 1994; Smith et al., 1994; Leone et al., 2001; Pedersen-Bjergaard et al., 2006).

Infrequently, ALL has been reported in patients following treatment with both alkylating agents and topoisomerase II-inhibiting drugs (Hunger et al., 1992; Andersen et al., 2001; Hijiya et al., 2009). Although t-ALLs occur infrequently, they seem to be more common in children under the age of 15 who have previously been treated with a topoisomerase II inhibitor (Andersen et al., 2001).

One of the unique features of t-AML, and to a lesser extent t-ALL, induced by the epipodophyllotoxin-type of topoisomerase II inhibitors, is the presence of clonal-balanced translocations involving the MLL gene (also known as ALL-1, HRX, and HTRX-1) located on the long arm of chromosome 11 (11q23) in the leukemic cells. Cytogenetic studies of patients with leukemias induced by these agents have shown that in over 50% of the cases, the leukemic clone involved a balanced translocation affecting the 11q23 region and another chromosomal partner, usually t(6;11), t(9;11), and t(11;19) (Pedersen-Bjergaard and Rowley, 1994; Smith et al., 1994; Canaani et al., 1995). In children previously treated with topoisomerase inhibitors, up to 90% of the treatment-related leukemias have an 11q23 alteration (Canaani et al., 1995). As indicated previously, as of 2006, 87 rearrangements involving the MLL gene have been identified, and 51 of the translocation partner genes had been characterized at the molecular level (Meyeret al., 2006). The four most common MLL translocation partner genes (i.e., AF4, AF9, ENL, and AF10) encode nuclear proteins that are part of a protein network involved in histone H3K79 methylation (Meyer et al., 2006) indicating a potentially important role for this pathway in epipodophyllotoxin leukemogenesis.

Numerous lines of evidence indicate that topoisomerase II as well as DNA-repair enzymes such as those involved in nonhomologous end joining play an important role in the formation of the 11q23 translocations. These have been summarized in a series of reviews (Greaves and Wiemels, 2003; Aplan, 2006; Felix et al., 2006; Zhang and Rowley, 2006). The presence of topoisomerase II recognition sites has been found in proximity to the translocation breakpoints as have recombinase recognition sites, Alu sequences, DNase hypersensitive sites, and scaffold attachment regions, and suggests that multiple types of damage and repair probably contribute to the generation of the observed translocations (Pui and Relling, 2000; Felix et al., 2006; Zhang and Rowley, 2006). Interestingly, in a recent report, Le et al. (2009) reported that

the translocation breakpoints within the MLL gene occurred in a highly specific fashion at the base of a secondary DNA structure formed from a palindrome. A stringent topoisomerase II consensus binding site was located at the apex of the secondary DNA structure. The authors proposed a model in which topoisomerase II facilitates the formation of a secondary structure that results in site-specific DNA strand breakage that is efficiently processed into translocations (Le et al., 2009). This processing most likely occurs through a mechanism involving nonhomologous end joining (Greaves and Wiemels, 2003; Zhang and Rowley, 2006). It should also be noted that the MLL gene has been shown to be prone to breakage during apoptosis, and it has been proposed that the observed translocations occur in hematopoietic cells rescued at an early, reversible stage during the apoptotic process (Stanulla et al., 1997; Betti et al., 2005; Vaughan et al., 2005; Basecke et al., 2006). A role for apoptosis has also been proposed in the formation of other leukemia-related translocations (Eguchi-Ishimae et al., 2001).

5.2.3. Other Likely Leukemia-Inducing Therapeutic Agents

In recent years, evidence has accumulated that other inhibitors of topoisomerase II, such as the anthracycline, anthracenedione, and bisdioxopiperazine derivatives, can also induce treatment-related leukemias (Xue et al., 1992; Zhang et al., 1993; Blatt, 1995; Andersen et al., 1998; Le Deley et al., 2003; Mistry et al., 2005; Mays et al., 2010). The leukemias induced by these agents are similar to the epipodophyllotoxins in that they have short latency periods and are infrequently preceded by myelodysplasia. However, they typically exhibit different types of clonal-balanced rearrangements (e.g., t[8;21], t[15;17], and inv[16]) and different FAB subtypes (M2 and M3).

Although azathioprine, 6-thioguanine, and 6-mercaptopurine are primarily associated with immunosuppression and the induction of NHL, a number of studies have suggested that patients treated with these 6-thioguanosine monophosphate-producing drugs are at an increased risk of developing ANLL (Bo et al., 1999; Karran, 2006; Yenson et al., 2008). It has been suggested that the risks are higher in patients with low thiopurine *S*-methyltransferase activity and may involve aberrant mismatch repair and microsatellite instability (Bo et al., 1999; Karran, 2006). Interestingly, the leukemias of many of these patients also show loss of chromosome 7 or part of chromosomes 5 or 7 (5q− or 7q−) (Yenson et al., 2008).

6. MECHANISMS INVOLVED IN THERAPY-RELATED ACUTE MYELOID LEUKEMIA (T-AML)

For more than 20 years, Pedersen-Bjergaard and colleagues have collected information on patients with t-AML and t-MDS who have been treated at their clinic in Copenhagen, Denmark. Based on the genetic alterations seen in this cohort of 140 patients as well as information in the literature, they have identified eight separate pathways that appear to lead either directly to t-AML or lead to t-MDS and then to t-AML. These pathways are illustrated in Figure 3 (from [Pedersen-Bjergaardet al., 2006]). The pathways have been classified into three groups largely based upon the therapeutic agent that was likely responsible for the leukemia. Each of the pathways is described below based on descriptions extracted from their 2006 article (i.e., Pedersen-Bjergaard et al., 2006).

Pathway I in the figure was characterized by patients who developed t-MDS prior to t-AML and exhibited either loss of chromosome 7 or part of the long arm of chromosome (7q−). These patients also did not exhibit the recurrent balanced translocations that are associated with AML. Most of these patients (35/39) had been treated with alkylating agents, and most (35/39) presented with t-MDS prior to t-AML. Methylation of the p15 promoter, considered a late event in leukemogenesis, was seen in 84% of the patients. In addition to the chromosome 7 changes, the leukemias of a significant portion (15/39 or 38%) of these patients had point mutations in the AML1 (also known as RUNX1) gene. A number of these patients also had mutations in either the TP53 tumor suppressor gene or an activating mutation in the RAS oncogene.

Pathway II predominated in patients that exhibited either loss of all (−5) or part of the long arm of chromosome 5 (5q−). Approximately half of the patients in this group also had −7 or 7q− alterations. As with Pathway I, onset of this disease is closely related to the use of alkylating agent chemotherapy (27/34) and an initial presentation as t-MDS (26/34). Seventy-seven percentage of these patients exhibited mutations in TP53. The patients also often present with a complex karyotype or loss of part of the short arm of chromosome 17 (17p−) or showed an amplification or duplication of chromosome bands 11q23 or 21q22. Methylation of the p15 promoter occurred in 80−90% of the patients of this group.

Pathway III was characterized by balanced translocations involving the 11q23 chromosome band and one of many partner chromosomes. These translocations frequently occurred in patients who had been previously treated with topoisomerase II inhibitors and

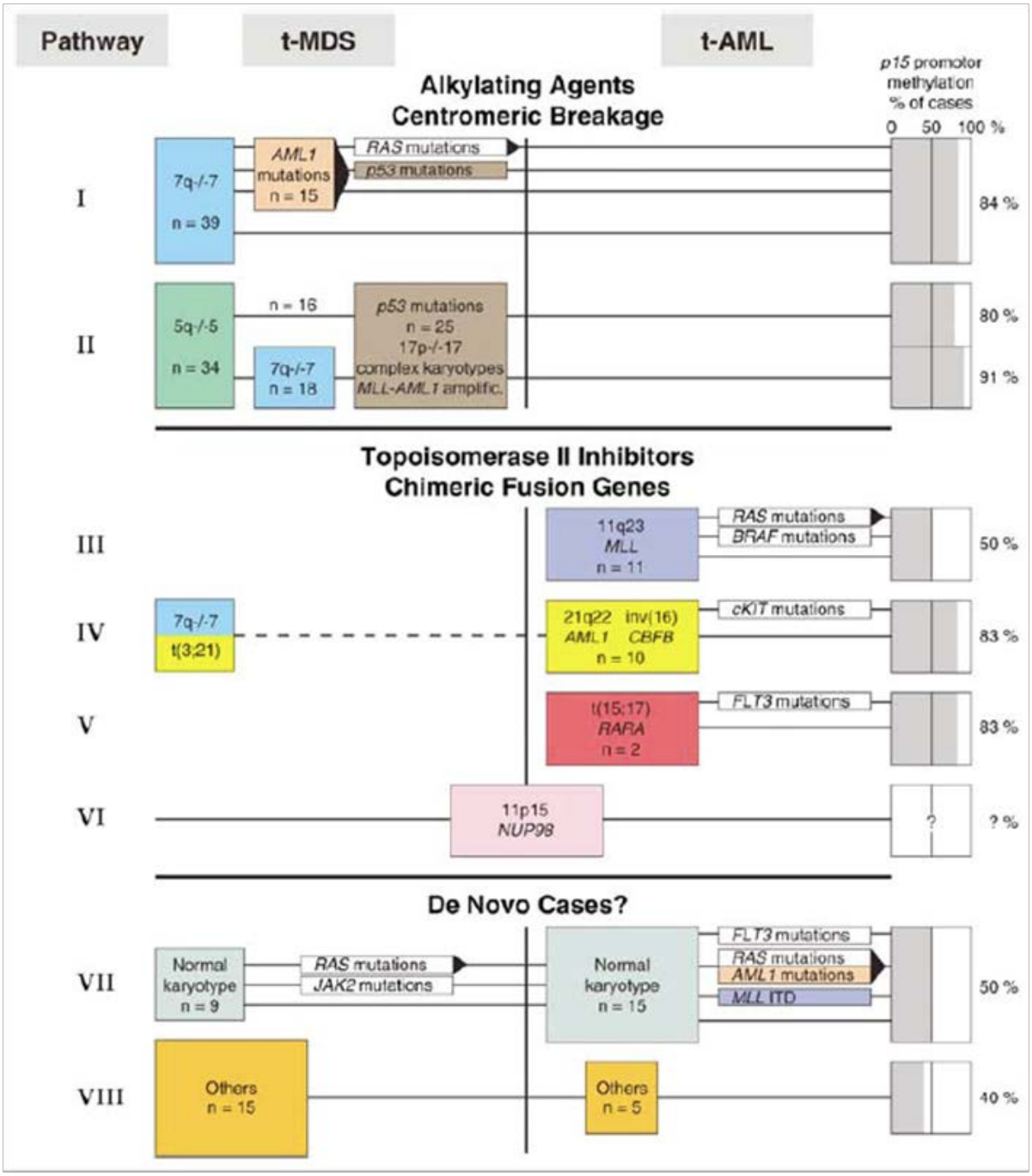

Figure 3. Genetic pathways of t-MDS and t-AML based on 140 cases from the Copenhagen study group. The black triangle indicates significant association with transformations from t-MDS to t-AML.

Source: Pedersen-Bjergaard et al. (2006). Reprinted by permission from Macmillan Publishers Ltd.

exhibited AML of the FAB subtypes M4 or M5. Three of the 11 patients in this group had a RAS mutation, and another 3 had a BRAF mutation. Methylation of the p15 promoter was seen in 50% of the patients.

Pathway IV occurred in patients with balanced translocations involving chromosome bands 21q22 or 16q22 leading to chimeric rearrangements involving the core binding factor genes AML1 or CBFB. These chromosomal changes are generally associated with previous treatment with topoisomerase II inhibitors, most commonly of the anthracycline class. While patients in this group with a t(3;21)(q26;q22) often presented as t-MDS, most of the others presented directly with t-AML. Chromosome 7 alterations were also seen in five of nine patients with 21q22 translocations. Point mutations in *KIT* (c-Kit) and PTPN11 were seen in a few patients. Methylation of the p15 promoter was seen in 83% of the patients.

Pathway V occurred in only two patients and is characterized by a translocation between chromosomes 15 and 17 involving the PML and RARA genes. Therapy-related promyelocytic leukemia (M3) that exhibits the characteristic t(15;17) has been reported to occur in patients treated with doxorubicin or mitoxanthrone. One of the patients in this group also had a mutation due to FLT3 internal tandem duplication.

Pathway VI is an uncommon pathway in which t-AML patients exhibit balanced translocations involving the NU98 gene at chromosome band 11p15. None of the 140 patients in the Copenhagen series exhibited this pattern.

Pathway VII exhibits a normal karyotype, and most often presents directly as t-AML. Approximately 17% of the patients in the Copenhagen series fell within this group and have not been consistently associated with any previous type of chemotherapy. The mutations seen are also commonly seen in de novo leukemias suggesting that these may represent sporadic cases of de novo leukemia occurring in these patients. Methylation of the p15 promoter was only seen in 50% of these patients.

Pathway VIII is composed of patients that exhibit unique or unusual chromosome alterations and represents 14% of the Copenhagen cohort. These cases do not show an association to any specific type of previous therapy and may also represent cases of de novo leukemia. Methylation of the p15 promoter was also uncommon, occurring in 40% of the cases.

Consistent with the description presented above, patients with t-AML with leukemic cells exhibiting loss of all or part of chromosomes 5 or 7, frequently present initially with t-MDS and

have often been previously treated with alkylating agent chemotherapeutic drugs. In contrast, t-AMLs exhibiting certain specific reciprocal translocations such as t(11q23) and t(21q22) occur in patients that were previously treated with a topoisomerase II-containing chemotherapeutic regimen and develop without a preceding MDS.

7. FACTORS CONFERRING AN INCREASED RISK OF INDUCED LEUKEMIA

7.1. MYELOSUPPRESSION

Myelosuppression and immunotoxicity frequently accompany exposure to leukemia-inducing agents, particularly those such as the chemotherapeutic drugs, ionizing radiation, and benzene, for which leukemogenicity has been clearly established (Ferguson and Pearson, 1996; Eastmond, 1997). The number of individuals affected and the magnitude of toxicity are influenced by the agent and dose-related factors such as total dose, dose per treatment, schedule, and route of administration as well as individual host factors (age, genetic susceptibility, prior therapy, health status, etc.) (Gale, 1988). A brief overview of the myelosuppressive effects of cancer therapeutic drugs has been written by Gale (1988) and is the basis for the following description. The severity of myelotoxicity induced by antineoplastic drugs varies considerably by chemical class. For drugs associated with t-AML, moderate-to-severe effects are generally seen. Epipodophyllotoxins, cisplatin, and procarbazine typically produce more moderate toxicity whereas severe effects are more common for the alkylating agents, the anthracyclines, and the nitrosoureas.

The period between dosing and the onset or appearance of the myelosuppression is also related to the class of agent. For some agents such as ionizing radiation, the onset of myelotoxicity occurs within 0 to 48 hours after exposure. For others, longer periods are required. The onset of myelosuppression by the alkylating agents and anthracyclines occurs 1 to 3 weeks following exposure and is believed to be due to the effect of these agents on immature hematopoietic cells that becomes more evident as the more mature blood cells die and require replacement. Myelosuppression induced by the nitrosoureas and mitomycin C is less frequent and occurs 4 to 8 weeks after treatment. This delayed effect is believed to be due to a relatively selective effect on immature stem cells. However, the onset of this delayed effect is dose-dependent. A two- to threefold increase in dose reduces the onset of myelotoxicity to 1 week. For some drugs such as busulfan, the manifestation of the myelotoxic effects is considered to be latent and may only be manifested under stress-related conditions.

In most cases, the induced myelotoxicity is transient with the blood cell counts returning to normal following the cessation of treatment or exposure (Hendry and Feng-Tong, 1995; Irons et al., 2005). However, in some cases, the induced myelosuppression can be more persistent and

progress to pancytopenia or infrequently to aplastic anemia, a condition that confers a much greater risk of developing leukemia (~10%) (Aksoy et al.,1984; Jandl, 1987; Ohara et al., 1997; Imashuku et al., 2003). Increased risks have also been seen for those who have previously exhibited less severe forms of bone marrow toxicity. Studies of benzene-exposed workers have shown that the leukemia mortality rate was much higher for workers who had previously been diagnosed with bone marrow poisoning (700 per 10^6 person-years) as compared to those exposed to, but not poisoned by, benzene (14 per 10^6 person-years), and particularly as compared to the general population (2 per 10^6 person-years) (Yin and Li, 1994). In one report, 36% of the benzene leukemia cases had a history of benzene poisoning with leukopenia or pancytopenia (Yin et al., 1994). However, this also indicates that for most cases, clinically detectable myelotoxicity (e.g., leucopenia or pancytopenia) may not be observed. It should also be noted that at lower exposure levels, the decreases in cell counts occurring in exposed groups may fall within what is considered the normal clinical range (Qu et al., 2002; Lan et al., 2004). This highlights one of the challenges in using myelotoxicity as a biomarker, as the normal range varies considerably in adults. For example, the mean white blood cell count in adults is $7{,}200 \times 10^3/\mu L$ with a 95% range from 3,900 to $10{,}900 \times 10^3/\mu L$ (Jandl, 1996).

7.2. GENETIC POLYMORPHISMS

Inherited polymorphisms in genes involved in xenobiotic metabolism and other cellular processes have been associated with increased risks of myelotoxicity or leukemia in numerous studies of patients or workers exposed to leukemogenic agents. In some instances, similar associations have been seen in follow-up studies by other investigators. However, in a significant number of cases, the results have either not been repeated or have not been reproducible. This is likely due to the limited nature of the studies that have been conducted to date. Consequently, it is difficult to make firm conclusions about many of the reported polymorphisms. Several recent reviews on polymorphisms and leukemia have been published that the reader may want to refer to for further details (Cheok and Evans, 2006; Cheok et al., 2006; Sinnett et al., 2006; Seedhouse and Russell, 2007). The following is a brief overview of some of the genetic polymorphisms involved in DNA metabolism, repair, and xenobiotic metabolism that have been repeatedly associated with altered risks of developing leukemia, particularly induced acute myeloid leukemias.

Because most leukemogens are genotoxic and exert their effects by damaging DNA, the DNA repair capacity of the bone marrow is likely to have a significant influence on the risk of t-AML. Indeed, individuals with inherited deficiencies in enzymes that are involved in DNA repair such as ataxia telangiectasia, Bloom syndrome, and Fanconi anemia are at significantly higher risk for developing leukemia and other lymphohematopoietic cancers (Segel and Lichtman, 2004). The associated risks can to be very high with up to 25% of the affected individuals developing leukemia/lymphoma during their lifetime. Individuals with these and other uncommon genetic syndromes such as neurofibromatosis 1 are also at an increased risk of t-AML following treatment with radiation, alkylating agents, and/or topoisomerase II inhibitors (Maris et al., 1997; Seedhouse and Russell, 2007).

Genetic polymorphisms in DNA repair genes that occur more frequently in the population can also confer an increased risk of t-AML. Defective mismatch repair is often manifested by microsatellite instability in cancer cells. Microsatellite instability is frequently seen in leukemias, particularly in t-AML, where it has been reported to occur in ~50% of the cases (Karran et al., 2003; Seedhouse and Russell, 2007). In contrast, less than 5% of de novo leukemias exhibit microsatellite instability. While the basis for this instability is still unknown, factors that may contribute are deficiencies in DNA-mismatch repair enzymes. In a fairly recent report, the hMSH2 mismatch repair variant was shown to be significantly overrepresented in t-AML patients that had previously been treated with 0^6-guanine-forming alkylating agents including cyclophosphamide and procarbazine, as compared with controls (Worrillow et al., 2003). Similarly, polymorphisms in genes involved in DNA repair (e.g., WRN, TP53, hOGG1, XRCC1, ERCC3, and BRCA2) have been reported by a number of investigators to be associated with a decrease in white blood cell counts or an increase in chromosomal damage in benzene-exposed workers (Shen et al., 2006; Kim et al., 2008; Wu et al., 2008; Hosgood et al., 2009; Sun et al., 2009).

Polymorphisms affecting DNA metabolism have also been reported to confer an increased risk of t-AML. Thiopurine methyltransferase catalyzes the *S*-methylation of thiopurine medications such as 6-mercaptopurine and 6-thioguanine, which are commonly used as chemotherapeutic agents. As summarized from Cheok and Evans (2006), the thiopurine methyltransferase pathway is the primary mechanism for inactivation of thiopurines in hematopoietic tissues. The link between thiopurine methyltransferase polymorphisms and

mercaptopurine toxicity has been extensively investigated, and studies have shown a strong relationship between thiopurine methyltransferase deficiency polymorphisms and hematopoietic toxicity. Three variant alleles are responsible for >95% of the cases with low or intermediate thiopurine methyltransferase activity. Patients homozygous or heterozygous for the low activity alleles are inefficient at detoxifying the mercaptopurines and accumulate high concentrations in their hematopoietic tissues. If their administered doses are not modified, they are at high risk for severe hematopoietic toxicity. Inherited thiopurine methyltransferase deficiency has also been associated with a higher risk of t-AML, particularly in ALL patients treated with topoisomerase II inhibitors (Thomsen et al., 1999; Gadner et al., 2006). It has been postulated that the increased risk may be due to an interference of 6-thioguanine or methylated 6-mercaptopurine with DNA repair after DNA damage has been induced by other chemotherapeutic agents.

The influence of polymorphisms in other xenobiotic metabolizing genes on the incidence of leukemia has been the subject of many investigations (Cheok et al., 2006; Leone et al., 2007; Seedhouse and Russell, 2007; Guillem and Tormo, 2008; Leone et al., 2010). In many cases, there was no difference between the frequencies seen in t-AML patients as compared to de novo leukemia patients or a control cohort. A number of studies have indicated that individuals with genes coding for nonfunctional or less active copies of various glutathione-*S*-transferases, CYP3A4, and NADPH quinone oxidoreductase 1 (NQO1) have an increased risk for developing t-AML following chemotherapy. However, these results have not consistently been seen. The relationship between NQO1 and leukemia is presented below as an example. However, it should be noted that these genes may have other important cellular functions in addition to their roles in xenobiotic metabolism. For example, NQO1 has recently been reported to also influence cellular signaling in the bone marrow niche (Ross et al., 2010).

Polymorphisms in NQO1, an enzyme involved with the reduction of quinones and protection against oxidative stress, have been repeatedly associated with the development of leukemia. Studies have reported that an inactivating polymorphism in NQO1 (the C609T, a functionally null variant) was overrepresented in patients with t-AML (Larson et al., 1999), in those with de novo AML (particularly those with translocations or an inv [16] clonal aberration [Smith et al., 2001]), in infant leukemias with a 11q23 karyotype, and in infants and children with the t(4;11) form of ALL (Wiemels et al., 1999; Smith et al., 2002). While these initial studies indicated a consistent association with a number of different leukemia types, more recent

studies have been less consistent with many not showing an association (Blanco et al., 2002; Sirma et al., 2004; Eguchi-Ishimae et al., 2005; Malik et al., 2006).

Genetic polymorphisms affecting NQO1 have also been associated with increased myelotoxicity and may confer an increased risk of leukemia. For example, benzene-exposed individuals who were rapid CYP2E1 metabolizers and had the C609T null variant for NQO1 had a 7.6-fold increased risk of benzene poisoning as compared to exposed individuals with the slow CYP2E1 metabolizer phenotype who had one or two of the wild type NQO1 alleles (Rothman et al., 1997). In another study by this research group, different NQO1 polymorphisms, as well as a polymorphism in myeloperoxidase, an enzyme implicated in the bioactivation of benzene's quinone metabolites in the bone marrow, were associated with lower blood cell counts in benzene-exposed workers (Lan et al., 2004). A similar association between NQO1 and chromosomal damage in the peripheral blood lymphocytes of workers exposed to benzene (as well as other potentially confounding chemicals) was also recently reported by another research group (Kim et al., 2008).

8. RISK ASSESSMENT IMPLICATIONS

As indicated above, approximately 25% of the agents established by IARC as human carcinogens induce lymphohematopoietic cancers, and approximately 22% are associated with the induction of leukemia, primarily AML. These agents have a wide variety of uses, and given their established carcinogenic effects, they have been the focus of many risk assessments and safety evaluations. The sections below focus on how mechanistic information on these carcinogenic agents can be used to inform risk assessment decisions. The following discussion is not intended to be comprehensive but rather will focus on some key issues related to contemporary risk assessment discussions.

8.1. HAZARD IDENTIFICATION

Hazard identification is the process by which hazardous substances—carcinogenic agents in this case—are identified and characterized. According to the IARC Preamble (IARC, 2008d), which describes the IARC groupings and the weight-of-evidence decision-making process, the Group 1 category "is used when there is *Sufficient Evidence of Carcinogenicity* in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than *sufficient* but there is *Sufficient Evidence of Carcinogenicity* in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity."

The Group 2A agents represent chemicals that are probable human carcinogens for which there "is *Limited Evidence of Carcinogenicity* in humans and *Sufficient Evidence of Carcinogenicity* in experimental animals. In some cases, an agent may be classified in this category when there is *Inadequate Evidence of Carcinogenicity* in humans and *Sufficient Evidence of Carcinogenicity* in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent may be classified in this category solely on the basis of *Limited Evidence of Carcinogenicity* in humans. An agent may be assigned to this category if it clearly belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A." Most of the Group 2A agents listed in Table 8 have mechanisms of action such as mutagenicity that are commonly associated with carcinogenesis. However, they

have not been adequately studied in humans, or for many of the chemotherapeutic agents, they were administered in combination therapy often with other carcinogenic agents so that the carcinogenic effects for the agent being investigated cannot be separately identified.

For most of the evaluations, studies of cancer in humans and animals have played the primary role in the IARC classification. In the past, information on the mechanism of action has typically only played a supportive role. More recently, however, the role of mechanism of action has increased such that insight into human relevance of observable mechanisms is more frequently used to reduce or elevate a carcinogenicity hazard characterization. As a result, it is reasonable to examine information on the mechanisms of action of the Group 1 and 2A carcinogens to help identify carcinogens and improve the hazard identification process. Below are several observations about how mechanistic information has and may be used in hazard identification for leukemia-inducing agents.

8.1.1. Utility of Short-Term Genotoxicity Tests and Human Biomonitoring

As seen in Table 8, most of the agents identified by IARC as human carcinogens are likely to act through a mutagenic or genotoxic mechanisms. The majority of the Group 1 and 2A carcinogens are alkylating agents, but other classes of genotoxic agents such as topoisomerase II inhibitors or nucleotide analogs are also present. The majority of the Group 1 and 2A leukemia- and lymphoma-inducing agents are active in short-term tests both in vitro (with metabolic activation as needed) and in vivo (IARC, 1987a). This is not surprising given their likely mechanisms of action. The exceptions to this tend to be the infectious agents and the immunosuppressive agents that act through nongenotoxic or indirect genotoxic mechanisms of action.

As also shown in Table 7, most of the leukemia-inducing agents have been shown to induce chromosomal aberrations or micronuclei in the peripheral blood lymphocytes of exposed humans and animals. There is increasing evidence that elevated frequencies of structural chromosomal aberrations and micronuclei in human lymphocytes can serve as predictive indicators (biomarkers) of cancer risk (Hagmar et al., 1998; Liou et al., 1999; Smerhovsky et al., 2001; Hagmar et al., 2004; Boffetta et al., 2007; Bonassi et al., 2007; Bonassi et al., 2008). In the recent Bonassi et al. (2008) study, the association between the frequency of chromosomal aberrations and the risk of lymphohematopoietic neoplasia, while elevated, did not achieve

statistical significance. The agents listed in Table 7 also cause significant bone marrow toxicity with noticeable decreases in blood cell counts of the exposed individuals. There are a few Group 1 agents that are exceptions such as ethylene oxide and 1,3-butadiene and do not appear to manifest these particular patterns. In summary, while hematotoxicity can be induced by most of the Group 1 and 2A leukemogens, this is not uniformly the case and clinically detectable hematotoxity does not need to occur in order for an agent to be classified as a human leukemogen.

8.1.2. Usefulness of Animal Bioassays

There is substantial evidence to indicate that chronic animal bioassays are effective in detecting human carcinogens. As described in the IARC Preamble (IARC, 2008d), "all known human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species." However, as further noted in the Preamble, "although this association cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, it is biologically plausible that agents for which there are *Sufficient Evidence of Carcinogenicity* in experimental animals also present a carcinogenic hazard to humans." Accordingly, the vast majority of the Group 1 leukemia- and lymphoma-inducing agents shown in Table 7 have been reported previously to be carcinogenic in rodent bioassays (Eastmond, 1997). Indeed, many of them have been shown to induce leukemias or lymphomas in mice and in rats. Most of these have been shown to occur in mice and were T-cell leukemias or lymphomas, rather than the ANLL, which is prevalent in humans. As a result, there appears to be fundamental differences between the types of lymphohematopoietic tumors induced in humans and rodents. The reason for this remains unknown but may be related to species differences in hematopoiesis and/or immune surveillance (Eastmond, 1997).

The good correlation seen for animals and humans mentioned above was based primarily on the results for chemical carcinogens and probably does not hold for many of the infectious agents, which are likely to only infect humans and closely related species. In addition, it is not clear at this point if this correlation will hold true for the topoisomerase II inhibitors, as most of them have not been adequately tested in experimental animals. While the fusion gene products have clearly been shown to affect hematopoiesis and can cause leukemias in mice (Corral et al.,

1996; Dobson et al., 1999; Lavau et al., 2000; Forster et al., 2003; So et al., 2003), it is not certain if the genes are located in regions in the rodent genomes that will allow the same chimeric genes to be formed. It should also be noted that the Group 2A carcinogens are also positive in rodent bioassays; however, this is to be expected, as this characteristic is one of the primary reasons that the chemicals have been listed.

8.1.3. Combining Different Types of Lymphohematopoietic Cancers for Analysis

In evaluating epidemiological studies, a decision has to be made about which specific types of disease should be combined for the analysis. Often the reporting of leukemias has been grouped into the four common categories of AML, CML, ALL, and CLL. Similarly, the lymphomas and myelomas have been grouped into NHL (lymphosarcoma, reticulosarcoma, and other malignant neoplasms of lymphoid and histocytic tissue), Hodgkin disease, and multiple myeloma (MM). More recent case-control studies of lymphoma have expanded the NHL grouping to include CLL, or to group lymphomas by cell type or by specific International Classification of Disease-Oncology category (e.g., diffuse lymphatic B-cell lymphoma, follicular lymphoma, etc.). However, many different types of groupings have been used in analyzing epidemiological data, primarily reflecting the classification system in use at the time of diagnosis or cause of death. An illustration of the various types of groupings used for studies of benzene has been described by Savitz and Andrews, 1997. In addition, evaluations of the induction of lymphohematopoietic diseases by an agent over time can be challenging due to changes in diagnosis, names of diseases, and classification schemes, as well as lack of detailed case and exposure information. Other issues such as misclassification errors and insufficient information on death certificates can also influence the outcome of the studies. The decision about how to analyze the data can also have a significant influence on the outcome of the analysis and the conclusions of the study.

As illustrated in Tables 1−3, there are a large number of distinct lymphohematopoietic cancers. By analyzing each separately, a study would only have the power to detect cancers that were very strongly associated with exposure to an agent, if detection was even possible. In addition, the large number of comparisons could lead to some associations being labeled as significant due simply to random chance. As a result, it is common to combine specific lymphohematopoietic cancers for analysis. Indeed, due to the low incidence of individual

lymphohematopoietic cancers, it is often necessary to combine subtypes to achieve the statistical power to detect exposure-related increases in disease. The key question, which has not fully been answered, is "Which categories of lymphohematopoietic cancer should be combined for analysis?" In theory, only the relevant disease entities would be combined for analysis. This might include combining specific types of hematopoietic cancer that share an underlying mechanism, that originate from a common cell, or that share key biological (morphologic, genetic, immunologic, etc.) features. However, in practice this becomes challenging, because all myeloid and lymphoid cells originate in the hematopoietic stem cells of the bone marrow, and it is often not clear at what stage in maturation the critical genetic and epigenetic events occurred. In addition, the delineation between myeloid and lymphoid lineages and the separation between lymphoid leukemias and lymphomas are not as distinct or dichotomous as once thought (WHO, 2008; IARC, 2008c; Doulatov et al., 2010; Dorshkind, 2010; Kawamoto et al., 2010).

The value of combining uncommon lymphohematopoietic cancers has been demonstrated for benzene (Savitz and Andrews, 1996, 1997). Benzene exposure is strongly associated with AML. However, its association with lymphoid cancers has been a source of ongoing discussion. Savitz and Andrews (1996, 1997) showed that by analyzing non-AML hematopoietic cancers together, a significant association between benzene exposure and these cancers could also be shown. IARC, in its most recent evaluation, has given more credence to an association between benzene and other lymphohematopoietic cancers as it concluded that there was evidence, albeit limited, for an association between benzene and ALL, CLL, NHL, and multiple myeloma (Baan et al., 2009; IARC, 2009). More recently, a similar association between benzene exposure and an increased incidence of lymphoid cancers (MM, ALL, and CLL) was seen in a metaanalysis of occupational benzene studies (Vlaanderen et al., 2011). These results suggest that the critical events induced by benzene that result in the development of leukemias and lymphomas occur either in the bone marrow hematopoietic stem cell and/or progenitor cells that give rise to both the myeloid and lymphoid lineages or that benzene can target both the hematopoietic stem and progenitor cells as well as the more mature lymphoid cells.

In addition to combining specific types of cancers for analysis during individual studies, it may be informative to see how various authoritative bodies such as IARC, the National Academies of Sciences, Biological Effects of Ionizing Radiation committee, and the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) have combined

the lymphohematopoietic cancers in making decisions about risk or for weight of evidence determinations for various leukemogenic agents. As indicated above for benzene, IARC chose to evaluate the major types of cancer separately. However, it is likely that the association between benzene and one type of cancer provided supportive evidence that benzene could be associated with another type of lymphohematopoietic cancer. This type of supporting evidence appears to have played an important role in the evaluations of butadiene where associations with CML, CLL, and to some degree, NHL, were combined in concluding that butadiene exposure caused cancer of the "haematolymphatic organs." Similarly, information on associations with different types of lymphohematopoietic cancers contributed to the recent IARC evaluation on formaldehyde (AML and CML) and ethylene oxide (NHL, MM, and CLL). It should be noted that for butadiene and probably benzene, supportive evidence came from associations with both myeloid and lymphoid tumors.

In evaluations of ionizing radiation, it has been common to use all leukemias or combine the results for ALL, AML, and CML in modeling the data and assessing cancer risks (UNSCEAR, 2000a, b; NRC, 2006 and references therein). Increases in CLL have not been seen in radiation-exposed study groups. When presenting the study results, the radiation-induced leukemias are frequently described in various documents as simply leukemias or non-CLL leukemias.

In contrast, in its recent review of U.S. Environmental Protection Agency's (EPAs) draft Integrated Risk Information System risk assessment for formaldehyde, a National Research Council review committee concluded that the broad grouping of "all lymphohematopoietic cancers" included at least 14 biologically distinct diagnoses in humans and should not be used in determinations of causality (NRC, 2011). The committee recommended that the EPA focus on the most specific diagnoses available in the epidemiological data.

Thus, as illustrated in the evaluation of the various cancer-inducing agents by the different authoritative groups, there is a range of opinions and no strict consensus on how specific lymphohematopoietic cancers should be combined for analysis and weight-of-evidence determinations. As discussed above, a variety of combinations including those combining acute and chronic myeloid neoplasms as well as lymphoid and myeloid neoplasms have been used in recent evaluations. With an increased understanding of the biology and mechanisms underlying

de novo and induced lymphohematopoietic cancers, a more accurate identification of the appropriate groupings for these cancers should be achievable.

8.1.4. Potential Influence of Latency Period in Identifying Leukemogens

The period between the initial exposure to a carcinogenic agent and the onset of cancer is known as the latency period. Latency periods for acute myeloid leukemias induced by chemotherapeutic agents tend to be much shorter than those seen for other induced cancers such as lung cancer induced by tobacco smoke, which has a median latency period of approximately 30 years (Weiss, 1997). As indicated above, the latency period for topoisomerase II inhibitors is quite short with median latency periods of 2−3 years. For radiation and chemical chemotherapy agents, the induced leukemias first appear 1−2 years after the beginning of treatment. The median incidence peaks at about 4−7 years, and by 10−15 years after the beginning of treatment; the incidence has frequently declined to control or near control levels (Casciato and Scott, 1979; Pedersen-Bjergaard et al., 1987; Kaldor et al., 1990; Davies, 2000; Schonfeld et al., 2006). This indicates that for leukemogens such as the chemotherapeutic alkylating agents, the risk of developing t-AML will most likely peak at ~4−7 years after the beginning of exposure and will begin to decline with additional follow-up. Continuing follow-up for many years after treatment would be expected to significantly reduce associations between exposure and leukemia and could mask risks that might be present and detectable at earlier times. Hence, adding person-years after exposure ends or is substantially reduced, as sometimes occurs in occupational studies, may significantly weaken true associations between exposure and the incidence of leukemia. The influence of latency or time since first exposure has been clearly demonstrated for benzene (Rinsky et al., 2002; Silver et al., 2002; Triebig, 2010) and has been postulated to have had an influence on recent formaldehyde results (Beane Freeman et al., 2009). Failure to account for latency period for weak carcinogens or under conditions of modest exposure for potent carcinogens could reduce the association so that the agent or exposure would no longer be considered carcinogenic. Two notes of caution are warranted: (1) The described latency periods have typically been seen with high doses of the leukemia-inducing agents. There is evidence that the latency period can be significantly influenced by the administered dose with higher doses producing shorter latency periods and lower doses producing longer latency periods (Cadman et al., 1977); (2) It should be emphasized that while latency periods are often reported as the

median or average number of years since first exposure, induced leukemias can occur many years after the reported median latency periods, and many years after exposure has ended (see Triebig, 2010 for examples). Similarly, treatment-related lymphomas have been reported to occur many years following radio- and/or chemotherapy with latency periods that are generally longer than those reported for t-AML (Krishnan and Morgan, 2007).

8.1.5. Metabolism and Bioactive Dose at the Target Organ

One of the fundamental principles of toxicology is that the toxicological response is related to the dose of the bioactive agent in the target tissue. However, the tissue dose of the bioactive agent may differ significantly from the external exposure dose. For many carcinogens, even if exposure occurs, the chemical may not arrive at the target organ in a form that enables it to exert a toxic or mutagenic effect due to pharmacokinetic or metabolic reasons. In fact, this is a fundamental difference between the risk assessment of ionizing radiation and chemical leukemogens. Because of its ability to directly penetrate tissues, radiation risk estimates are almost always based on the dose of radiation that reaches the bone marrow. In contrast, risks for chemical leukemogens such as benzene, butadiene, or ethylene oxide are typically based on the exposure dose and may not accurately reflect the bioactive dose that reaches the bone marrow.

Studies have also shown that pharmacokinetic and metabolic factors can significantly influence the risks from leukemia-inducing agents. For example, the peak plasma concentrations of melphalan have been shown to vary by over 50-fold after oral dosing due to variability in absorption, first pass metabolism, and rapid hydrolysis (GlaxoSmithKline, 2007). Indeed, for a few patients, detectable plasma levels of melphalan were not seen even after the administration of a high dose (1.5 mg/kg) of the drug (Choi et al., 1989). The differences in plasma concentration would undoubtedly have an effect on its efficacy as well as its risk of inducing t-AML.

Metabolism has also been shown to exert a major effect on the mutagenic and carcinogenic risks of leukemia-inducing agents. For example, metabolic activation by the cytochrome P450 enzymes (CYP450) has been shown to play an important role in the toxic and genotoxic effects of benzene and cyclophosphamide (Snyder, 2004; Rooney et al., 2004). Other xenobiotic metabolizing enzymes such as the epoxide hydrolase, myeloperoxidase, glutathione transferases, and other detoxification enzymes, as well as efficient repair of the induced adducts,

may also influence the toxicity and carcinogenicity of leukemogenic agents. In addition, population variation in metabolism may also have contributed to the mixed results reported in epidemiological studies (Doughety et al., 2008).

8.1.6. DNA-Adduct Type, Metabolism, and Repair

The type of DNA adducts formed and their repair can have a major impact on the toxicity, mutagenicity, and probably the cancer risk of leukemia-inducing agents. As indicated earlier, three major classes of alkylating agents have been identified that have significantly different mutagenic and carcinogenic potencies (Vogel et al., 1998). Category 1 agents are mono-functional alkylating agents such as ethylene oxide and methyl methane sulfonate, which primarily react at the N7 and N3 moieties of purines in DNA. These adducts are efficiently repaired, and as a result, these agents tend to be relatively weak mutagens. Category 2 agents such as procarbazine and ENU induce *O*-alkyl adducts and *N*-alkyl adducts in DNA, which are often involved in base pairing and are slowly repaired. These agents are generally potent mutagens and carcinogens. Category 3 agents, such as melphalan and busulfan induce DNA breaks and structural aberrations through their ability to cross-link DNA (Vogel et al., 1998). They are highly toxic, mutagenic, and carcinogenic. For example, butadiene can be metabolized to diepoxybutane, a cross linking agent, so it has been classified as a Category 3 agent. However, its potency for rats and probably humans has been reported to be much lower than the other Category 3 agents. This might be due to the need for two separate bioactivation steps to form diepoxybutane as indicated above as well as the nature of the adducts formed by butadiene. The butadiene crosslinks tend to be intrastrand and are more efficiently repaired. Consistent with this explanation is the fact that diepoxybutane is believed to be the reactive intermediate formed from treosulfan, a chemotherapeutic agent that appears to be somewhat less potent in inducing t-AML in humans than other Category 3 chemotherapeutic agents when considered on a mg/kg-basis (Kaldor et al., 1990). Interestingly, it should be noted that treosulfan administration to humans has been associated with AML whereas, as described above, occupational exposure to 1,3-butadiene has been associated with CLL and CML. One possible explanation is that the epoxybutene metabolite may play a more important role than diepoxybutane in inducing the butadiene-related leukemias in humans. It should be noted,

however, that there is also considerable overlap in the carcinogenic potency of chemicals in each of the three categories.

8.1.7. Age-Related and Individual Susceptibility

Many factors such as age, sex, genetic composition, and general health status have been shown to influence susceptibility to leukemia-inducing agents. For example, age has been shown to be a factor in susceptibility to radiation-induced leukemia. Studies of the atomic bomb survivors, who were exposed to both gamma, and to a lesser extent, neutron radiation, indicated that the highest leukemia risks were seen in children (Preston et al., 1994, 2004). The leukemia risk also decreased more rapidly in children as compared to those in older age groups (Preston et al., 1994). Interestingly, a similar increased susceptibility has not been seen for children treated with alkylating agent-based chemotherapy as their leukemia risks do not appear to be greater than those seen in adults, and in some cases, may be less (Levine and Bloomfield, 1992; Pyatt et al., 2005, 2007). However, direct comparisons are difficult because in most studies, the children and adults have been treated with different therapeutic regimens. For the nontherapeutic classes of leukemia-inducing agents, little is known about the relative susceptibility of children to leukemia, as the critical studies have almost always been conducted on adults. However, a number of studies on related biomarkers have indicated that children or adolescents are also susceptible to the toxic and genotoxic effects of these leukemogenic chemicals (Aksoy et al., 1974; Aksoy, 1988; Niazi and Fleming, 1989; Neri et al., 2006).

Genetic polymorphisms in genes coding for xenobiotic metabolizing enzymes, DNA-metabolizing or repair enzymes, and drug transporter proteins are other factors that have been implicated as significantly influencing an individual's risk of developing leukemia when exposed to genotoxic agents. A considerable number of studies have been conducted to identify enzymes involved in the bioactivation and the inactivation of carcinogenic agents (for reviews, see Cheoket al., 2006; Leone et al.,2007, 2010, and Guillem and Tormo,2008). Individuals with genes coding for nonfunctional or less active copies of various glutathione-*S*-transferases, CYP3A4, and NQO1 have been reported to be at increased risk for developing t-AML following chemotherapy. Similar increased risks have been seen for individuals that lack efficient DNA-metabolizing or repair enzymes such as enzymes involved in nucleotide excision repair or mismatch repair. However, further research in this area is needed as similar associations for the

metabolizing enzymes and DNA-repair enzymes have not been seen in other studies. As discussed by Lutz and colleagues, from a population risk perspective, the increased susceptibility associated with the various genetic polymorphisms can have a significant impact on cancer risks, particularly among those exposed to lower doses (Lutz, 1990, 2001).

8.1.8. Summary

As reported in Table 8 and from the above discussion, leukemia-inducing agents act through different mechanisms to induce their carcinogenic effects. While most of these have been determined by IARC to likely act through mutagenic or genotoxic mechanisms, different leukemogens have different potencies and different associated risks, which appear to be significantly influenced by the specific mechanisms involved. Even among the alkylating agents, different chemicals can have different potencies that are likely due to the nature of the DNA adducts formed and their repair as well as metabolic and pharmacokinetic factors. In addition, polymorphisms in genes related to xenobiotic metabolism and DNA repair can lead to increased susceptibility among groups in the population. Furthermore, identifying the specific types of cancer-causing agents with their associated mechanism and using that information to inform key steps in the risk assessment process remains one of the ongoing challenges.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

This report on "*Lymphohematopoietic Cancers Induced by Chemicals and Other Agents: Overview and Implications for Risk Assessment"* has undergone an external peer review performed by expert scientists in accordance with the EPA guidance on peer review (U.S. EPA, 2006). The external peer reviewers were tasked with providing written answers regarding their general impression of the document as well as specific charge questions. A summary of significant comments made by the external peer reviewers and EPA's response to these comments arranged by charge question follow. In many cases, the comments of the individual reviewers have been synthesized and paraphrased in the development of Appendix A. The public comments and responses have been summarized in the subsequent section following the external peer review comments and disposition.

EPA determined not to include Section 8, examples of specific environmental agents inducing leukemia, because of ongoing chemical assessments (ethylene oxide and formaldehyde) and previously completed assessment (1,3-butadiene). Therefore, any external reviewer's or public comments on these chemical agents as well as melphalan are not addressed in this final revised report.

A.1. EXTERNAL PEER REVIEW COMMENTS

The reviewers have made editorial and sentence modification suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further. When the external peer reviewers have similar comments on multiple charge questions, the comments were organized under the most appropriate charge question.

A.1.1. General Comments

Comments: Reviewers considered the overall report to be of excellent quality, well-written, well organized, comprehensive, thoughtful, and insightful and of exceptional accuracy. The reviewers commented that the clarity of presentation was excellent and the document had compiled a huge amount of epidemiological and mechanistic information and synthesized into a comprehensible and succinct framework. The reviewers also thought that the report addressed an important question on how mechanistic information could be used in the assessment of risk for lymphohematopoietic cancers. One reviewer had a concern that there was little discussion of *de novo* myelodysplastic syndromes (MDS) which are an increasingly prevalent type of hematopoietic cancer.

Response: EPA thanks the reviewers for their constructive comments. With respect to the myelodysplastic syndrome, it should be noted that the focus of this document is on treatment-related MDS rather than de novo MDS. The discussion of t-MDS has been integrated with the t-AML discussion throughout the document (e.g., see Section 2, Tables 5 and 6, Figure 3).

A.1.2. Specific Charge Questions

1. **Evaluate and comment on the draft report organization, content, and clarity of the presentation. Please make specific suggestions on how to improve the draft report.**

   Comment: Most reviewers thought that the report was well organized and the contents in the report were clear, complete and the presentation was excellent. One reviewer suggested inclusion of a section on benzene as an example of a specific leukemia inducing chemical. This reviewer also suggested that the authors should consider data from both myeloid and lymphoid origins. A comment was made to differentiate individual susceptibility from age related susceptibility. A suggestion was also made for adding a table for easy reference which would summarize the DNA alkylating agent categorization scheme of Vogel and colleagues. Reorganization of text under Section 8 was also suggested. The same reviewer also commented that the report mostly focuses on leukemia and limited information was provided on lymphoma and that the mechanism of immunomodulation was not comprehensively described. Discussion of hematoxicity and other biological observations was suggested to be included in the report. One reviewer suggested that we delete Table 9. There were other minor editorial changes recommended.

   Response: The chemicals discussed for illustrative purposes were selected from among those that had been recently reviewed by the International Agency for Research on Cancer or similar authoritative body. Such a recent review was not available for benzene. However, as noted, benzene is used as an example in many places throughout the document. Sections 4 and 5 provide the background on leukemia and lymphoma-inducing agents and as such serve as introductions to Section 6. Additional information on the mechanisms underlying induced lymphomas to Section 4 is now included. In response to one of the reviewer's comment, rather than create a separate section on age-related susceptibility, EPA has changed the heading of Section 8.1.7 to note this difference.

   With respect to summarizing the DNA alkylating agent categorization, given that it would be difficult to include all of the relevant information in a table, EPA prefers not to include one. Furthermore, regarding comments on Section 8 of the external peer review draft (examples of Specific Leukemia-inducing Agents), Section 8 has not been included in the revised document so as to not have the appearance of certifying or further debating controversial topics associated with WHO hazard characterizations of the exampled agents. In reference to the comment on additional information on lymphomas, while there is an introductory material regarding lymphohematopoietic cancers in general, the primary emphasis of the document is on myeloid leukemias where most information on chemical etiology is available. The summary and introduction have been modified to emphasize this point. Similarly, with respect to addition of hematotoxicity, the document is intended as an overview, such a detailed discussion is outside the scope of this document.

2. **Within the goals of the draft report, evaluate and comment on the description of the types of induced lymphohematopoietic neoplasms, their characteristics and the**

mechanisms underlying their development as well as the general implications of the presented information for risk assessment.

Comment: Overall, the comments by the reviewers were "excellent description of the types of lymphohematopoietic neoplasms and defines to the average scientific reader and toxicologist the origins of these neoplasms and the characteristics and mechanisms that are similar as well as different in their development. Both the text and the figures that follow within the document are excellent, easy to read, and provide, in exceptional clarity". One reviewer appreciated the simplified scheme of hematopoietic neoplasm classification shown in Table 1. Another reviewer endorsed this report to be an excellent overview that would serve as a useful resource for use in the identification and risk assessment of chemicals with the potential to cause LHP cancer. Specific comments on this charge question included clarity on the issue of latency as it might be applied to risk assessment. Additional references were provided for inclusion in the document, a suggestion was made to elaborate on the implications of risk assessment as it pertains to chemical specific metabolism, DNA adduct type, metabolism and repair, discussion on DNA reactive metabolites of butadiene. One reviewer thought there was missing information on MDS.

Response: EPA thanks the peer reviewers for the positive and constructive comments. The section on latency has been revised in response to reviewers' comments. Information as well as references about other mechanisms that are likely to contribute to induced leukemogenesis has been added. The text has been modified to address the comment on risk assessment implications regarding certain chemical-specific metabolism, implications on DNA adduct type are addressed and information on other metabolism and repair has been addressed in other sections. The focus of this document is on treatment-related MDS rather than de novo MDS. The discussion of t-MDS has been integrated with the t-AML discussion throughout the document (e.g., Section 2, Tables 5 and 6, Figure 3). Other editorial changes recommended have been incorporated.

3. **Evaluate and comment on the quality and completeness of the information and literature discussed in the draft report. Please identify any additional relevant published information in the peer-reviewed literature, which may enhance the quality of the draft report. Please justify their inclusion in relevance to the goals of the draft report.**

Comment: Overall, the reviewers were impressed with the quality and completeness of information in the document. Reviewers thought that EPA had compiled a huge amount of data and literature into the document in an efficient and meaningful way, appropriately focused on the stated purpose and goals. Specific comments included additional description of bone marrow niches and stem cell cycling, potential influence of stroma and styromal factors on leukemic clones, consideration of stereochemistry in butadiene metabolism and relationship to DNA damage, evidence of systemic genotoxic effects of formaldehyde, genotoxic actions of LHP cancer-inducing agents, and discussion on lymphomas.

Response: Again, EPA thanks the reviewers for the positive and constructive comments. Specific comments are addressed in the revised document. For example, additional information on stem cell cycling has been added, although EPA believes that a detailed description of the bone marrow niche is outside the scope of this document; mention of styromal cells and factors has been added, additional explanation has been provided regarding why p53 does not easily fit into the classification scheme, text has been changed to address the polymorphism issue. A full discussion regarding the genotoxic actions of LHP cancer-inducing agents that don't seem to be myelotoxic and the implications of these data for indentifying chemicals with similar properties and actions is outside the scope of this document. Again, a sentence has been added to indicate the agents do not have to exhibit hematotoxicity in humans to be considered human leukemogens. A clarifying statement on lymphomas has been incorporated to specify this information. Suggested and appropriate references have been added and editorial comments have been considered.

4. **Considering the stated goals and objectives, and scope of the draft report, were the goals of the draft report met? If not, what specific recommendations would help meet the goals of the draft report?**

Comment: Most reviewers agreed that the report met the stated goals and objectives. They agreed that this was an excellent overview of what is know about the mechanisms of LHP carcinogenesis. One reviewer thought that the report falls short of making strong conclusions and recommendations.

Response: EPA believes that the strength of the recommendations and conclusions are appropriate for this type of "overview" report. This is not a guidance or policy report, but an overview of the existing literature.

A.2. PUBLIC COMMENTS

Several public comments were received during the public comment period including American Chemistry Council, International Institute of Synthetic Rubber Producers, Dr. Richard Albertini, and Sielken and Associated Consulting. Following the public comment period, the report was subject to external peer review including several experts in the field of leukemia, mode of action and general toxicology.

A.2.1. General Comments:

Comments: Most comments by the public focused on three specific chemical examples (1,3-butadiene, ethylene oxide and formaldehyde) discussed in Section 8 of the external peer review draft. Other comments included providing additional information and/or clarification on different physiological functions of many lymphoid and myeloid cells, mechanism of lymphoid malignancies, developmental differences between myeloid and lymphoid lineage, information quality act etc.

Response: EPA, when appropriate, has considered the public comments and made necessary changes to the document. EPA considers a detailed discussion of lymphopoiesis, full discussion of stem cell mobilization and homing, different

physiological functions of the many lymphoid and myeloid cells is outside the intended scope of this document. The objective of this document is to provide an overview of lymphohematopoietic cancer; in particular, the document focuses primarily on acute myeloid leukemias.

A.2.2. Specific Comments:

EPA has determined not to include Section 8 of the external peer review draft, i.e., examples of specific environmental agents inducing leukemia, because of ongoing chemical assessments (ethylene oxide and formaldehyde) and previously completed assessment (1,3-butadiene). Therefore, public comments on these chemical agents as well as melphalan are not addressed in this final revised report. Response to information quality act has been provided.

Comment: Detailed description of different types of physiological functions of the different cell types that lead to different leukemia endpoints was suggested. Furthermore, extensive information on origin of spontaneous/non-xenobiotic-induced leukemias was recommended. Grouping or combining various lymphohematopoietic subtypes was suggested.

Response: As indicated in the abstract and introduction, the objective of the document is to provide an overview of lymphohematopoietic cancers. In particular, the document focuses primarily on acute myeloid leukemias induced by chemical, and to a lesser degree, radiation, where the majority of information in induced leukemias is available. The abstract, the executive summary and the introduction have been modified to clarify this point. Correspondingly, a detailed description of the different physiological functions of the many lymphoid and myeloid cells is outside the intended scope of this document. Similarly, detailed information on the origin of spontaneous/nonxenobiotic-induced leukemias, both lymphoid and myeloid, where there is also extensive information, is outside the intended scope of the document. As described in the report, there is currently no consensus on how lymphohematopoietic cancers should be grouped. In specific instances, different authoritative bodies have used different groupings or have made different recommendations. In response to the public and peer reviewers' comments, EPA has made substantial changes to current Section 8.1.3 to discuss in more detail issues related to the combining various lymphohematopoietic subtypes. In addition, EPA has also added the recent 2011 National Research Council's recommendation on this subject with regard to formaldehyde.

Comment: A comment suggested considering lymphoid malignancies and its physiological mechanisms of DNA double-strand breaks, translocations and potential malignant transformation and to expand such information in the document.

Response: Recent information on the mechanisms involved in the development of induced leukemias is presented for all of the IARC Group 1 and Group 2A carcinogens in presented in Table 8. In addition, detailed information on specific classes of agents is presented in Sections 6−8. While there is a large database on the role of DNA

double-strand breaks, translocations and potential malignant transformation in spontaneous lymphoid malignancies or those of infectious origins, there is much less information on those induced by chemical agents. As indicated above, the primary focus of this document is on chemical-induced leukemias. Because of the paucity of mechanistic information on chemically induced lymphoid leukemias, this topic was not selected as an example in the document. As clearly documented in Table 8 of the document and discussed in the text, most leukemia-inducing agents induce AML through a mechanism involving genotoxicity and/or mutagenicity. In contrast, most lymphoma-inducing agents are immunomodulating and/or specifically target lymphoid cells. These agents are believed to act as either direct carcinogens acting on the cellular DNA, indirect carcinogens acting through chronic inflammation, or through immune-suppression. To supplement the information found in Table 8, additional information on these lymphoma-inducing agents has been added to Section 4.

Comment: A detailed discussion of developmental differences between myeloid and lymphoid lineage cells is recommended. Furthermore, a discussion of migration of pluripotent stem cells from the bone marrow to the blood is suggested. The public comment also suggested including description and discussion of the myelodysplastic syndrome and its relationship to myeloid leukemia. Detailed description of the genotoxic mechanism (MOA) of lymphohematopoietic malignancies is suggested. Furthermore, public comments suggest addressing the potential implications on the dose response phase of risk assessment for this MOA.

Response: While there is an introduction of lymphohematopoietic cancers in general, the primary emphasis of the document is on myeloid leukemias where the most information on chemical etiology is available. The summary and introduction have been modified to emphasize this point. A detailed discussion of lymphopoiesis, full discussion of stem cell mobilization and homing is outside the scope of this document. However, the discussion of mobilization has been modified to reflect the observation that only a small to very small percentage of the mobilized stem cells appear to return to the bone marrow. The focus of this document is on treatment-related MDS rather than de novo MDS. The discussion of t-MDS has been integrated with the t-AML discussion throughout the document (e.g., Section 2 and 6, Tables 5 and 6, Figure 3). As indicated previously, the primary focus of this document is on chemically induced acute myeloid leukemia. As shown in Table 8 and throughout the text, the vast majority of the IARC Group 1 leukemogens listed are believed to cause leukemia through a mechanism involving mutation. These can be chromosomal mutations or point mutations. As described in Sections 4−6, the major genetic changes in t-AML induced by chemotherapeutic alkylating agents are interstitial deletions of the long arms of chromosomes 5 and/or 7. These chromosomal mutations are likely the result of DNA alkylation by the alkylating agent leading to chromosome deletion rather than the result of endogenous processes. Only a small portion of t-AML exhibit reciprocal translocations and these are due to an inhibition of the enzyme topoisomerase II and are not likely to be the direct result of endogenous

processes. The focus of the Section 8 risk assessment discussion is on hazard identification and not dose-response assessment.

Comment: A comment on EPA report that certain sections were extracted from respective IARC monographs, thus resulting in misunderstandings of relevant literature was mentioned. This comment was particularly on a section that mentions ….. "a chemical that induces structural chromosome aberrations causes cancer". This comment was focused on publications by Bonassi et al. (2008).

Response: EPA appreciates the comment in bringing the recent Bonassi et al. (2008) paper to its attention. A citation to this paper as well as the descriptor "nonspecific" has been added to the discussion of chromosomal aberrations in Section 8.1. However, the Agency disagrees with the reviewer's interpretation of the ramifications of the Bonassi results. The Bonassi study indicated that the association between chromosomal aberrations and cancer was independent of the origin of the chromosomal aberrations. Whether the chromosomal aberrations were formed from exposure to a genotoxic agent or whether the aberrations were formed by genetic or endogenous factors, the risk is similar. As a result, the results of this study clearly support the association between exposure to genotoxic agents and cancer risk that is indicated in the document. In the Bonassi et al. (2008) article, the association between the frequency of chromosomal aberrations and lymphohematopoietic diseases, while elevated, did not achieve statistical significance. This is not surprising given the uncommon nature of the lymphohematopoietic cancers, the heterogeneity of exposures and the populations studied, and the imprecision in scoring chromosomal aberrations.

Comment: Use of rodent T-cell leukemias/lymphomas as evidence that a particular agent causes LHC cancer in humans while it is known fact that mice harbor viruses in these cells that themselves are leukemogenic was questioned. Also, a public comment suggested that EPA better define the statements that 'chemicals that cause leukemia or lymphomas in humans do so by a mutagenic mode of action'.

Response: In spite of the potential influence of murine viruses, T-cell leukemias and lymphomas induced by radiation and chemical agents in mice are considered by authoritative bodies to provide useful information for assessing the risk of chemical agents. The observed site concordance is mentioned in the document as being notable, but is not considered necessary for the animal data to be useful for risk assessment. Furthermore, the statements regarding the involvement of mutations or genotoxicity in the mechanism of action of the listed carcinogens is largely based on the conclusions of the recent IARC Volume 100 review. However, to avoid possible policy implications associated with using "mutagenic mode of action", EPA has changed the descriptions throughout the document. The basis for this conclusion with reference can be found in Table 8 of the document. As indicated above, the wording has been changed.

Comment: The final report should fully comport with the explicit information quality act (IQA) guidelines by assessing the best available science and using a weight-of-evidence approach as indicated in the OMB guidelines and EPA-issues IQA guidelines. The document should undergo independent scientific peer review.

Response: EPA has followed OMB Information quality Act Guidelines as well as EPA guidelines for ensuring and maximizing the quality, objectivity, utility and integrity of information. The objective of this report is to provide an overview of the types and mechanisms underlying the lymphohematopoietic cancers induced by chemical agents and radiation in humans, with a primary emphasis on acute myeloid leukemia and agents that induce this type of cancer. A weight of evidence approach has been used including giving the most weight to the peer-reviewed publications, evaluations and monographs which are consensus documents that have been written and reviewed by knowledgeable experts in the field. EPA conducted an independent peer-review of the external review draft and the peer-reviewers comments were considered and the revised final document reflects response to their comments (see Appendix A).

Comment: Extensive information on polymorphism in metabolic genes as its relevance for human susceptibility to butadiene was provided. Furthermore, information was provided on "estimating an upper bound on the added number of leukemia mortalities in 2010 possibly due to butadiene exposure to an ambient concentration of 0.6ppb rather than 0ppb"

Response: EPA thanks the public for the in depth analysis of mouse-human interspecies comparison and human intraspecies differences by genotypes and also quantitative risk assessment information for butadiene exposure. The information provided is of interest, though beyond the scope of this document.

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