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Chemical and Radiation Leukemogenesis in Humans and Rodents and the Value of Rodent Models for Assessing Risks of Lymphohematopoietic Cancers

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LIST OF KEY ABBREVIATIONS

Acute lymphoblastic leukemia	ALL
Acute lymphoblastic leukemia with B-cell lineage	B-ALL
Acute lymphoblastic leukemia with T-cell lineage	T-ALL
Adult T-cell leukemia	ATL
Acute myeloid leukemia	AML
Acute nonlymphocytic leukemia	ANLL
Burst-forming unit - erythroid	BFU-E
Burst-forming unit - megakaryocyte	BFU-meg
Butadiene monoepoxide (monoepoxybutene)	BDO
Butadiene diepoxide (diepoxybutane)	BDO2
Chronic lymphocytic leukemia	CLL
Chronic myelogenous leukemia	CML
Chronic myelomonocytic leukemia	CMML
Colony-forming units of basophils	CFU-baso
Colony-forming units - eosinophil	CFU-Eos
Colony-forming units of granulocytes and macrophages	CFU-GM
Colony-forming units - monocytes	CFU-M
Colony-forming units - neutrophil	CFU-G
Erythroleukemia M6	
Fluorescence in situ hybridization	FISH
Glycophorin A	GPA
Gray	Gy
Interleukin-3	IL-3
Immunoglobulin	Ig
Megakaryoblastic leukemia	M7
Monocytic leukemia	M5
Multipotent myeloid progenitor cell	CFU-GEMM
Myelodysplastic syndromes	MDS
Myeloblastic leukemia with maturation	M2
Myeloblastic leukemia with minimal differentiation	M0
Myeloblastic leukemia without maturation	M1
Myelomonocytic leukemia	M4
Natural killer cells	NK cells
Non-Hodgkin's lymphoma	NHL
Promyelocytic leukemia	M3

Radiation leukemia virus	RadLV
Refractory anemia	RA
Refractory anemia with excess blasts	RAEB
Refractory anemia with ring sideroblasts	RARS
Refractory anemia with excess blasts in transformation	RAEB
Sister chromatid exchange	SCE
Sievert	Sv
Stem-cell factor	SCF
T-cell receptor	TCR

PREFACE

The National Center for Environmental Assessment-Washington Office (NCEA-W) was responsible for having this document prepared under contract by an expert in the field of leukemogenesis.

This document consists of the following sections: (1) a brief overview of human hematopoiesis and lymphopoiesis, (2) a comparison of these processes to those seen in rodents, (3) a description of selected characteristics of chemical- and radiation-induced hematopoietic neoplasia in humans and rodents, (4) a summary of the current information on the leukemias and lymphomas induced by six major classes of leukemia-inducing agents, and (5) a description of the key genetic alterations and genes involved in chemically induced leukemias. In the final section, a number of key issues related to chemical leukemogenesis and the use of rodent models for human risk assessment are discussed. This document is intended to serve as useful background information for risk assessors who are dealing with cancers of the hematopoietic system. The literature search for this review is current through December 1996.

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EXECUTIVE SUMMARY

Lymphohematopoietic neoplasia are characterized by an uncontrolled proliferation or expansion of cells originating from the bone marrow or lymphoid tissues that do not retain the capacity to differentiate normally to form mature blood cells. These neoplasms represent clonal expansions of hematopoietic cells within either the myeloid or lymphoid lineage and are further identified as chronic or acute depending on the rate of clonal expansion and the stage of differentiation that dominates the leukemic clone. In recent years, a large amount of information has emerged that is providing an increased understanding of the mechanisms underlying the development of hematopoietic neoplasia in humans and rodents. This report is intended to provide an up-to-date overview of the lymphoid and hematopoietic diseases induced in humans and rodents following exposure to chemical and physical agents.

Following a brief introduction to hematopoiesis and lymphopoiesis in humans and rodents, selected characteristics of known leukemia-inducing agents and their effects in mice and rats are compared, allowing some generalizations to be made about leukemogenesis and the value of rodent models. Four main patterns are outlined as follows: (1) The primary type of lymphohematopoietic cancer induced by chemicals and radiation in humans is myeloid leukemia, with the exception of the immunosuppressive agents, which are associated almost exclusively with the development of lymphomas. (2) Potent human leukemia-inducing agents induce significant myelotoxicity and structural chromosomal aberrations in exposed humans. Similar effects are seen when these agents are administered to animal models. (3) Administration of human leukemia-inducing agents to mice results in more lymphohematopoietic tumors. However, in contrast to the human, these tumors are primarily lymphoid in origin. (4) The rat is considerably less responsive than the mouse to the induction of lymphohematopoietic neoplasia following administration of human leukemogens. When induced, resulting neoplasms in rat also are primarily lymphoid in origin.

In the next section of the report is a more detailed examination of leukemias and related effects seen following treatment by a number of classes of human leukemia-inducing agents. The objective of this section is to identify similarities and differences between classes of agents and to provide insights into mechanisms underlying their hematopoietic effects. Six different classes of established leukemia-inducing agents were selected; ionizing radiation; alkylating agents, epipodophyllotoxin-type topoisomerase inhibitors; dioxopiperazine-type topoisomerase inhibitors; benzene; and 1,3-butadiene, a probable human leukemia-inducing agent.

Although similarities are seen among various agents (or classes of agents), each of these agents exhibits a unique profile of lymphoid or hematopoietic neoplasia. Ionizing radiation is unique among the six agents in that it is associated with increased incidence of chronic myelogenous leukemia and acute lymphoblastic leukemia, in addition to acute nonlymphocytic leukemia. The other agents are associated primarily with acute nonlymphocytic leukemias, although some associations between butadiene exposure and the development of lymphoid neoplasia in humans have been seen. Leukemias induced by radiation and alkylating agents are characterized by latency periods of about 5 years, the common appearance of a

myelodysplastic phase, and cells that frequently exhibit loss of all or part of chromosomes 7 and 5. In contrast, leukemias induced by the topoisomerase inhibitors have latency periods of around 3 years, do not exhibit a myelodysplastic phase, and are characterized by cells that contain balanced translocations rather than deletions or losses. Benzene exhibits some similarities to the other classes but possesses enough unique characteristics to indicate that it is induced by additional or different mechanisms. Butadiene is quite different from other specific agents examined. Although detailed information on types of induced leukemia and related genetic changes is lacking, butadiene appears to have a significant number of similarities to ethylene oxide and vinyl chloride. All three are small-molecular–weight compounds that appear to act through the formation of DNA adducts by epoxide intermediates. Similarities between these agents in their metabolism, DNA adducts, mutational spectra, and the nature of the induced rodent tumors have been noted previously.

In the report's latter portion, the current understanding of mechanisms underlying acute nonlymphocytic leukemia (the major leukemia type seen in chemically exposed individuals) in humans and thymic lymphoma and myeloid leukemia in mice is presented. The relevance of mouse models for human leukemias and lymphomas is then discussed. These sections indicate that chemical- and radiation-induced lymphomagenesis and leukemogenesis are complex processes involving multiple genes, chromosomal alterations, and, probably, altered differentiation. In addition, such other factors as metabolic capabilities, DNA repair, and genetic susceptibilities also have been shown to influence cancer incidence. Given the complexity and multiplicity of steps, animal models are unlikely to reproduce precisely all the critical stages involved in development of chemical-induced leukemias or lymphomas in humans. Indeed, significant interspecies differences can be seen in humans and rodents for such basic biological processes as hematopoiesis and in response to leukemia-inducing agents. In spite of these limitations, rodent models have proved valuable for identifying metabolic pathways and immunotoxic and myelotoxic effects as well as the processes and genes involved in hematopoiesis and leukemogenesis. Furthermore, chronic animal bioassays using mouse models have been shown to be effective in identifying human leukemia-inducing agents. However, in evaluating results of animal bioassays, consideration should be given to processes or mechanisms that do not operate in humans but may be responsible for or contribute to the incidence of cancer in rodents.

In the final section, a series of recommendations is made for future research in this area.

1. INTRODUCTION

Lymphohematopoietic neoplasia can be defined as an uncontrolled proliferation or expansion of lymphohematopoietic cells that do not retain the capacity 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). The myeloid clones are designated as chronic or acute leukemias, depending on 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 or remain confined to lymphoid proliferative sites such as the lymph nodes or spleen; they 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 that progresses over a period of months or years to a blast or acute leukemic phase. Within these general classifications, leukemias and lymphomas represent a heterogeneous group of diseases. Heterogeneity can be seen even within such discrete clinical or pathological types of leukemia as acute lymphoblastic leukemia or acute myelogenous leukemia, which include subgroups involving different molecular mechanisms and etiologies. The myelodysplastic syndromes (MDS) are a series of blood disorders characterized by maturation defects resulting in ineffective hematopoiesis. These syndromes are commonly considered as preleukemic because a variable, but significant, proportion (8 to 60%) of various disorders progress to frank leukemia (Wright, 1995). An outline of major types of human leukemias and lymphomas with their subtypes is shown in Table 1. For the most common of these human cancers, tumors affecting the same cell type and exhibiting similar characteristics have been identified in rodents (Pattengale, 1994; Pattengale and Taylor, 1983; Perkins, 1989; Ward et al., 1990).

As seen with other cancers, leukemogenesis and lymphomagenesis are multistep processes involving a series of genetic and possibly epigenetic alterations in the transformation of a normal cell into a malignant cell. Because of the heterogeneous nature of the lymphohematopoietic neoplastic diseases, a large number of genes are likely to be involved. As many as 200 separate genes may be involved in the origin of all leukemias (Greaves, 1995). Some of these genetic alterations can be detected only at the molecular level. However, a large number of these genetic changes can be found directly through the use of cytogenetic and molecular cytogenetic techniques. Nonrandom chromosomal alterations are detected in the neoplastic cells of a majority of patients withleukemias or lymphomas, and identification ofgenes involved in these alterations has provided

Table 1. Classification of major lymphohematopoietic neoplastic diseases in humans

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 possibly originating in myeloid-committed precursors	
Acute nonlymphocytic leukemia or Acute myeloid leukemia	ANLL/AML
Myeloblastic leukemia with minimal differentiation	M0
Myeloblastic leukemia without maturation	M1
Myeloblastic leukemia with maturation	M2
Promyelocytic leukemia	M3
Myelomonocytic leukemia	M4
Monocytic leukemia	M5
Erythroleukemia	M6
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's lymphoma	NHL
Nodal/splenic phase	
Leukemic phase	
B-cell lineage	
Non-Burkitt's	
Burkitt's L3	
T-cell lineage	
Lymphoblastic lymphoma	
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	
V. Neoplasms of uncertain (possibly lymphoid) origin: Hodgkin's Disease	

Modified from Sullivan (1993).

valuable insights into leukemogenesis and lymphomagenesis in humans (Rowley, 1990; Sandberg, 1990).

Leukemias induced by therapeutic, occupational, or environmental exposure to chemicals and radiation appear to have characteristics different from those typically seen in individuals without a history of chemical or radiation exposure, suggesting variations in origin and etiology (Pedersen-Bjergaard and Rowley, 1994). In recent years, a number of animal and human studies provided significant new information on mechanisms underlying hematopoietic neoplasia induced by chemical and physical agents. This information has important implications for assessing the risk of leukemias, and to a lesser degree lymphomas, resulting from chemical exposure; it also provides insights into the value of rodent models for predicting chemically induced leukemias and lymphomas in humans.

The objective of this article is to provide an overview of the types of lympho-hematopoietic neoplasia induced by chemical agents and radiation in humans and rodents and to summarize current information on chemical leukemogenesis mechanisms in these organisms. Due to the complexity of leukemogenesis, the large number of potential agents involved, and the extensive literature on leukemogenesis, this review will focus primarily on the types and mechanisms underlying leukemias induced by six extensively studied classes of leukemia-inducing agents. These classes are ionizing radiation; alkylating agents; epipodophyllotoxin-type and dioxopiperazine-type topoisomerase inhibitors; benzene; and 1,3-butadiene, a probable human leukemia-inducing agent.

The body of the review consists of the following sections: (1) A brief overview of human hematopoiesis and lymphopoiesis; (2) A comparison of these processes to those seen in rodents; (3) A description of selected characteristics of chemical- and radiation-induced hematopoietic neoplasia in humans and rodents; (4) A summary of current information on the leukemias and lymphomas induced by six major classes of leukemia-inducing agents; and (5) A description of the current understanding of mechanisms involved in chemically and radiation-induced leukemias in humans and mice. In the final section, a number of key issues related to chemical leukemogenesis and the use of rodent models for risk assessment are discussed.

2. OVERVIEW OF HUMAN HEMATOPOIESIS AND LYMPHOPOIESIS

2.1. HUMAN HEMATOPOIESIS AND LYMPHOPOIESIS

With the exception of lymphocytes, blood-cell formation in normal human adults occurs exclusively in the bone marrow. All mature blood cells have a finite life, with the majority of cells being terminally differentiated and unable to replicate (Bagby, 1994). To maintain steady-state levels, 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. Estimates of cell turnover range from 200 billion to 1 trillion cells per day in a 70-kg man (Irons, 1991; Ogawa, 1993). Furthermore, the hematopoietic system must respond to a variety of environmental stresses by increasing the blood-cell counts of specific lineages when needed (Bagby, 1994). For example, upon exposure to a hypoxic environment, erythrocyte production will increase without a change in neutrophil production. Similar lineage-specific responses are required following exposure to myelotoxic agents. To maintain steady-state blood-cell levels and respond to environmental pressures, hematopoiesis must be a highly regulated process.

This 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. These cells also can 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 multi potent and committed progenitor cells that represent about 2 to 5 per 1000 marrow cells (Mihich and Metcalf, 1995). [The exact definition and numbers of primitive hematopoietic stem cells are still the subject of ongoing research and debate (Orlic and Bodine, 1994).] Each progenitor cell can generate 100,000 or more maturing progeny (Mihich and Metcalf, 1995). The process of proliferation and differentiation is regulated by more than 25 growth factors, cytokines, and other regulators that may act directly on one or more of the major lineages of blood cells or interact to influence cell growth (Mihich and Metcalf, 1995). A diagram illustrating the hierarchical relationships among major cell types involved in hematopoiesis is shown in Figure 1.

Under the control of various growth factors, the primitive hematopoietic stem cell either can self replicate or replicate to form pluripotent stem cells. These cells, in turn, can divide

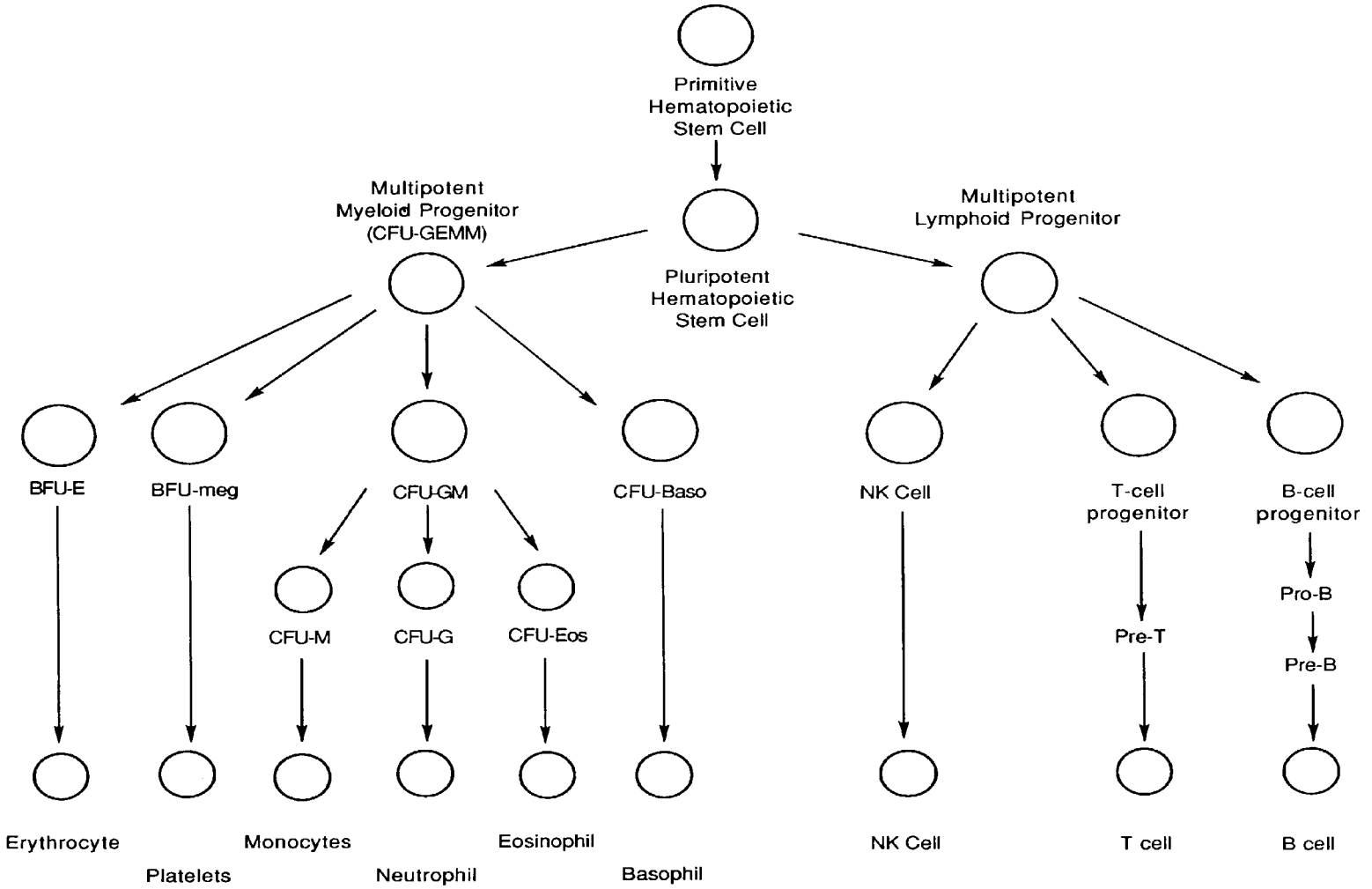

Figure 1. Hierachical relationships between major cell types involved in hematopoiesis. For simplicity, a number of steps in maturation pathways have been omitted.

to form multi potent progenitor cells that are committed to either the myeloid or lymphoid lineages (Bagby, 1994). The multi potent myeloid progenitor cell (CFU-GEMM) can give rise to colony-forming cells of each myeloid lineage (erythrocyte, BFU-E; megakaryocyte, BFU-meg; monocyte/neutrophil/eosinophil, CFU-GM; basophil, CFU-baso). The CFU-GM cell can undergo further differentiation to form colony-forming cells that are restricted to the monocyte (CFU-M), neutrophil (CFU-G), and eosinophil (CFU-Eos) lineages.

The multi potent lymphoid progenitor cell undergoes further specialization to form natural killer (NK) cells, T-cells, and B-cells (see Burns et al., 1996, for additional details). Shortly after commitment to the T-cell lineage, pre-T-cells migrate from the bone marrow to the thymus where they begin T-cell receptor (TCR) rearrangement. Through a process of sequential positive and negative selection, cells that can properly recognize major histocompatibility class surface proteins and foreign peptides survive and are released to the blood. These mature T-cells then migrate to the lymph nodes and elsewhere through the circulatory system and body. Following commitment to the B-cell lineage, the pro-B-cells in the bone marrow begin a process of rearranging their V (variable), D (diversity), J (joining), and C (constant) gene segments to form antigen-receptor genes. The resulting pre-B-cells expressing: heavy chains in their cytoplasm undergo further maturation and eventually are characterized by the presentation of IgM and IgD surface immunoglobulins. These mature B-cells then migrate from the bone marrow to the lymph nodes and other secondary lymphoid organs. Relatively little is known about development of NK cells, apart from the observation that mature NK cells are localized primarily in the spleen, blood, and peritoneal exudate.

2.2. COMPARISON OF HEMATOPOIESIS AND LYMPHOPOIESIS IN RODENTS AND HUMANS

In general, hematopoiesis and lymphopoiesis in rodents is similar to that described above for humans. All blood cells originate from a pluripotent hematopoietic cell and become committed into both myeloid and lymphoid lineages. These individual cells then differentiate into their respective T- and B-cells for lymphocytes and neutrophils, eosinophils, and monocytes for myeloid cells. Rodent models, and in particular mouse models, are thought to be highly relevant for understanding most aspects of hematopoiesis (Bagby, 1994). However, a number of differences between hematopoiesis and lymphopoiesis in humans and rodents are significant. Hematopoiesis in adult humans is restricted to the bone medullary spaces. Extramedullary hematopoiesis involving the spleen, liver, and lymph nodes rarely occurs except under conditions of extreme demand (Irons, 1991). In contrast, small groups of hematopoietic cells commonly are

found in mouse and rat spleen, and occasionally small clusters of these cells can be located in the liver (Hall, 1992; Irons, 1991). In addition, compensatory hyperplasia of various cell lineages in rodents can confound the diagnosis of lymphoma and other diseases. Furthermore, the relative number of lymphocytes and macrophages in rodents can vary considerably, depending on the amount of splenic hematopoiesis (Hall, 1992).

There are also significant differences in the composition of nucleated cells in the blood and bone marrow of adult rodents and humans. Illustrated in Table 2 are average values reported for humans, rats, and mice from a variety of sources. For example, lymphocytes make up about 35% of nucleated cells in adult human blood and around 70% of nucleated cells in rodent blood. This frequency also can be influenced by age and strain. For example, in rats the percentage of lymphocytes decreases from 90% at 2 months of age to about 65% at 30 months (Valli et al., 1990). In a corresponding fashion, the percentage of neutrophils increases with age from about 9% at 2 months to around 30% at 30 months. A similar but less-pronounced change in the percentage of lymphoid cells can also be seen in the bone marrow. The proportion of myeloid and erythroid cells in the marrow increases over the first year of life, whereas the proportion of lymphocytes and monocytes decreases between 2 and 12 months (Valli et al., 1990).

This high frequency of lymphocytes in the peripheral blood of rats and mice is among the highest percentage seen in mammals. In a report by Smith (1990), in which around 100 mammalian species were compared, the frequency of lymphocytes reported for the rat ranked among the highest 5 species when compared on a per-liter basis. Among the top 5, the rat had the highest percentage of circulating white blood cells (71%) compared with the pig (51%), the crab-eating macaque (63%), the guinea pig (65%), and the elephant (54%) (Smith, 1990). Correspondingly lower percentages of neutrophils, eosinophils, and basophils are seen in the rat as compared to the human.

In addition to this high percentage of lymphocytes, other differences in the blood profile can be seen. Both mice and rats have smaller erythrocytes than humans, with a survival time of 40 to 68 days compared to about 120 days in humans (Hall, 1992; Irons, 1991). A large proportion of the neutrophils of both mice and rats exhibit a "ring" type of nucleus with a donut shape rather than the multilobulated type seen in humans (Andrew,1965; Hall, 1992; Hulse, 1964). Furthermore, unlike human neutrophils, the granules are small and difficult to stain (Bannerman, 1983). In addition, platelet counts in rats and mice are very high, averaging about 1 million/:l in the rat and 1.5 million/:l in the mouse (Hall, 1992).

Table 2. Inspecies comparison of the composition of major nucleated cell types in bone marrow and blood					
	Bone Marrow				
	Myleloid		**Erythroid**		**Lymphoid**
Human	56		26		13
Mouse	44		24		31
Rat	36		34		23
	Blood				
	Neutrophilis	**Monocytes**	**Eosinophils**	**Basophils**	**Lymphocytes**
Human	54	7	3	1	36
Mouse	19	5	0.7	0	76
Rat	22	3	2	0.1	73

[a]All values represent percentages of nucleated cells. Values are averages obtained from the following sources: Bannerman, 1983; Bergemann and Rastletter, 1979; Chervenick et al., 1968; Hulse, 1964; Parmley, 1988; Ringler and Dabich, 1979; Smith, 1990; Snyder et al., 1975; and Valli et al., 1990.

The different characteristics of rodent blood and bone marrow combined with differences in splenic hematopoiesis indicate that in spite of their similarities, significant differences in hematopoiesis exist among mice, rats, and humans. A recent article by Irons and associates also pointed out that species differences are likely to exist in the organization of the hematopoietic stem-cell compartment, particularly in regard to the stage at which the primitive hematopoietic progenitor cells become restricted to a specific differentiation pathway (Irons et al., 1995). Primitive human hematopoietic progenitor cells are supported in Go by IL-3 or GM-CSF but not SCF, whereas the comparable murine primitive hematopoietic progenitor cells require IL-3 or SCF for survival. These differences in cell composition, number, proliferation rates, or organization of the stem-cell compartment are likely to influence the hematotoxic and carcinogenic effects observed in human and rodent systems following exposure to carcinogenic agents.

3. OVERVIEW OF CHEMICALLY AND RADIATION-INDUCED HEMATOPOIETIC NEOPLASIA IN HUMANS AND RODENTS

Over the past 50 years, considerable evidence has demonstrated the involvement of radiation, selected therapeutic drugs, and occupational chemicals in the etiology of human leukemias and lymphomas. Agents recognized by the International Agency for Research on Cancer (IARC) as Group 1 carcinogens, meaning that sufficient evidence exists of their carcinogenicity in humans, are listed in Table 3. This list comprises

only the IARC Group 1 chemicals associated with lympho- or hematopoietic neoplasia. Also listed are ionizing radiation and several topoisomerase II inhibitors not reviewed by IARC but for which there is convincing evidence of leukemogenicity in humans. In addition, a group of chemicals is classified by IARC as probable human carcinogens (Group 2A) based on either animal studies, mechanistic information, or limited epidemiology; these chemicals are likely to be associated with lymphohematopoietic cancers. For each agent, information summarizes the primary type of leukemia or lymphoma observed, whether the agent induces significant myelotoxicity in humans, and whether increases in structural chromosomal aberrations have been seen in the peripheral blood lymphocytes of humans exposed to each agent. In addition, the primary types of lymphohematopoietic tumors seen in various mouse and rat cancer bioassays are listed. For most, a summary description from a recent IARC monograph is also presented.

At present 16 agents show sufficient evidence of lymphohematopoietic neoplasia in humans, and 10 are probable human leukemogens. A number of patterns emerge from the information in the table. Of the Group 1 carcinogens, 12 of the 16 are associated primarily with acute nonlymphocytic leukemia (ANLL). The two strong immunosuppressive agents, cyclosporin and azathioprine, are associated primarily with Non-Hodgkin's lymphoma (NHL) and two agents, ethylene oxide and vinyl chloride, have been associated with both ANLL and lymphoid malignancies. However, the leukemogenic effects of these last two appear to be relatively weak, and their classification as Group 1 carcinogens seems to rely primarily on tumors seen in other tissues (vinyl chloride) or on mechanistic data (ethylene oxide). In addition to its ability to induce ANLL, radiation also has been strongly associated with the induction of chronic myelogenous leukemia and acute lymphoblastic leukemia in humans (BEIR V, 1990; UNSCEAR, 1994).

Table 3. Characteristics of selected known and probable human leukemia-inducing agents

	Human[v]	Myelotoxic[c]	Aberrations[g]	Mouse	Rat	Source
Agents carcinogenic to humans (IARC Group 1)						
DNA-reactive						
1,4-Butanediol dimethanesulphonate (Busulfan, Myleran)	ANLL[w]	H[y]	SCA	Leukemia/lymphoma Thymic lymphoma Thymic lymphoma	NLHT[i] NLHT[i]	IARC, 1987a Conklin et al., 1965 Robin et al., 1981 Schmahl and Osswald, 1970
Chlorambucil	ANLL	H	SCA[m]	Hematopoeitic[a] Lymphosarcoma Lymphosarcoma Lymphoma + granulocytic leukemia	Hematopoietic + Lymphatic Leukemia Lymphoma Hematopoietic + Lymphatic[a]	IARC, 1987a Kaldor et al., 1988[n] Weisburger et al., 1975 Berger et al., 1985 Cavaliere et al., 1990
(1-(2-)Chlorethyl)-3-(4-methylcyclohexyl) nitrosurea (Methyl-CCNU, Semustine)	ANLL	H[o]	NA	NLHT[a,l]	NLHT[a,l] –[e]	IARC, 1987a Schmahl and Habs, 1982
Cyclophosphamide	ANLL	H	SCA	NLHT Leukemia Lymphosarcoma Lymphocytic leukemia	NLHT Lymphoid hematopoietic leukemias Variety[p] Lymphold + hematopoietic leukemias Hematopoietic + lymphoid tumors NLHT[i]	IARC, 1987a Kaldor et al., 1988[n] Petru et al., 1989 Schmahl and Habs, 1978 Schmahl and Osswald, 1970 Schmahl and Habs, 1979 Schmahl and Habs, 1982 Schmahl and Habs, 1976
Melphalan	ANLL	H	SCA	Lymphosarcoma Lymphosarcoma Lymphosarcoma	Lymphosarcoma Lymphosarcoma NLHT[i] Lymphosarcoma	IARC, 1987a Kaldor et al., 1988[n] Weisburger et al., 1975 Gold et al., 1984[n]
Treosulphan	ANLL	H	NA	NA	NA	IARC, 1987a
Vinyl chloride	Mixed[x]	–[g]	SCA	NLHT[i]	NLHT[f,l] –[d]	IARC, 1987a Radike et al., 1981

Table 3. Continued

	Human[v]	Myelotoxic[c]	Aberrations[g]	Mouse	Rat	Source
DNA-reactive (continued)						
Thio-TEPA (tris(1-aziridinyl)-phosphine)	ANLL	H	SCA	Lymphoid Lymphoid	Lymphoid Lymphoid + granulocytic leukemia NLHT[d]	IARC, 1990d NCI, 1978c Schmahl and Osswald, 1970
Ethylene	Mixed[x]	–[g]	SCA	Lymphoma Lymphoma	Lymphoid[r] Lymphoid[r] Lymphoid[r]	IARC, 1994a Lynch et al., 1984 Snellings et al., 1984 NTP, 1987
Topoisomerase II inhibitors						
Etoposide	ANLL[z]	H	Na[q]	NA	NA[i]	
Teniposide	ANLL[z]	H	NA	NA	NA	
Bimolane	ANLL[z]	Na[h]	SCA	Granulocytic leukemia	NA	Ye et al., 1994
Immunosuppressive agents						
Cyclosporin	NH Lymphoma	–[g]	SCA	Lymphoma[k]	–[j]	IARC, 1990c
Azathioprine	NH Lymphoma	H	SCA[s]	Lymphoma[a]	Lymphoma Thymic lymphoma	IARC, 1987a Cohen et al., 1983
Other						
Benzene	ANLL	H	SCA	Lymphoma Lymphoma Lymphoma	NLHT[t] NLHT[t] NLHT[b,c]	IARC, 1987a NTP, 1986b (see text)
Ionizing radiation	ANLL CML ALL	H	SCA	Thymic Lymphoma Myeloid leukemia Myeloid leukemia Thymic Lymphoma	 NLHT[b,c] NLHT[t] Lymphoid+Myeloid (see text)	Yokoro et al., 1986 Storer et al., 1982 Conklin et al., 1965 (see text) Gross and Dreyfuss, 1979 Schmahl and Osswald, 1970
Agents probably carcinogenic to humans (IARC Group 2A)						

Table 3. Continued

	Human[v]	Myelotoxic[c]	Aberrations[g]	Mouse	Rat	Source
DNA-reactive						
Bischloroethyl nitrosourea (BCNU)	ANLL[a]	H	Na[q]	NLHT[t]	NLHT[t] –[d] –[e]	IARC, 1987a Schmahl and Habs, 1978 Schmahl and Habs, 1982
1-(2-)Chlorethyl)-3-cyclohexyl-1-nitrosurea (CCNU)	ANLL[a]	H	NA	Lymphosarcoma[a]	NLHT[t] Hematopoietic & Lymphatic[c]	IARC, 1987a Schmahl and Habs, 1982
Cisplatin	ANLL[a]	H	Na[q]	NLHT[t]	Leukemia Myeloid or stem-cell leukemias Myeloid leukemias	IARC, 1987a Kempf and Ivankovic, 1986b Kempf and Ivankovic, 1986a
Nitrogen Mustard (Mechlorethamine)	ANLL[a]	H	SCA	Lymphoma Thymic lymphoma	NLHT[t] NLHT[d,l]	IARC, 1987a Conklin et al., 1965 Schmahl and Osswald, 1970
Procarbazine	ANLL[a]	H	Na[q]	Lukemias[c] Lymphosarcoma Lymphocytic Leukemia Lymphoma + Leukemia	NLHT[t] Lymphoma + Leukemia Lymphoma NLHT[t] NLHT[t]	IARC, 1987a Weisburger et al., 1975 Kelly et al., 1964 NCI, 1979 Schmahl and Osswald, 1970 Kelly et al., 1968
Chlorozotocin	ANLL[a]	H	NA	NA	NLHT[t]	IARC, 1990b
1,3-Butadiene	Mixed[a,x]	–[g]	–	Lymphoma Lymphoma	NLHT[t] NLHT[t]	IARC, 1987a (see text)
Topoisomerase II inhibitor						
Adriamycin	ANLL[a]	H	SCA[a]	NA	NLHT[t]	IARC, 1987a

Table 3. Continued

	Human[v]	Myelotoxic[f]	Aberrations[g]	Mouse	Rat	Source
Other						
Azacytidine	ANLL[a]	H	Na[q]	Granulocytic + Lymphocytic Granulocytic sarcoma + Lymphoma Lymphoma Lymphatic leukemia + Lymphoma + Myeloid leukemia	NLHT[l] –[a,d]	IARC, 1990a NCI, 1978a Cavaliere et al., 1987 Schmahl et al., 1985
Chloramphenicol	ANLL[a]	H	NA	Lymphoma[a]	NA	IARC, 1987a

[a]Limited evidence.
[b]Influenced by strain.
[d]Hemopoietic and lymphatic tumors showed a statistically nonsignificant increase.
[e]No data presented, increases reported at other sites.
[f]Reported to induce lymphomas in hamster [Registry of Toxic Effects of Chemical Substances (RTECS)].
[g]Myelotoxicity is either not seen or infrequently seen (Arky, 1996; ATSDR, 1992b; Handschumacher, 1990; IARC, 1994a).
[h]Reported to alter leukocyte count in treated dogs (RTECS, 1996).
[i]Minor increases of tumors at various sites have been reported (RTECS, 1996).
[j]Study had limited sensitivity.
[k]Increases observed in AKR mouse, a strain highly susceptible to lymphomas. Cyclosporin also has been shown to accelerate lymphoma development in mice treated with radiation or N-methyl-N-nitrosourea and accelerate the formation of lymphomas in grafted macaques, a species of monkey with an extremely low frequency of lymphoma (IARC, 1990c).
[l]No or minimal increase in lymphohematopoietic tumors reported. In many cases, tumors in other tissues were reported.
[m]Information from Seiber and Adamson, 1975.
[n]Secondary reference.
[o]Information from Calabresi and Chabner, 1990.
[p]Slight increase in lymphohematopoietic neoplasia.
[q]Increase seen in experimental animals (RTECS,1996).
[r]Mononuclear cell leukemia classified as lymphoid based on the classification scheme of Ward et al., 1990.
[s]From IARC, 1981.
[t]Myelotoxic to humans. Information from Arky, 1996, unless otherwise indicated.
[u]SCA - Increases in structural chromosomal aberrations observed in human lymphocytes. Information from Sorsa, et al., 1992, unless otherwise indicated.
[v]Primary classification based on IARC Monographs or BEIR V unless otherwise specified.
[w]ANLL - Acute nonlymphocytic leukemia.

Table 3 Footnotes (continued)

[x]Leukemias of both myeloid and lymphoid lineages frequently reported.
[y]Effect frequently observed in humans.

[z]Classification based on Pedersen-Bjergaard and Rowley, 1994, for etoposide and teniposide and Zhang et al., 1993, for bimolane.

In regard to myelotoxicity, 12 of the 16 agents for which information was available showed significant evidence of myelotoxicity in humans who either received the drug therapeutically or were exposed to the agent occupationally. Most of these agents, such as benzene, busulfan, chlorambucil, and radiation, are well known for their myelotoxic effects. Ethylene oxide, vinyl chloride, and cyclosporin were not classified as myelotoxic; although bone marrow toxicity is seen occasionally following exposure to these agents, it typically is an infrequent event (ATSDR, 1992b; IARC, 1994a). Of the Group 2 leukemogenic agents, nine of the ten have been observed to induce significant myelotoxicity in humans. The one agent for which myelotoxic effects commonly have not been seen in humans is 1,3-Butadiene. Although not listed in the table, most of these Group 1 and 2 carcinogens have been shown to exhibit myelotoxic effects in rodent models.

Of the Group 1 agents for which data could be found (11 total), all were reported to induce structural chromosomal aberrations in the peripheral blood lymphocytes of exposed individuals. Reports of structural chromosomal aberrations in humans were rarely found for the Group 2 agents. However, most Group 1 and Group 2 carcinogens have been shown to induce chromosomal aberrations in rodent models.

Testing results for most Group 1 and 2 agents in rodent bioassays for carcinogenicity are also shown in Table 3. Based on the number of studies and reported frequencies for various lymphohematopoietic tumor types, the predominant tumor type associated with each agent was determined. For simplicity and because of the close relationship between tumor types such as T-cell lymphomas and T-cell leukemias (Pattengale, 1990), the tumors are summarized as primarily lymphoid, myeloid, or of multiple lineages. In addition, many of these studies were conducted using a variety of strains and administration routes and were done before the implementation of standardized testing protocols. For the 16 Group 1 agents, the primary tumor type in the mouse was lymphoid for 10 of the chemicals and myeloid for 1 agent, bimolane. Either no information was available or the studies were of inadequate quality for 4 agents (methyl CCNU, treosulphan, etoposide, and teniposide). Only for vinyl chloride had significant increases in lymphohematopoietic tumors not been reported. [An increase in lymphoid tumors has been seen for vinyl chloride in hamsters (ATSDR, 1992b)]. Radiation was listed as inducing primarily lymphoid tumors because this is the most frequently observed tumor type in most strains of mouse (Storer et al., 1982; Yokoro et al., 1986). However, myeloid leukemia is induced by ionizing radiation in some mouse strains (Riches, 1995; Yokoro et al., 1986). Of the 10 Group 2 agents, 5 induced primarily lymphoid neoplasms in the mouse, 0 myeloid, and 1 mixed (azacytidine). Two agents showed no evidence of lymphohematopoietic cancers (BCNU, and cisplatin), and inadequate information was available for two agents (chlorozotocin and adriamycin).

An overview of tumor types induced in rat by Group 1 carcinogens revealed that three agents—melphalan, ethylene oxide, and azathioprine—caused primarily lymphoid neoplasms, and three—chlorambucil, cyclophosphamide, and thio-TEPA—induced tumors with both lymphoid and myeloid lineages. No rat cancer bioassay data was available for treosulphan, etoposide, teniposide, and bimolane. Significant increases in lymphohematopoietic cancers were not generally seen for six agents: busulfan, methyl CCNU, vinyl chloride, cyclosporin, benzene, and radiation. Radiation has been reported to induce

lymphoid and myeloid leukemias in rats in a few reports, but most studies using this species have failed to see significant increases in these tumor types (Gross and Dreyfuss, 1979; Ward et al., 1990). Similar results have been reported for the cancer bioassays of benzene performed in the rat (Maltoni et al., 1989; NTP, 1986a).

Based on the above information, a number of patterns seem apparent. (1) The primary type of lymphohematopoietic cancer induced by chemicals in humans is myeloid leukemia (ANLL), with the exception of immunosuppressive agents associated almost exclusively with lymphoma development. (2) Potent leukemia-inducing agents also induce significant myelotoxicity and structural chromosomal aberrations in exposed humans. (3) Administration of human leukemia-inducing agents to mice results in more lymphohematopoietic tumors. However, in contrast to human, these tumors are primarily lymphoid in origin. (4) The rat is considerably less responsive than the mouse for the induction of lymphohematopoietic neoplasia following administration of human leukemogens. When induced, resulting neoplasms in the rat are primarily lymphoid in origin.

4. LEUKEMIAS INDUCED BY SELECTED CLASSES OF ENVIRONMENTAL AND THERAPEUTIC AGENTS IN HUMANS

4.1. GENERAL COMMENTS

A number of studies have shown an association between exposure to petroleum, solvents, pesticides, and other chemical agents and increased risks of hematopoietic neoplasia (Brandt, 1992). In most of these studies, the increased risks have been for ANLL, but increases in other types of leukemias have also been reported (Brandt, 1992; Malone et al., 1989; Persson et al., 1989; Weisenburger, 1994). In some cases, increases have been attributed to benzene exposure whereas in other cases, the agent responsible for the neoplastic effects is not readily apparent (Checkoway et al., 1984; Ott et al., 1989; Persson et al., 1989). There is also evidence that such other chemicals as ethylene oxide, vinyl chloride, and 1,3-butadiene may have been responsible for some increases in observed lymphohematopoietic cancers (Finch and Linet, 1992; IARC, 1987a; IARC, 1992; IARC, 1994a). However, for most studies on these agents, the hematopoietic effects have been relatively weak, and some inconsistencies have been seen (Cole et al., 1993; Shore et al., 1993). Although not a chemical, ionizing radiation has been included because it has been extensively studied and represents an important leukemia-inducing agent. Differences in effect have been seen for different types of ionizing radiation and for applications at different dose rates. However, for simplicity and ease of presentation, radiation has been treated as one agent with uniform characteristics. For a more detailed description of the effects of ionizing radiation, the reader is referred to the following sources: BEIR V, 1990; Hendry and Lord, 1995; UNSCEAR, 1993; and UNSCEAR, 1994. The following section will review six agents, for five of which there is strong and consistent epidemiological evidence. The six agents are ionizing radiation; alkylating agents; epipodophyllotoxin-topoisomerase and dioxopiperazine-topoisomerase inhibitors; benzene; and 1,3-butadiene, an agent for which human data is more limited and controversial.

However, before describing what is currently known about the mechanisms of genotoxicity and leukemogenesis for the individual agents, some general observations about solvent and chemically induced lymphohematopoietic cancers will be discussed.

Over the past 20 years, cytogenetic studies of bone-marrow cells of leukemia patients have become important for diagnosing the disease, as prognostic indicators, and for mechanistic information (Pedersen-Bjergaard and Philip, 1987). Karyotype comparison between cases with de novo leukemia (ANLL) and patients previously treated with alkylating chemotherapeutic agents revealed that the latter groups had a significantly higher frequency of leukemic cells with abnormal karyotypes, primarily loss and deletions of chromosomes 5 and 7 (Rowley, 1983). Based on these results, a series of studies was conducted to determine if a similar pattern could be seen in patients with a history of exposure to occupational and environmental chemicals (Golomb et al., 1982; Mitelman et al., 1978; Mitelman et al., 1979; Mitelman et al., 1981). These studies generally have shown that patients with a history of chemical exposure have significantly higher frequencies of karyotypically aberrant leukemic cells than nonexposed patients. A variety of abnormal karyotypes were originally reported, including -5/5q-, -7/7q-, +8, +21, t(8;21) and t(9;22) (Mitelman et al., 1978; Mitelman et al., 1981). Differences in frequency between exposed and nonexposed were substantial (83% to 24%) in initial studies. In follow-up studies, the differences, although still significant, generally have not been as strong. This issue was addressed at the Fourth International Workshop on Chromosomes in Leukemia, and a significantly higher frequency of karyotypically abnormal leukemic cells was seen in the exposed (64%) when compared to the nonexposed (49%) (Mitelman et al., 1984). In this study, -5/5q-, -7/7q- and t(8;21) appeared to be associated with previous occupational exposure. More recent studies generally have seen similar associations for chromosomes 5 and 7 (Cuneo et al., 1992; Fagioli et al., 1992; Zedginidze et al., 1990). Association of lifestyle exposures such as smoking and alcohol consumption with leukemic karyotype have been reported in several studies (Crane et al., 1989; Sandler et al., 1993). In both these studies, cigarette smoking was associated with -7/7q-. Although less information is available for other types of lymphohematopoietic cancers, one study reported that higher frequencies of cytogenetic abnormalities were seen in the lymphoma cells of patients with non-Hodgkin's lymphoma who had a history of exposure to organic solvents (Brandt et al., 1989). The exposed patients had a higher frequency of translocations involving the 14q32 band. Aberrations of 6q- appeared to occur more frequently in the nonexposed, but the difference was not statistically significant ($p=0.08$).

One recent morphologic, immunologic, and cytogenetic study of leukemia (AML) patients with history of exposure to pesticides and organic solvents is particularly noteworthy (Cuneo et al., 1992; Fagioli et al., 1992). In this study, clonal chromosomal aberrations involving chromosomes 5 or 7 were seen more frequently among exposed patients. Myelodysplasia involving multiple cell lineages was seen in assessable patients with chemical exposure but was seen only in a minority of nonexposed individuals. In addition, immunological studies revealed that leukemic cells of 80% of exposed patients were positive for the CD34 stem-cell marker, whereas only 22% of leukemic cells of nonexposed patients were positive for this marker. Exposed patients showed a much lower frequency of remission following conventional chemotherapy. These

studies suggest that leukemias caused by chemical exposure have higher frequencies of chromosomal abnormalities, affect multiple lineages, and more frequently involve the more primitive hematopoietic stem cells.

Activation of one of the *ras* oncogenes, primarily N-*ras* or K-*ras*, has been observed frequently and consistently in MDS and human leukemias (Bishop, 1991; Bos, 1989; Sandberg, 1993). Although mutations in *ras* were detected in only a minority of leukemias induced by alkylated agents (Pedersen-Bjergaard et al., 1988; Yunis et al., 1989), there is some evidence that leukemic cells in other chemically exposed individuals exhibit higher frequencies of *ras* activation than similar cells from nonexposed individuals (Taylor et al., 1992). In the case control studies by Taylor and associates (Taylor et al., 1992), patients with *ras*-mutation–positive AML were more likely to have worked in an occupation with chemical exposure, and the risk was higher in those who had worked for 5 or more years in an exposed profession. The *ras*-positive patients also were more likely to have had dermal exposures to chemicals, to have breathed chemical vapors, and to have worked in a dusty environment.

4.2. IONIZING RADIATION

4.2.1. Background

Shortly after the discovery of X rays by Roentgen in 1895, injuries resulting from overexposure to radiation became apparent. Initial reports primarily were skin reactions. Since that time, ionizing radiation has been shown to affect a wide range of tissues and organs, with the bone marrow and lymphoid tissues among the most severely affected (Upton, 1993). A rapidly delivered whole-body dose of 2 to 3 Sievert (Sv.; 1 Sv = 100 rem) results in extensive killing of lymphocytes and their precursors, with manifestations of severe lymphopenia and immunosuppression within 48 hours (Upton, 1993). Extensive killing of hematopoietic cells also occurs, leading to aplasia within the marrow and a decrease in granulocyte and platelets. Regeneration of the bone marrow varies markedly among individuals and is related to the dose, type of radiation, and extent of exposure. A number of studies have shown that regeneration takes place primarily during the first 1 to 2 years following radiotherapy, but in some cases the irradiated area may show hypoplasia for as long as 13 years after exposure (Parmentier et al., 1988).

4.2.2. Hematopoietic and Lymphoid Neoplasia Seen in Humans

Chronic exposure to lower levels or high exposures over a short time period has been associated with a variety of cancers: skin carcinomas were seen in X-ray workers; leukemias, breast cancer, and thyroid cancer in radiologists; osteogenic sarcomas in radium dial painters; and lung cancer in miners (Upton, 1993; Wang et al., 1988). The strongest association between radiation and cancer has been seen for the induction of leukemia. Numerous population studies have shown an association between ionizing radiation and various types of leukemia and lymphoma (BEIR V, 1990; UNSCEAR, 1994). The actual risk of these malignancies following exposure to radiation appears to be complex and is related to the type of radiation, the dose, the proportion of the body exposed, and the extent of cell killing and DNA repair (Curtis et al., 1994). Such

factors as age, gender, genetic background, and physiological condition of exposed individuals also can influence the risk of radiation-induced cancer (BEIR V, 1990). In lymphoid or hematopoietic tissues that are distributed diffusely, a significant proportion of tissue must be irradiated to increase the incidence of neoplasia (Storer et al., 1982). Reduced risks of leukemia also have been seen in atomic-bomb survivors who received high doses [above 3 to 4 Gray (Gy)] of primarily gamma radiation that has been attributed to extensive killing of marrow-containing progenitor cells (BEIR V, 1990). A similar reduced risk at high doses has been seen in recent years; changes in therapeutic strategy employing high doses within limited fields appear to have reduced the risk of leukemia and altered the shape of the dose-response curve in the high-dose region (Boice et al., 1987; Curtis et al., 1994).

The most extensive information on induction of leukemias and lymphomas has come from studies of Japanese atomic-bomb survivors who were exposed to both gamma, and to a lesser extent, neutron radiation. The latest update of this study group, which was concluded in 1987, included 93,696 survivors and encompassed 2,778,000 person-years of study (Preston et al., 1994). Unfortunately, data collection began 5 years after the bombing, so little information is available about leukemias occurring during that period or about the minimum latency period (Mole, 1990; Preston et al., 1994). In addition, dose estimates for many individuals remain imprecise. In spite of this, a great deal of valuable information on types of neoplasia and variables affecting responses is available from this population. In the 1980s, most cases in the leukemia registry were reclassified using more modern criteria and nomenclature such as the French-American-British (FAB) classification scheme. This reclassification allowed a more accurate identification of the types of lymphoid and hematopoietic neoplasia induced by radiation. Results of previous and recent analysis indicate that acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML), and acute myelogenous leukemia (AML) were the major contributors to the total leukemias seen (Matsuo et al., 1988; Preston et al., 1994). No increase in risk was seen for adult T-cell leukemia (ATL), chronic lymphocytic leukemia (CLL) or Hodgkin's disease (HD) (BEIR V, 1990; Matsuo et al., 1988; Preston et al., 1994; UNSCEAR, 1994). A summary of the hematopoietic and lymphoid neoplasia seen in the Nagasaki A-bomb survivors is shown in Figure 2. The types of leukemia

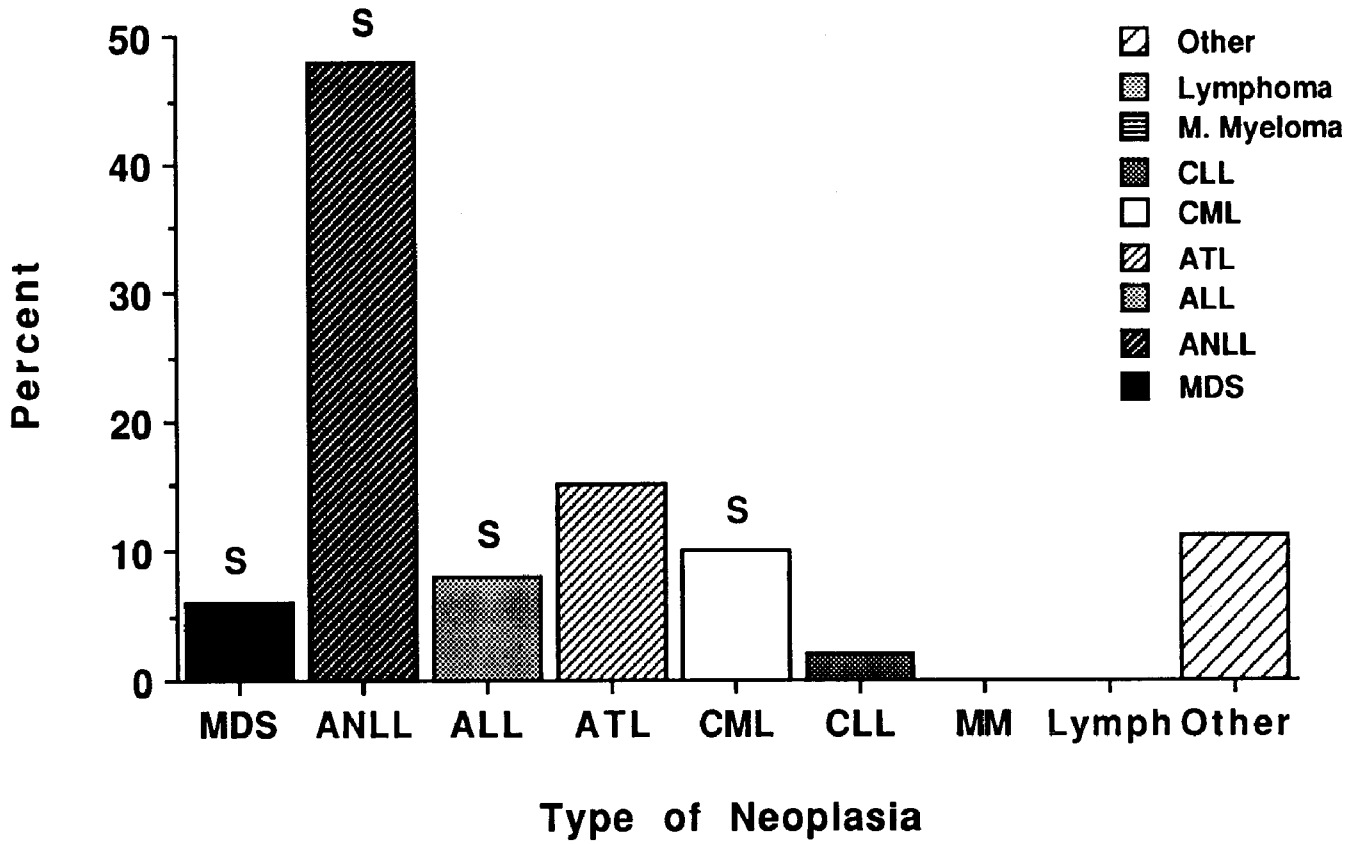

Figure 2. Types of lymphohematopoietic neoplasia in Nagasaki survivors of the atomic-bomb blast. Types of cancer for which evidence is sufficient for association with radiation are labeled with an S. Data are from Matsuo et al., 1988. No information was available from this source for multiple myeloma or lymphomas.

and related disorders that have been consistently or occasionally associated with radiation exposure are indicated.

In bombing survivors, the incidence of total leukemia appeared to increase in a nonlinear fashion and was influenced both by gender and age at exposure (Preston et al., 1994). Young men had the highest excess risks during the period 5 to 10 years after exposure, but these risks decreased rapidly with time. Excess risk during the early time period was not as high in older men but decreased more slowly. Exposed women tended to have lower excess risks than men until about 20 years after exposure. Interestingly, the risk of leukemia appears to decrease for women who were young at the time of exposure, whereas no decrease in risk has been seen in women who were older then (Preston et al., 1994). Evidence also shows that the latency period can be affected by the intensity of radiation exposure (Cadman et al., 1977). Latency periods of around 5 years were observed in survivors located within 1500 meters of the hypocenter, whereas latency periods of 10 years or longer were seen for individuals located at greater distances (Cadman et al., 1977). Additional details for each type of hematopoietic or lymphoid malignancies are described below.

4.2.2.1. *Acute Nonlymphocytic Leukemia (ANLL)*

There is strong evidence for the induction of ANLL in individuals exposed to ionizing radiation as a result of atomic-bombing or therapeutic uses of radiation (BEIR V, 1990; Preston et al., 1994; UNSCEAR, 1994). All FAB subtypes were represented at lower and intermediate doses. However, at the highest doses promyelocytic leukemia (M3) and erythroleukemia (M6) were not seen among those exposed. Acute myelogenous leukemia (M2) was the FAB subtype most strongly associated with radiation exposure (Matsuo et al., 1988). Recent studies conducted on atomic-bomb survivors have indicated that risk was highest in those exposed when young, and those risks decreased with time. Considerably lower risks were seen in survivors who were over 20 years of age at the time of the bombing, but there is no evidence that those risks have decreased with time (Preston et al., 1994). Similar age-related findings have been seen in other studies (UNSCEAR, 1994).

4.2.2.2. *Chronic Myelogenous Leukemia (CML)*

Significant increases in CML were seen in survivors of both Hiroshima and Nagasaki, and the risk appears to be linear with dose (Preston et al., 1994). Men had greater CML risks that decreased rapidly with time. Risks for females were much lower but continued to be elevated 25 years after exposure. Similarly to ANLL, CML occurrence was pronounced in the young (Ichimaru et al., 1986). In addition, the risk of CML, which was higher for residents of Hiroshima than Nagasaki, may be related to differences in the background incidence of CML in the two areas (Preston et al., 1994). Increased risks of CML also have been seen in patients treated with X rays for ankylosing spondylitis during the mid-1930s to 1950s, as well as in radiologists using X rays before the implementation of modern safety standards (BEIR V, 1990; Finch and Linet, 1992).

4.2.2.3. *Acute Lymphocytic Leukemia (ALL)*

Significant increased risks for ALL were seen in exposed survivors. Both age of exposure and gender (to a lesser extent) seemed to influence the excess risk that was seen, with somewhat lower risks observed in older individuals and in women (marginal significance)(Preston et al., 1994). The increases in ALL occurred most frequently in those who were less than 15 years old at the time of the bombing, with the incidence peaking within about 8 years and then decreasing. In adults, the induced ALL occurred late in life at about half the peak frequency of children. These periods of elevated risk correspond to periods during which ALL typically is seen in humans (Pendergrass, 1985).

4.2.2.4. *Adult T-Cell Leukemia (ATL)*

No significant association was seen between radiation exposure and adult T-cell leukemia (Preston et al., 1994). This is particularly interesting in that the human T-cell leukemia virus, HTLV-1, is endemic to the Nagasaki region. About 30% of leukemia cases diagnosed in Nagasaki as part of the long-term survivor study were diagnosed as ATL whereas about 0.5% of leukemias in Hiroshima were ATL (Preston et al., 1994). The lack of an observed association between ATL and radiation exposure suggests that radiation exposure does not interact with HTLV-1 to increase the incidence of leukemia. In addition, recent studies have seen no evidence for an association between radiation exposure and infection with HTLV-1 (Matsuo et al., 1995).

4.2.2.5. *Multiple Myeloma*

At present, there appears to be limited evidence for an association between multiple myeloma and radiation exposure. Although a considerable number of studies have reported increased frequencies of multiple myeloma in radiation-exposed individuals, the increases generally have been modest and frequently have not achieved statistical significance (BEIR V, 1990; UNSCEAR, 1994). More recent evaluations and reanalyses of previous studies have found much less evidence for an association (Preston et al., 1994; UNSCEAR, 1994). As an example, recent analysis of atomic-bomb survivors have found no significant association between radiation exposure from the bombs and the risk of multiple myeloma (Preston et al., 1994). This finding is in contrast to previous reports that were based on mortality rather than incidence. The differences are believed to be related to reliance on questionable diagnoses in the earlier studies as well as the inclusion of high-dose cases and cases with second primary tumors that were excluded from the more recent analysis (Preston et al., 1994). A more detailed review of myeloma and lymphoma cases is planned to clarify these issues (Preston et al., 1994).

4.2.2.6. *Non-Hodgkin's Lymphoma (NHL)*

There is limited evidence that exposure to high doses of radiation increases the risk of NHL (Boice et al., 1988; Ichimaru et al., 1986; Kobayashi et al., 1990; Neglia et al., 1991; Preston et al., 1994). Initially, some evidence was provided from early prevalence studies (Ichimaru et al., 1986). More recent incidence

studies also have provided some evidence for an association between male atomic-bomb survivors and the development of lymphoma (Preston et al., 1994). No association was seen for females, and little evidence was seen for an influence of time or age at exposure (Preston et al., 1994). Elevated risks of lymphoma also have been seen following radiotherapy to treat cervical cancer (Boice et al., 1988) and ankylosing spondylitis (Darby et al., 1985). However, other studies have not seen any increase (UNSCEAR, 1994). The background frequency of lymphoma is significantly influenced by age and gender (Preston et al., 1994). Although the specific etiology of NHL is currently unknown, it is likely to be related to disturbances of the immune system. Increased risks of NHL have been seen in individuals with immunosuppression resulting from HIV infection, Hodgkin's disease, genetic syndromes, and treatment with immunosuppressive agents associated with organ transplantation (Tucker, 1993). Recent results have indicated that atomic-bomb survivors exhibit an increased prevalence of Epstein-Barr virus reactivation (Akiyama et al., 1993). This is of interest, as previous studies have suggested an association between infection with Epstein-Barr virus and subsequent development of secondary NHL following radiotherapy (Evans and Mueller, 1990; List et al., 1986). This suggests that immunosuppression and immunomodulation (Akiyama et al., 1991) induced in radiation-exposed individuals may play a role in the etiology of radiation-induced NHL. However, studies in laboratory animals have failed to demonstrate a direct relationship between immunomodulating agents and the development of cancer (Schmahl, 1986).

4.2.3. Chromosomal Alterations Observed in Model Systems and Human Biomonitoring Studies

Radiation is a highly potent clastogen that induces structural chromosomal aberrations animals and in human cells both in vitro and in vivo (Bender et al., 1988; Rithidech et al., 1995; Tanaka et al., 1983; Wald and Conner, 1988). Elevated frequencies of chromosomal aberrations such as acentric fragments, micro nuclei, dicentric chromosomes, inversions, and translocations have been seen immediately in lymphocytes and bone marrow of individuals exposed to high levels of radiation (Bender et al., 1988; Lucas et al., 1992; Tanaka et al., 1983). Unstable alterations such as acentric fragments, micro nuclei, and dicentric chromosomes decrease with time, whereas the frequency of stable alterations, primarily translocations and inversions, remains relatively stable for years and may be detected 30 to 40 years later (Lucas et al., 1992; Straume et al., 1992). Studies of chromosomal alterations in peripheral blood lymphocytes of highly exposed atomic-bomb survivors conducted many years after bombing have shown that the breakpoints in lymphocyte chromosomes of these individuals did not occur randomly throughout the genome (Kamada et al., 1988; Tanaka et al., 1983). Of particular note is that a number of these nonrandom breakpoints (e.g., 5q31, 7q32, 11q23, 21q2) lie within or immediately adjacent to regions that are altered in therapy-related and de novo leukemias and are believed to indicate the location of genes involved in leukemogenesis (Pedersen-Bjergaard and Rowley, 1994). The 7q32 region also has also been associated with lymphomagenesis (Offit et al., 1991; Offit et al., 1995). Elevated frequencies of alterations affecting 5q31, 7q22, and 21q22 also have been seen in the lymphocytes of ankylosing spondylitis patients many years after radiotherapy (Buckton, 1983). Translocations involving 7q32-36 also were seen in the lymphocytes of technicians with long-term radiation

exposure (Kumagai et al., 1991). In addition, nonrandom breaks have been observed at 6q21 in lymphocytes of atomic-bomb survivors. This region has been implicated in a variety of lymphoid malignancies (Johansson et al., 1993).

4.2.4. Genetic Alterations in Cancer Patients

4.2.4.1. *Chromosomal Alterations*

Cytogenetic analysis were performed on 25 leukemic patients with a history of radiation exposure greater than 0.01 Gy (Kamada et al., 1988). The ten patients with the highest exposure (>2 Gy) exhibited abnormal clones, and the aberration frequency was higher than that of patients with lower exposures. Abnormalities affecting chromosomes 5 and 8 seemed to predominate. There is also a report of a radiation-exposed preleukemia case that exhibited a 7q- (Ichimaru et al., 1986). Other studies have been performed on patients who have developed ANLL following radiotherapy. Although relatively few studies are available, karyotypeas of these patients generally exhibit the same pattern of nonrandom aberrations (-7, -5, 5q-, 7q-) as seen in patients treated with alkylating agents (Kantarjian and Keating, 1987; Le Beau et al., 1986; Philip and Pedersen-Bjergaard, 1988; Sandberg, 1990). Although alterations affecting chromosomes 5 and 7 have been reported to occur less frequently than in patients treated with chemotherapy (Kantarjian and Keating, 1987), this is difficult to evaluate because relative risks are low following radiotherapy as currently performed (Philip and Pedersen-Bjergaard, 1988). Consequently, many observed cases are likely to represent de novo leukemias. In the relatively few cases for which cytogenetic analysis are available, the 9;22 translocation generally seen in leukemic cells of radiation-exposed CML patients is similar to what is commonly seen in de novo CML (Ichimaru et al., 1986; Kamada et al., 1988; Philip and Pedersen-Bjergaard, 1988; Sandberg, 1990). Interestingly, a 9;22 translocation also was seen in a radiation-exposed leukemia patient with AML (M2)(Kamada et al., 1988).

4.2.4.2. *Other Genetic Changes*

The association between chromosomal abnormalities and leukemia development has been investigated further. The transforming activity of DNA extracted from bone marrow of A-bomb survivors with high frequencies of nonclonal chromosomal aberrations and nine leukemia patients previously exposed to radiation, (three with CML, five with AML, and one with ALL) was tested using an in vivo selection assay (Kamada et al., 1988). The DNA was transfected into NIH3T3 cells and subsequently injected into nude mice. Tumors were formed in all mice injected with DNA from the leukemic patients and in 3 of 4 mice injected with DNA from heavily exposed survivors. Activated N-*ras* genes were detected in 7 of 9 tumors formed from leukemic patient DNA and 2 of 3 tumors from exposed-survivor DNA. As a control 5 DNA samples from healthy medical students were tested in the same way, and no positive results were obtained.

4.2.5. Hematopoietic and Lymphoid Neoplasia Seen in Rodent Models

4.2.5.1. *Thymic Lymphoma*

Although ionizing radiation is myelotoxic in all tested mammalian models, considerable variability has been observed in the incidence of tumors and types of lymphohematopoietic neoplasia observed in animal experiments (see UNSCEAR, 1977, UNSCEAR, 1993, and references therein for reviews). Differences in response have been seen for different species, genders, and strains, thus raising questions about the appropriateness of any one strain for predicting response and dose-response in humans (UNSCEAR, 1993). The mouse has been used most widely to study radiation-induced leukemias and lymphomas, whereas the rat has been used more widely for studies of radiation-induced breast cancer (Yokoro et al., 1986). In general, the rat is considered to be a poor model for radiation-induced lymphohematopoietic cancers (Gross and Dreyfuss, 1979; Ward et al., 1990). As a result, this article will focus primarily on the induction of leukemias and lymphomas in mice.

As observed in humans, lymphoid and hematopoietic tissues exhibit severe damage following high doses of whole-body irradiation to rodents (Storer et al., 1982). Decreases are seen in all cell lineages, with lymphocytes showing high sensitivity and related effects on both humoral and cellular immune responses. Cell regeneration begins rapidly and takes place primarily during the first 2 years after exposure (Parmentier et al., 1988). However, studies have shown that the bone-marrow multipotential stem-cell pool is reduced 6 to 18 months after whole- or partial-body irradiation and that hematopoietic activity of irradiated areas does not recover completely (Parmentier et al., 1988).

The thymus is also one of the most sensitive tissues to tumor induction by radiation in many mouse strains (Yokoro et al., 1986). For example, doses of certain types of ionizing radiation as low as 0.25 Gy can induce thymic lymphoma in sensitive mouse strains such as the RFM strain (Storer et al., 1982). The C57Bl/6 strain is another radiation-sensitive strain that has been widely used for mechanistic studies. Following whole-body X irradiation administered in fractionated doses, a high incidence of thymic lymphoma (>90%) was observed in C57BL/Ka mice (Sen-Majumdar et al., 1994b). Although the lymphoma develops in the thymus, the initial step in leukemogenesis occurs in bone marrow (Kotler et al., 1994; UNSCEAR, 1977). The relationship between thymus and bone marrow appears to be complex. As described by Furmanski and Rich (1982), irradiation of the thymus itself does not result in lymphocytic leukemias, nor does whole-body irradiation of thymus-intact and bone marrow-shielded–mice. Implants of normal thymuses in previously irradiated, athymic mice serve as the focus for development of thymic leukemia. Preleukemic bone-marrow cells (but not thymocytes) from irradiated mice can transfer the disease to normal recipients. Based on these observations, it appears that the primary leukemogenic event occurs in the bone marrow; the progenitor cells then migrate to the thymus, where maturation and expression of neoplasia occur.

Initially, a viral hypothesis was presented to explain the high incidence of thymic lymphoma following fractionated whole-body irradiation. In initial studies by Kaplan and associates, a thymotropic and lymphomagenic retrovirus, the radiation leukemia virus (RadLV), was isolated from cell extracts of one of the lymphomas (Kaplan, 1967). The original isolate was weakly leukemogenic upon intrathymic injection into

C57Bl/Ka mice. However, following serial passage, the virus became highly leukemogenic and capable of inducing lymphomas in adult mice (Yefenof and Kotler, 1995). Based on the work of Kaplan, the key steps in lymphomagenesis following radiation were outlined by Janowski as described below (Janowski et al., 1990):

1. Activation of a potentially leukemogenic retroviral genome from endogenous proviruses of the host.
2. Involution of the thymus, followed by a regeneration that leads to the reappearance of virus-susceptible cells, normally present in neonates.
3. Impairment of immune surveillance;
4. Possible functional impairment of bone-marrow cells that might allow "normal" reconstruction of the thymus.

However, follow-up studies by a number of investigators failed to demonstrate retroviral expression during X-ray lymphomagenesis (Kotler et al., 1994). It currently appears that activation of RadLV may be critical for only a minority of lymphomas observed following X irradiation and may not be the causative agent (Sen-Majumdar et al., 1994a). As a result, two additional theories have been postulated to explain the induction of T-cell lymphomas by radiation (Kotler et al., 1994): (1) Haran-Ghera proposed that irradiation induces a genetic lesion in premature bone-marrow cells that, upon migration to the thymus, undergo malignant transformation. (2) Lieberman and associates suggested that fractionated radiation activates a leukemogenic factor in the bone marrow that is transmitted to target cells in the thymus. In both these cases, the thymus provides the right microenvironment that permits the development of a mature lymphoma.

More recent studies also have indicated significant differences between murine lymphomas induced by X rays and by RadLV (Kotler et al., 1994; Sen-Majumdar et al., 1994a). Initiation and maturation of virally induced lymphomas appear to occur within the thymus, whereas X-ray lymphomagenesis involves a gradual efflux of progenitor cells (or the leukemogenic factor) from the bone marrow to the thymus (Kotler et al., 1994). One of these bone-marrow–derived preleukemic cells then becomes dominant and eventually develops into a lymphoma. Recent studies have indicated that $1C11^{high}CD3^{+}4^{-}8^{-}$ cells that represent a very small subset of normal thymocytes are either targeted for neoplastic transformation after irradiation or represent the earliest identifiable thymocyte population after the transforming event (Sen-Majumdar et al., 1994a).

4.2.5.1.1. *Genetic alterations in animals.*

4.2.5.1.1.1. *Chromosomal alterations*. Cytogenetic studies of radiation-induced murine thymic lymphomas indicate that trisomy of chromosome 15 is a frequent nonrandom alteration (Adler et al., 1994; Janowski et al., 1990; Klein, 1981). Trisomy 15 has been reported to appear late in the development of neoplastic cells, indicating that this alteration may contribute to tumor progression (McMorrow et al., 1988). McMorrow and associates also have reported the presence of a t(1;5) in lymphomas obtained from irradiated mice (McMorrow et al., 1988). This alteration occurred in 43% of tumors from irradiated animals and

occurred at an earlier stage than trisomy15, suggesting that it may be a relatively early event in radiation lymphomagenesis.

4.2.5.1.1.2. ***Other genetic changes*****.** Similar to that observed in humans, the involvement of the *ras* oncogene has been seen in murine lymphomas induced by radiation in a variety of mice strains (Newcomb et al., 1988). For example, in the C57Bl/6J strain, thymic lymphomas developed in over 90% of mice exposed to gamma radiation, with a latency period of 6 months (Newcomb et al., 1988). Of 12 radiation-induced lymphomas, 2 showed transforming activity in nude mice or in a focus-forming assay. Southern-blot analysis of these transformants demonstrated K-*ras* transforming sequences in 2 of 12 radiation-induced lymphoma DNAs and N-*ras* transforming sequences in 5 of the 12 (Newcomb et al., 1988). Interestingly, exposure of this same strain to neutron radiation induced a lower frequency of activated *ras* oncogenes (4 of 24) and induced a spectrum of mutations different from that seen with gamma irradiation (Sloan et al., 1990). Overexpression of the N-*ras* proto-oncogene in mice has been associated with an increased formation of thymic lymphomas (Mangues et al., 1994). Additional studies of irradiated C57Bl/6J mice showed that 2 of 15 tumors induced by ionizing radiation exhibited mutations in the *p53* tumor-suppressor gene, suggesting that *p53* mutations played only a minor role in development of radiation-induced thymic lymphomas (Brathwaite et al., 1992). However, a more recent study has provided evidence for mutant *p53* involvement in a large proportion of radiation-induced lymphoid cancers (10 of 17 evaluated), particularly in those that are disseminated into a leukemic form (Hsiao et al., 1995).

4.2.5.2. ***Myeloid Leukemia***

In certain strains of mice such as RFM, SLJ/J, or CBA/H, significant increases of myeloid leukemia have been seen at doses in the range of 1 to 5 Gy (Storer et al., 1982). These leukemias generally are myeloid or myelomonocytic in origin, although induction of erythroblastic and megakaryoblastic leukemias has been reported following irradiation (Riches, 1995). The incidence of these leukemias is influenced by genetic background, hormonal status, and rearing environment (UNSCEAR, 1993). In addition, induced frequencies generally are higher in males than in females and tend to be relatively low (Janowski et al., 1990; UNSCEAR, 1993). Peak incidences of 20 to 25% were seen at doses of about 3 Gy and declined at higher doses (UNSCEAR, 1993). At this time, little evidence indicates the involvement of murine retroviruses or other indirect mechanisms in induction of myeloid leukemias following radiation treatment (Janowski et al., 1990).

4.2.5.2.1. ***Genetic Alterations in Animals.***

4.2.5.2.1.1. ***Chromosomal alterations*****.** Alterations affecting mouse chromosome 2 have been seen consistently in myeloid leukemias induced in a number of strains (Hayata, 1983; Hayata et al., 1983; Rithidech et al., 1995; Trakhtenbrot et al., 1988). For example, Hayata evaluated the chromosomes of 52 myeloid leukemias (5 myeloblastic, 22 granulocytic, 17 myelomonocytic, and 8 monocytic) occurring in 5 different strains of male and female mice, 2 of which developed spontaneously and 50 that developed

following radiation exposure (Hayata, 1983; Hayata et al., 1983). In 49 of these cases including the 2 nonirradiated animals, a partially deleted copy of chromosome 2 was observed. The chromosomal segment lying between the C and D regions was commonly missing from all deleted No. 2 chromosomes. In addition, an alteration affecting chromosome 6 was observed in 16 cases. More recent studies by Rithidech and coworkers have shown that a clone of bone-marrow cells containing deletions and translocations affecting mouse chromosome 2 could be seen in mice at various times after radiation exposure (Rithidech et al., 1995). This fraction of cells with stable chromosome 2 lesions progressively increased from 8% of analyzed cells at 3 months to 38% at 24 months, suggesting an expansion of cells containing the chromosome 2 abnormality. All nine mice that developed myeloid leukemia exhibited a deletion of the D and E regions of chromosome 2. These results suggest that genes within or close to mouse chromosome regions D and E play a significant role in developing radiation-induced myeloid leukemia in the mouse. In addition to chromosome 2, loss of the Y chromosome frequently occurs as a late event in leukemogenesis in male mice (Wright, 1995).

Little is known about the genes involved in myeloid leukemogenesis in the mouse. A homeobox gene *Hox 4.1* has been mapped to the D region of mouse chromosome 2, and a potential role for its involvement in myeloid transformation has been suggested (Janowski et al., 1990; Rithidech et al., 1995). In addition, Silver and colleagues have reported a deregulation of *IL-1*β in 3 murine AMLs involving a rearrangement of mouse chromosome 2 (Janowski et al., 1990; Silver et al., 1989). No such effect was seen in the closely linked *IL-1 alpha* gene, the *beta* 2 *microglobulin* gene, or the *c-abl* oncogene located on chromosome 2 (Janowski et al., 1990; Silver et al., 1988). Based on the genetic homology of D and E regions on mouse chromosome 2 with the 2q24-32 and 11p11-13 regions of human chromosomes, the possible involvement of the *WT1* tumor-suppressor gene located at 11p13 has also been proposed (Rithidech et al., 1995). In addition, some homology between the distal portion of mouse chromosome 2 and the long arm of human chromosome 20 suggests a possible relationship to the 20q13 deletion seen occasionally in de novo and therapy-related human leukemias (Pedersen-Bjergaard and Rowley, 1994; Williamson et al., 1995).

4.3. ALKYLATING AGENTS

4.3.1. Background

Several years after the successful initiation of intensive chemotherapy with genotoxic agents, an increase in treatment-related cancers began to appear (Seiber and Adamson, 1975). Initially, this increase consisted primarily of ANLL. However, with increased periods of follow-up, increases in other types of solid tumors have been observed (Boffetta and Kaldor, 1994; Loescher et al., 1989; Tucker et al., 1988; van Leeuwen et al., 1994). With implementation of new treatment combinations and therapeutic strategies, an increasing number of agents have been recognized as inducing hematopoietic and lymphopoietic neoplasia in humans. Indeed, drugs used in cancer chemotherapy comprise most agents identified by IARC and other groups as human leukemia-inducing agents (see Table 3). Currently therapy-related leukemias comprise 10 to 20% of leukemia cases seen at major institutions (Pedersen-Bjergaard et al., 1995). Identification of specific agents involved in leukemogenesis and interpretation of many of the studies can be challenging due

to the use of multiple therapeutic agents, varying dosing regimens, supplemental use of radiotherapy, and variable periods of patient follow-up. Based on a large number of studies, two main classes of leukemogenic chemotherapeutic agents have been identified—alkylating agents and topoisomerase inhibitors (Pedersen-Bjergaard and Philip, 1991; Pedersen-Bjergaard and Rowley, 1994). This section will focus on the types and unique characteristics of hematopoietic neoplasia induced by alkylating agents, whereas the following two sections will describe leukemias resulting from treatment with two different classes of topoisomerase inhibitors.

4.3.2. Hematopoietic and Lymphoid Neoplasia Seen in Humans

Numerous studies conducted over many years have demonstrated that patients treated with alkylating chemotherapeutic agents are at an increased risk of MDS and ANLL (Levine and Bloomfield, 1992; Smith et al., 1994a). These agents exhibit varying degrees of myelotoxicity and immunotoxicity, which are affected by the agent, dose, and route of administration (Gale, 1988). Increased risks of leukemia appear 1 to 2 years after treatment and remain elevated for 6 to 8 years after chemotherapy ends (Pedersen-Bjergaard and Philip, 1991). An example of the types of lymphohematopoietic neoplasia observed in Hodgkin's disease patients following treatment with alkylating agents is shown in Figure 3. FAB subtypes M1 and M2, acute myeloblastic leukemia with or without maturation, have been associated most commonly with prior treatment with alkylating agents although all FAB subtypes of

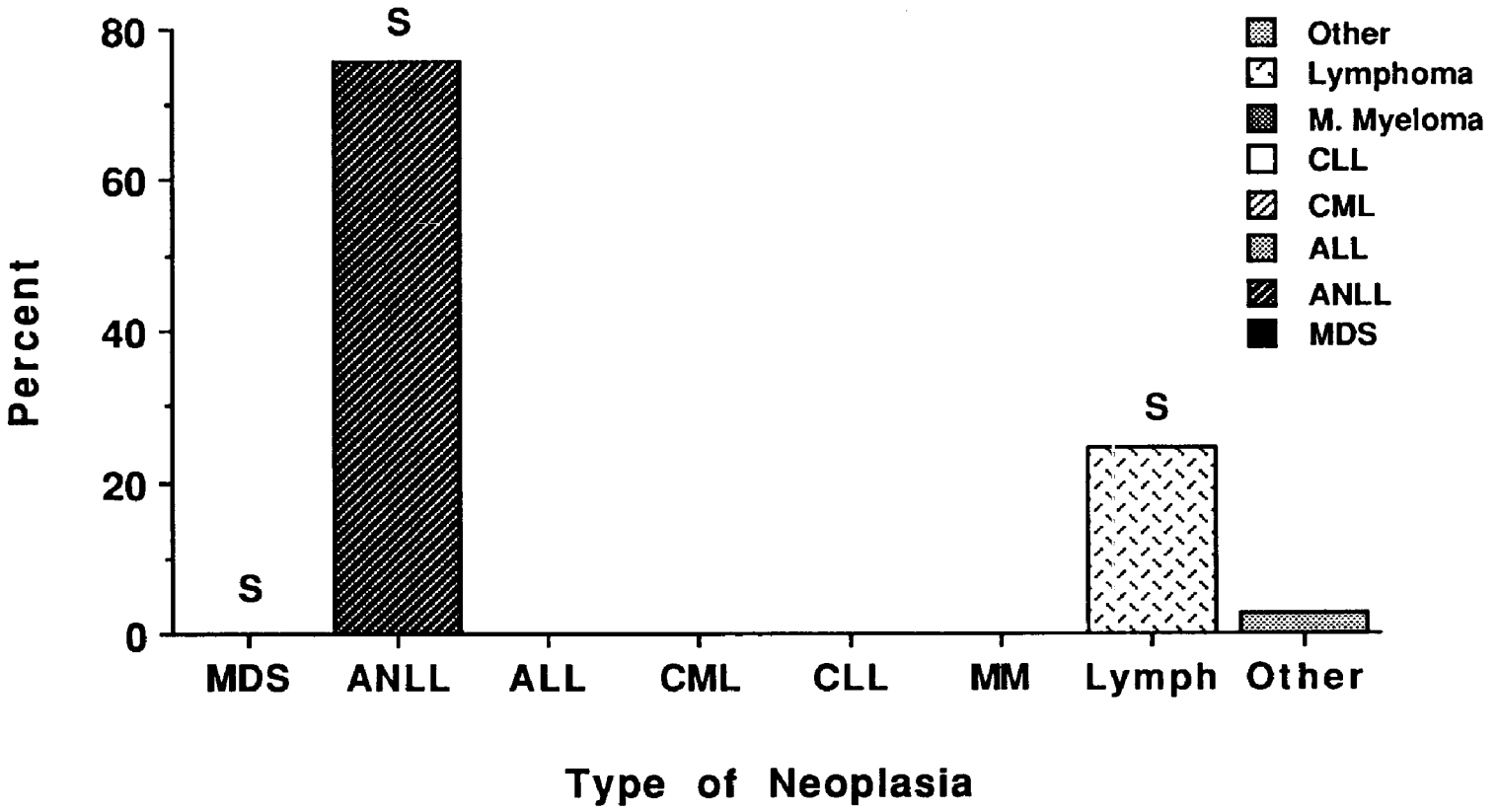

Figure 3. Types of lymphohematopoietic neoplasia observed in Hodgkin's disease patients following chemotherapy and radiation. Alkylating agents commonly are used for this type of chemotherapy. Types of cancer for which evidence is sufficient for association with alkylating agent–based chemotherapy are labeled with an S. Data are from Kaldor et al., 1990. No information was available from this source for myelodysplastic syndromes, multiple myeloma, or lymphomas.

ANLL, with the exception of promyelocytic leukemia (M3), have been observed periodically (Ellis et al., 1993; Pedersen-Bjergaard and Rowley, 1994). A weak but significant increase in NHL has been reported in numerous studies of Hodgkin's disease patients following treatment with chemotherapeutic agents and radiotherapy (Boffetta and Kaldor, 1994; Boivin et al., 1995; Koletsky et al., 1986; Sont et al., 1992; van Leeuwen et al., 1994; van Leeuwen et al., 1989). Although elevated risk may be related to genetic susceptibility and related immunosuppression (Tucker, 1993), a significant role for chemotherapy (particularly in combination with radiotherapy) has been seen in a number of studies (Boivin et al., 1995; Koletsky et al., 1986; Sont et al., 1992; van Leeuwen et al., 1994; van Leeuwen et al., 1989). Therapy-related NHL generally has a latency period of 5 to 6 years, similar to that seen with myelogenous leukemias (Weisenburger, 1994). However, treatment-related NHL cases can be seen 15 or more years after treatment (Henry-Amar and Somers, 1990; Tucker et al., 1988; van Leeuwen et al., 1989; Weisenburger, 1994). In addition, a recent review of the literature has indicated that therapy-related ALL makes up 5 to 10% of observed cases of secondary leukemias (Hunger et al., 1992).

As described by Ellis and colleagues (Ellis et al., 1993), clinically the myelogenous leukemias can be described as panmyelosis with trilineage involvement. A significant proportion of patients exhibit pancytopenia, and examination of bone marrow reveals features of myelodysplastic syndrome. Transformation into an acute leukemia typically occurs within 12 months following the diagnosis of myelodysplastic phase. Observed leukemias generally are refractory to treatment, progress rapidly, and are often lethal. Secondary ANLL is preceded by MDS much more commonly (30 to 80% of the time) than seen with de novo ANLL (20%) (Levine and Bloomfield, 1992). The median latency period for this type of therapy-related ANLL is about 4 to 5 years including the myelodysplastic phase (Levine and Bloomfield, 1992; Smith et al., 1994a). However, evidence shows that the latency period can be affected by treatment intensity, with shorter latency periods observed in higher doses and more intensive chemotherapy, whereas less intensive treatment is associated with longer latency periods (Cadman et al., 1977).

4.3.2.1. *Factors Influencing Risk of Leukemia*

The risk of developing ANLL has been shown to be proportional to dose; at least for patients treated for Hodgkin's disease, increasing age (over 40) is associated with increased risk (Levine and Bloomfield, 1992; Pedersen-Bjergaard and Rowley, 1994). The risk appears to be less in children than adults, with younger children exhibiting lower risks than older children (Levine and Bloomfield, 1992). In many cohorts of patients treated with alkylating agents, the cumulative risk of developing treatment-related leukemias has been shown to increase by about 0.5 to 1% per year (over the first 8 to 10 years) (Pedersen-Bjergaard and Rowley, 1994). Patients receiving alkylating agents have a much higher risk of therapy-related leukemia than de novo leukemia, with reported relative risks of 100 to 300 (Pedersen-Bjergaard and Philip, 1991; Pedersen-Bjergaard and Rowley, 1994). Recent studies have investigated the potential role of genetic polymorphisms affecting genes involved in detoxication of alkylating agents or in repair of DNA adducts and their relation to risks of treatment-related myelodysplastic syndrome (MDS) and ANLL (Chen et al., 1996; Kyrtopoulos,

1995). In one recently conducted study, individuals exhibiting the glutathione-S-transferase theta 1 null phenotype exhibited a 4.3-fold increased risk of MDS following chemotherapy (Chen et al., 1996; Chen et al., 1995). No increased risk was seen for patients with the glutathione-S-transferase M1 genotype. In other studies, a protective role of O^6-methylguanine-DNA methyltransferase, an enzyme involved in repair of alkylating agent–induced DNA damage, was seen in the accumulation of O^6-methylguanine, a premutagenic lesion, in patients treated with alkylating agents (Kyrtopoulos, 1995). In addition, Sagher and associates reported that Hodgkin's disease survivors who were treated with an alkylating agent and later developed ANLL tended to have lower blood lymphocyte levels of O^6-methylguanine-DNA methyltransferase than similarly treated patients who did not develop ANLL and untreated control subjects (Kyrtopoulos, 1995; Sagher et al., 1988).

4.3.3. Chromosomal Alterations Observed in In Vitro and Human Biomonitoring Studies

Cytogenetic investigations of cancer patients following administration of alkylating agents have detected significantly elevated frequencies of structural chromosomal aberrations in peripheral blood lymphocytes at various times following chemotherapy (Lambert et al., 1984; Mamuris et al., 1989a; Reeves et al., 1985; Robison et al., 1982; Seiber and Adamson, 1975). Aberration frequency has been reported to be related to chemotherapy intensity and duration (Lambert et al., 1984; Lawler et al., 1982). As with radiation, many of these aberrations are unstable and decrease with time (Haglund et al., 1980; Robison et al., 1982). However, elevated frequencies of chromosomal aberrations, principally translocations, can be detected in patients' cultured lymphocytes for years following treatment (Haglund et al., 1980; Lambert et al., 1984). Some studies have indicated that induced aberrations occurred nonrandomly, with higher frequencies in chromosomes 5, 7, 9, and 11 (Mamuris et al., 1989a). A few of these persistent breakage-prone regions (7q31, 5q23) were same or immediately adjacent to regions implicated in therapy-related leukemogenesis (Mamuris et al., 1989a). Similar findings have been reported in in vitro studies showing that alkylating agents used clinically can be highly clastogenic, with breakage affecting regions implicated in carcinogenesis (Aurias, 1993; Brogger, 1977; Mamuris et al., 1989b; Seiber and Adamson, 1975).

Alkylating agents generally exhibit a wide range of such genotoxic effects as potent mutagenicity in standard mutation assays, sister chromatid exchanges (SCEs), and clastogenicity in cytogenetic assays (Ashby, 1992; IARC, 1987b; Sorsa et al., 1992). Alkylation of both proteins and nucleotides can occur, but binding to DNA, particularly at such sites involved with base pairing as the O^6 position of guanine, is believed to play a critical role in carcinogenesis (Dumenco et al., 1993). Recent studies have shown the importance of DNA alkylation and repair in formation of murine thymic and splenic lymphomas (Dumenco et al., 1993). The expression of a single human O^6-alkylguanine-DNA alkyltransferase gene in transgenic mice reduced lymphoma frequency from 71% in the MNU-treated nontransgenic mice to 16% in the treated transgenic littermates (Dumenco et al., 1993). A relationship between human O^6-alkylguanine-DNA alkyltransferase activity and SCE formation in human lymphocytes treated in vitro was also seen for bifunctional, but not monofunctional, alkylating agents (Schwartz et al., 1989).

4.3.4. Genetic Alterations in Cancer Patients

4.3.4.1. *Chromosomal Alterations*

Cytogenetic studies of therapy-related leukemias resulting from treatment with alkylating agents have shown that a high frequency (70 to 97%) of leukemic cells exhibit clonal chromosomal aberrations (Levine and Bloomfield, 1992; Pedersen-Bjergaard and Philip, 1991). In contrast, a large percentage (~45%) of cells from de novo MDS and ANLL exhibit a normal karyotype (Pedersen-Bjergaard et al., 1995; Pedersen-Bjergaard et al., 1993). Karyotypes of these therapy-related leukemias are characterized by unbalanced chromosome aberrations, most commonly loss of chromosomes 7 and 5 or loss of their long arms (5q- and 7q-) (Kantarjian and Keating, 1987; Pedersen-Bjergaard et al., 1995). Deletions affecting 17p, 12p, and 20q; loss of chromosome 18; trisomy 8 (usually as a subclone); and a 1q duplication (usually associated with a chromosome 7 alteration) also have been reported (Pedersen-Bjergaard et al., 1995). These alterations, with the exception of trisomy 8, typically are seen in a much smaller proportion of de novo leukemias (Pedersen-Bjergaard and Rowley, 1994; Walker et al., 1994). Deletions breakpoints are variable but almost always include one region believed to be critical for leukemogenesis (Pedersen-Bjergaard and Rowley, 1994). The chromosome 5 critical region has been determined to be 5q31, whereas several regions including 7q22 and 7q31-qter have been proposed for chromosome 7 (Johansson et al., 1993; Kantarjian and Keating, 1987; Kere et al., 1989; Pedersen and Ellegaard, 1994). Identification of critical genes deleted in these regions is currently the focus of intensive investigation. Interestingly, a large number of genes involved in hematopoiesis and lymphopoiesis have been localized within or adjacent to these critical regions (Huebner et al., 1985; Lemieux et al., 1994; Logan et al., 1989; Offit et al., 1995; Pedersen-Bjergaard et al., 1995; Pedersen-Bjergaard and Rowley, 1994; Pettenati et al., 1987; Simeone et al., 1994; Willman et al., 1993).

4.3.4.2. *Other Genetic Changes*

Among the many genes involved in carcinogenesis, activation of one of the *ras* oncogenes, primarily N-*ras* or K-*ras*, has been frequently and consistently observed in MDS and human leukemias (Bishop, 1991; Bos, 1989; Sandberg, 1993). Mutations in N- and K- *ras* genes observed in about 20 to 30% of de novo leukemias, represent some of the most frequent genetic aberrations in these tumors (Neubauer et al., 1994). However, there is much less evidence for the involvement of *ras* oncogenes in chemical-induced leukemias; activation of these genes does not appear to play an essential role in leukemogenesis (Levine and Bloomfield, 1992; Yunis et al., 1989). In a study of patients with treatment-related ANLL, Pedersen-Bjergaard and colleagues detected a *ras* mutation—a G-T transversion in codon 13 of the N-*ras* gene—in only 1 of the 13 patients studied (Pedersen-Bjergaard et al., 1988). Consistent with this is the observation that *ras* mutations are found preferentially in myelomonocytic (M4) and monocytic (M5) subtypes of leukemia rather than the myelogenous forms (M1 and M2) that are more commonly seen following chemotherapy with alkylating agents (Bos, 1989; Pedersen-Bjergaard and Rowley, 1994).

4.3.5. Hematopoietic and Lymphoid Neoplasia Seen in Rodent Models

Many alkylating agents used therapeutically have been studied in chronic animal cancer bioassays. As summarized in Table 3, significant increases in lymphoid and hematopoietic cancers have been seen in most bioassays. As indicated above, lymphohematopoietic effects seem to be seen more consistently in mice than rats, and the tumors seen are more commonly lymphoid in lineage rather than myeloid.

4.3.5.1. *Genetic Alterations in Animals*

4.3.5.1.1. ***Chromosomal alterations*.** Limited information is available about types of chromosomal alterations seen in these leukemias or lymphomas. Following treatment with chemical agents, trisomy 15 and trisomy 2 (as well as other alterations affecting this chromosome) have been reported in mouse lymphomas and rat leukemias, respectively (McMorrow et al., 1988; Sugiyama et al., 1978; Wiener et al., 1978). However, an absence of trisomy 15 and the occurrence of a diploid karyotype have been reported in other studies of murine tumors induced by chemical agents (Goodenow et al., 1986).

4.3.5.1.2. ***Other genetic changes*.** Although a number of oncogenes have been reported in spontaneous or transgenic rat and mouse leukemias, much less information is available from studies of chemically induced leukemias and lymphomas. The *ras* oncogene has been the gene most commonly associated with chemically induced murine leukemias and lymphomas. Following a single injection of a potent alkylating agent, N-methylnitrosourea (NMU), to C57Bl/6J mice, thymic lymphomas with a latency period of 3 months developed in over 90% of the mice (Newcomb et al., 1988). Eight of the ten NMU-induced lymphomas showed transforming activity in nude mice and in a focus-forming assay. Southern-blot analysis of these transformants demonstrated K-*ras* transforming sequences in all eight NMU-induced lymphoma DNAs. A single-base transition from a G to A at the second position of codon 12 (exon I) was seen in five of the six tumors that could be evaluated. Follow-up studies indicated that in about half the tumors, the mutant allele was expressed predominantly, suggesting that the normal allele had been lost or that the mutant allele had been amplified (Corominas et al., 1991). Overexpression of the N-*ras* protooncogene in transgenic mice has also been associated with an increased formation of thymic lymphoma (Mangues et al., 1994). Additional studies of NMU-treated C57Bl/6J mice for p53 mutations showed detectable mutations in 2 of 15 tumors induced by NMU (Brathwaite et al., 1992). Based on these results, the authors concluded that *p53* mutations were not likely to play a major role in NMU-induced thymic lymphomas.

4.4. EPIPODOPHYLLOTOXIN-TYPE TOPOISOMERASE INHIBITORS

4.4.1. Hematopoietic and Lymphoid Neoplasia Seen in Humans

Toward the end of the 1980s, patients began to appear with therapy-related leukemia that exhibited features different from those previously seen following treatment with alkylating agents and radiation (Pui et al., 1989). These patients had been treated with etoposide or teniposide, two of the recently developed epipodophyllotoxin-type chemotherapy agents (Pedersen-Bjergaard and Philip, 1991). Since then a large

number of studies have been published indicating an association between these drugs and the subsequent development of leukemia (Haupt et al., 1993; Pedersen-Bjergaard et al., 1995; Smith et al., 1994a). These agents exhibit a moderate degree of myelosuppression and genotoxicity that is mediated by inhibition of topoisomerase II through a process that involves stabilization of the DNA-enzyme complex (Gale, 1988; Smith et al., 1994b). Topoisomerase II is an enzyme involved in a wide variety of cellular functions, including DNA replication, recombination, and repair (Anderson and Berger, 1994). In recent years, evidence has indicated that DNA-intercalating topoisomerase inhibitors, such as the anthracycline derivatives, also may induce secondary leukemias (Blatt, 1995; Quesnel et al., 1993). Leukemias developing after treatment with epipodophyllotoxin-type topoisomerase inhibitors appear from 10 months to 8 years following the initiation of chemotherapy, with a median latency period of 24 to 36 months (Smith et al., 1994b). In addition, the leukemias were primarily of the monocytic or myelomonocytic subtype (M4 and M5) and were rarely preceded by a clinically observed myelodysplastic phase, a pattern that differs significantly from leukemias seen following therapy with alkylating agents or radiation (Pedersen-Bjergaard and Rowley, 1994; Smith et al., 1994b). Occasionally, myelogenous (M2) and promyelocytic leukemias (M3) have been seen (Bhavnani et al., 1994; Haupt et al., 1993; Hoffmann et al., 1995; Horibe et al., 1993; Quesnel et al., 1993). Less frequently, treatment-related ALL has been reported (Felix et al., 1995a; Pui, 1992; Zhang et al., 1996). Although these lymphocytic leukemias are not common, molecular and cytogenetic evidence indicates that they were caused by treatment with topoiSomerase inhibitors (Felix et al., 1995a; Haupt et al., 1993; Kobayashi et al., 1995; Quesnel et al., 1993; Zhang et al., 1996). A profile of the types of hematopoietic neoplasia observed in children following treatment with epipodophyllotoxin-type topoisomerase II inhibitors is shown in Figure 4.

4.4.2. Factors Influencing Risk of Leukemia

Leukemia risk following therapy was reported in to be very high in early studies, with cumulative incidences approaching 19% in some treatment groups (Pui et al., 1989). Interestingly, the risk of a

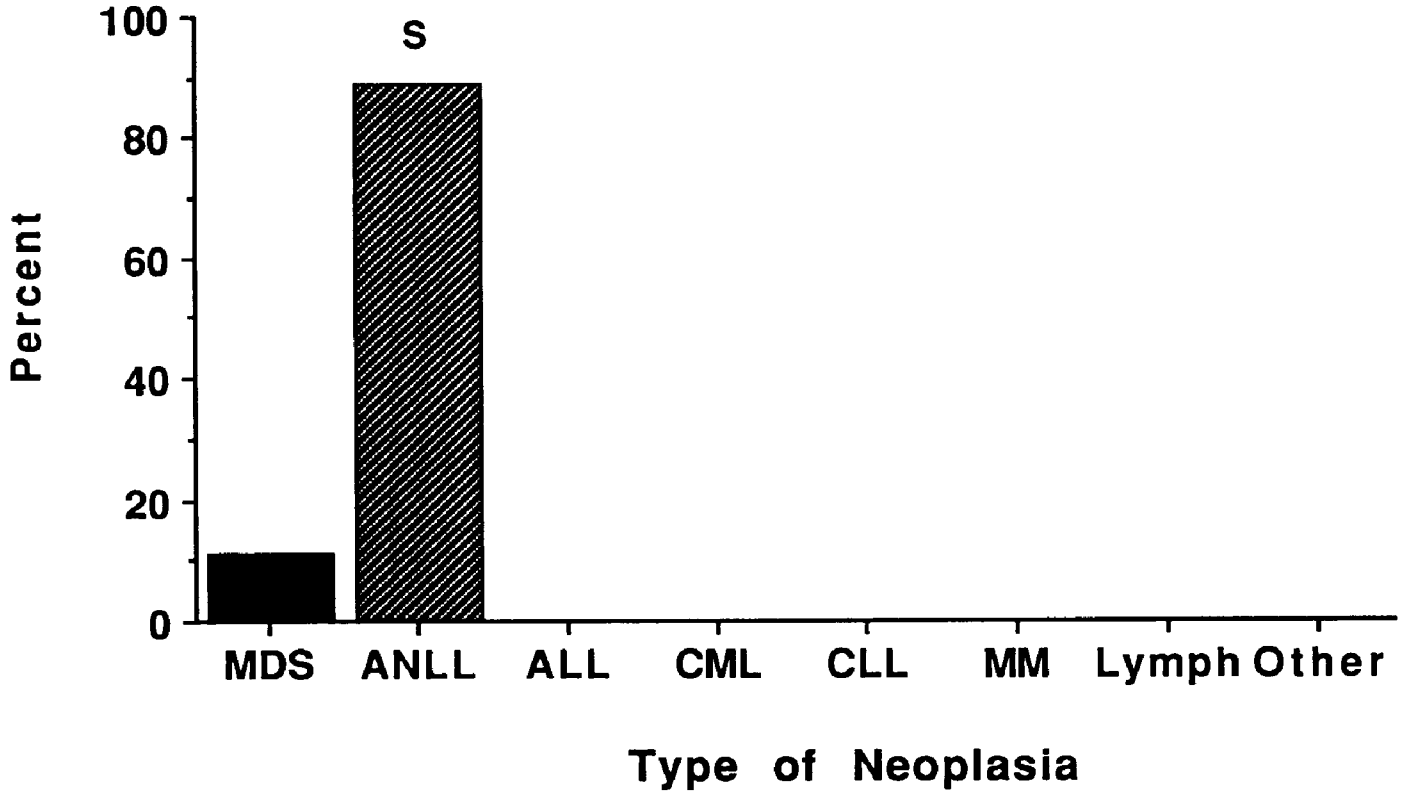

Figure 4. Types of lymphohematopoietic neoplasia observed in children treated with epipodophyllotoxin-type antineoplastic drugs, etoposide, and teniposide. Types of cancer for which evidence is sufficient for association with these drugs are labeled with an S. Data are combined from Stark et al., 1994, and Sandoval et al., 1993. No information was available from this source for multiple myeloma or lymphomas.

secondary cancer appears to be more closely related to the treatment regimen than to total dose (Pui et al., 1991). 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%). With implementation of newer treatment protocols, the risk of ANLL is lower and within the range of that seen with alkylating chemotherapeutic agents (Smith et al., 1993). There also is some evidence that the combined treatment with topoisomerase inhibitors and alkylating agents (or cisplatin) confers a greater risk of secondary leukemia than with either type of chemotherapeutic agent (Blatt, 1995; Sandoval et al., 1993; Smith et al., 1994a).

4.4.3. Chromosomal Alterations Observed in In Vitro and Human Biomonitoring Studies

In vitro studies and short-term animal bioassays have shown that these agents are highly clastogenic, induce aneuploidy, and are effective at inducing deletion-type mutations (Anderson and Berger, 1994; Ashby et al., 1994; Ferguson and Baguley, 1994). Cytogenetic studies of peripheral blood lymphocytes have shown that in vitro etoposide induces a high frequency of reciprocal translocations with elevated alterations affecting chromosomes 1, 11, and 17 (Maraschin et al., 1990; Pedersen-Bjergaard and Rowley, 1994). More recently, in vitro studies have investigated the mechanisms underlying chromosomal breakage at band 11q23 in human chromosomes (Felix et al., 1995b). In these studies, etoposide and teniposide were assayed in the presence of limiting dilutions of the topoisomerase enzyme using subclones containing the normal 11q23 homologue and a t(9;11) translocation breakpoint junction. The strongest cleavage sites coincided with the t(9;11) and two other translocation breakpoint sites within the normal homologue, indicating a direct relationship between DNA cleavage by topoisomerase II and the 11q23 translocation.

4.4.4. Genetic Alterations in Cancer Patients

4.4.4.1. *Chromosomal Alterations*

Cytogenetic studies of patients with these epipodophyllotoxin-related leukemias have shown that the leukemic clone in over 50% of these cases involved a balanced translocation affecting the 11q23 region and another chromosomal partner, usually t(6;11), t(9;11), and t(11;19) (Canaani et al., 1995; Pedersen-Bjergaard and Rowley, 1994; Smith et al., 1994b). In children who have received prior treatment with topoisomerase inhibitors, 80 to 90% of secondary leukemias have a 11q23 alteration (Canaani et al., 1995). Identification of topoisomerase II recognition sites adjacent to translocation breakpoints of participating chromosomes has provided evidence for direct involvement of topoisomerase enzymes in translocation formation (Domer et al., 1995; Gu et al., 1994; Negrini et al., 1993). In addition, the presence of recombinase recognition sites and *Alu* sequences in some cases suggests that aberrant recombination also may contribute to generation of observed translocations (Domer et al., 1995; Gu et al., 1994; Gu et al., 1992; Negrini et al., 1993; Schichman et al., 1994). Less frequently, such translocations involving the 21q22 region as t(8;21) and t(3;21), as well as those affecting chromosomes 15 and 17 (t15;17), have been seen (Haupt et al., 1993; Hoffmann et al., 1995; Horibe et al., 1993; Pedersen-Bjergaard and Rowley, 1994). The

high frequency of translocations affecting the 11q23 region is particularly interesting in that translocations involving this region are frequently seen in de novo leukemias, particularly in children with ALL (Canaani et al., 1995). Translocations affecting the 11q23 region also occur in about 5% of de novo cases of ANLL (Smith et al., 1994b). The 11q23 alterations affecting both treatment-related and de novo leukemias appear to involve common breakpoints, although as many as 27 different cytogenetic abnormalities have been seen (Canaani et al., 1995; Smith et al., 1994b). The 8;12 translocation also is seen frequently in de novo leukemias (Nucifora and Rowley, 1995; Walker et al., 1994).

4.4.4.2. *Hematopoietic and Lymphoid Neoplasia Seen in Rodent Models*

Few, if any, chronic cancer bioassays have been performed to assess the carcinogenic activity of these types of topoisomerase inhibitors in laboratory animals (Anderson and Berger, 1994). Clearly there is a need for animal bioassays, particularly in mice, for these classes of topoisomerase II inhibitors.

4.5. DIOXOPIPERAZINE-TYPE TOPOISOMERASE INHIBITORS

4.5.1 Hematopoietic and Lymphoid Neoplasia Seen in Humans

In recent years, a large number of case reports have been published indicating that a new pattern of therapy-related leukemia occurs in patients who have been administered bis-dioxopiperazine derivatives. These agents also have been shown to be effective inhibitors of topoisomerase II (Ishida et al., 1995; Tanabe et al., 1991) but are believed to exhibit their inhibitory effects through direct effects on the topoisomerase enzyme,rather than through formation of the cleavable complex (Tanabe et al., 1991). Razoxane (ICRF 159), bimolane, and related derivatives (ICRF 154, ICRF 187, ICRF 193, MST-16) have been shown to exhibit antipsoriatic and antineoplastic activity and currently are being used therapeutically or in clinical trials (Caffrey et al., 1985; Gilbert et al., 1986; Li et al., 1989; Xue et al., 1992c; Zhang et al., 1993). Razoxane and related compounds, also reported to reduce cardiotoxicity in cancer patients who were administered adriamycin, are being used increasingly in this manner (Bu'Lock et al., 1993; Jelic et al., 1995; Kolaric et al., 1995).

Bimolane has been used widely in China as an antipsoriatic agent. Over the past 10 years, more than 140 cases of bimolane-induced leukemias have been reported following the therapeutic use of this drug (Zhang et al., 1993). These leukemias exhibit a latency period of 12 to 96 months after therapy initiation, with a median of about 36 months (Xue et al., 1992c; Zhang et al., 1993). However, in a few cases leukemias have developed with latency periods of more than 108 months (9 years). A profile of the types of hematopoietic neoplasia observed in Chinese patients taking bimolane for psoriasis is shown in Figure 5. In this large case series, the subtypes of leukemia reported were promyelocytic (M3 in 67

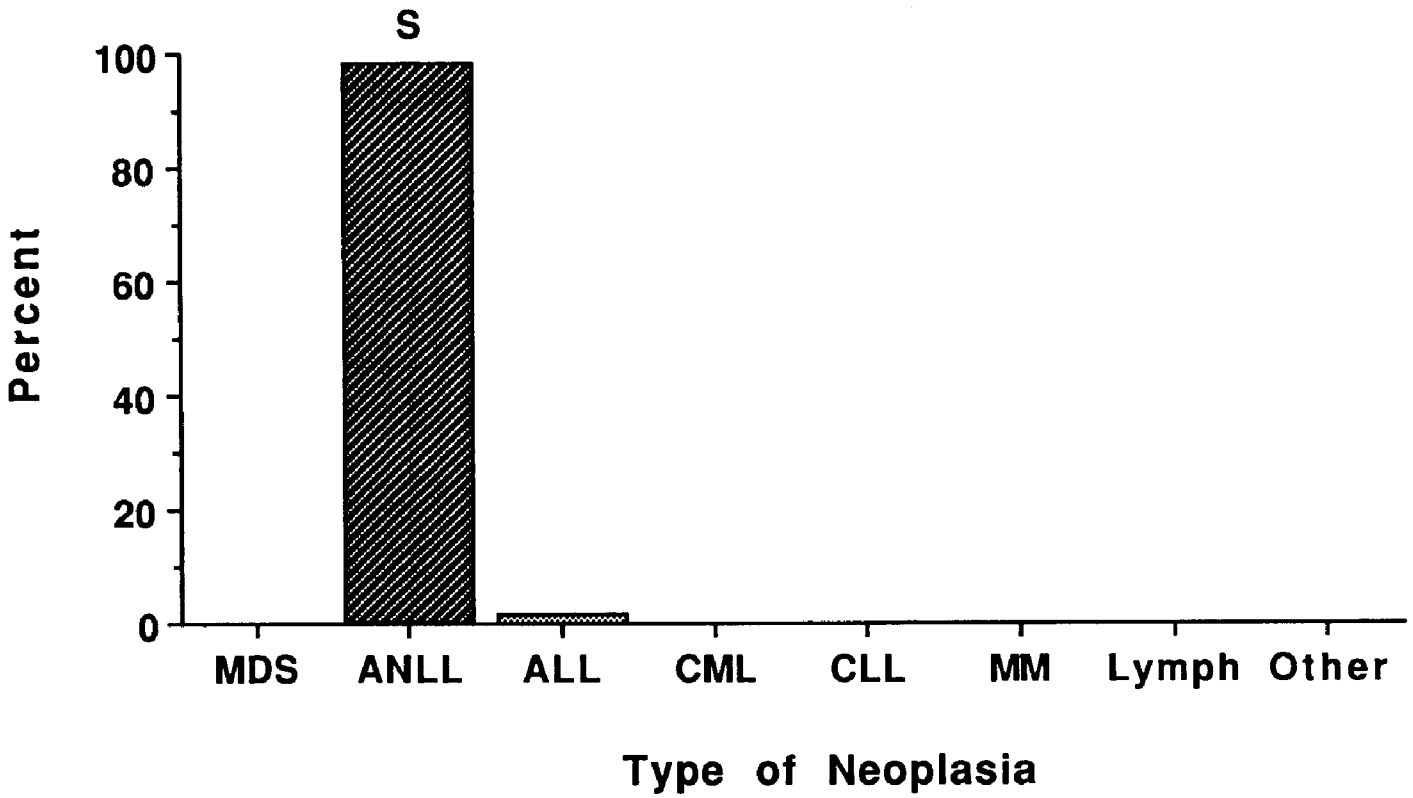

Figure 5. Types of lymphohematopoietic neoplasia observed in psoriasisare patients treated with bimolane. Types of cancer for which evidence is sufficient for association with this dioxopiperazine-type drug are labeled with an S. Data are from Zhang et al., 1993. Data for multiple myeloma and lymphomas do not appear to be presented.

cases for 48%) followed by acute myeloblastic with maturation (M2 in 35 cases for 25%), monocytic (M5 in 24 cases for 17%), myelomonocytic (M4 in 7 cases for 5%), acute myeloblastic without maturation (M1 in 4 cases for 3%), acute lymphocytic (ALL in 2 cases for 1.4%), and acute erythroblastic (M6 in 1 case for 0.7%). An association between bimolane treatment and the development of acute myeloblastic leukemia (M2a) also was reported based on a case control study involving more than 1200 leukemia patients from China (Anemia, 1992). Elevated nonsignificant risks also were seen in this study for other types of ANLL as well as ALL using a conditional logistic model. Although the number of reported cases is much higher for bimolane, similar reports also have indicated an association between therapy with razoxane and ICRF 154 and the development of ANLL (Bhavnani et al., 1994; Caffrey et al., 1985; Gilbert et al., 1986; Kingston et al., 1993; Li et al., 1989). Subtypes reported in these studies were the M3, M2, M4, and M5 subtypes similar to those reported in bimolane-treated leukemic patients.

4.5.2. Chromosomal Alterations Observed in Model Systems and Human Biomonitoring Studies

A number of Chinese studies have demonstrated that bimolane exhibits cytotoxic and genotoxic effects in human lymphocytes treated in vitro (Xue et al., 1992a; Xue et al., 1992b; Xue et al., 1994). Following bimolane treatment, increased frequencies of micronuclei were seen, as were cells exhibiting increases in nuclear abnormalities, cell cycle delay, and abnormal numbers of nuclei indicating that this agent interferes with cytokinesis. Consistent with these observations, increased frequencies of chromosomal aberrations and micronuclei have been observed in lymphocytes of psoriatic patients taking bimolane (Ye et al., 1994). In addition to cytogenetic changes, treated patients were reported to have reduced levels of CD4 T-lymphocytes and IgM levels in their blood (Ye et al., 1994).

4.5.3. Genetic Alterations in Cancer Patients

4.5.3.1. *Chromosomal Alterations*

Cytogenetic analyses were performed on a series of 14 consecutive patients whose leukemia was believed to be caused by bimolane therapy (Xue et al., 1992c). Clonal karyotypic abnormalities were seen in all cases. Eight cases exhibited the t(15;17) characteristic of M3 leukemias and t(8;21) was seen in four cases of M2 leukemia. A deletion of 7q was seen in one case of M4 and one case of M5 leukemia. Preleukemia was observed in only one instance. The reported presence of topoisomerase recognition sites immediately adjacent to t(15;17) translocation breakpoints indicates the direct involvement of topoisomerase II inhibition in the formation of this abnormality (Dong et al., 1993).

4.5.4. Hematopoietic and Lymphoid Neoplasia Seen in Rodent Models

Two studies were located in which the dioxopiperazine-type of topoisomerase II inhibitors were tested in experimental animals. A chronic cancer bioassay on ICRF-159 conducted under the auspices of the National Cancer Institute was published in 1978 (NCI, 1978b). In this study, B6C3F1 mice and Sprague-Dawley rats were administered ICRF-159 by intraperitoneal injection three times a week

for 52 weeks and sacrificed at 86 weeks. A significant increase in uterine adenocarcinomas was seen in female rats. No increase in tumors was seen in the male rats. In the female mice, a significant dose-related increase in lymphoid neoplasms (histiocytic lymphomas, lymphocytic lymphomas, and lymphocytic leukemias) was observed. No significant increase in tumors was seen in the male mice. In a recently published study from China, a significant increase in leukemia was reported (Ye et al., 1994). Of the 40 mice treated with bimolane, 10 developed granulocytic leukemia whereas no leukemias were seen in the control group of 20 mice. Seven of the ten leukemias were described as the immature granulocytic type.

4.6. BENZENE

4.6.1. Background

Benzene is a widely used industrial chemical and ubiquitous environmental pollutant (IARC, 1982; IPCS, 1993) with an annual production of 11.8 billion pounds in the United States. Due to benzene's presence in gasoline and tobacco smoke as well as other consumer products, a large proportion of the population experiences low-level exposure to this agent. Chronic exposure to benzene is associated with pancytopenia, aplastic anemia, and lymphohematopoietic neoplasia in humans (ATSDR, 1992a; IARC, 1982; IPCS, 1993). Chronic exposure of laboratory animals to benzene results in myelotoxicity as well as the formation of tumors at multiple sites, including lymphoid neoplasia (Huff et al., 1989; IARC, 1982; IPCS, 1993).

4.6.2. Metabolic Studies Utilizing Animal and *In Vitro* Systems

Although benzene have a simple structure, identification of the mechanisms by which benzene exerts its toxic and carcinogenic effects has remained elusive (see Kalf, 1987; Snyder et al., 1981; and Subrahmanyam et al., 1991 for more detailed description of the metabolism of benzene). Early animal studies showed that benzene was unlikely to be the actual toxicant but rather required metabolism to exert its toxic effects (Andrews et al., 1977; Gad-El Karim et al., 1986; Sammett et al., 1979; Sawahata et al., 1Õ85). The metabolism of benzene is more rapid and extensive in the mouse than in the rat, and this appears to correlate with the sensitivity of these two species to benzene's myelotoxic and genotoxic effects (Henderson et al., 1992; Sabourin et al., 1988). Early animal studies by Sammett and associates showed that partial hepatectomy resulted in higher levels of benzene in the bone marrow yet protected against toxicity (Sammett et al., 1979). In addition, Irons and coinvestigators showed that very little metabolism of benzene occurred in the bone marrow (Irons et al., 1980; Sawahata et al., 1985). This combination of metabolism in the liver and toxicity in bone marrow suggests that a relatively stable metabolite formed in the liver is transported to bone marrow and exerts its toxic effects.

Subsequent studies have shown that bioactivation in the liver occurs primarily via oxidation by the cytochrome P450 2E1 isozyme leading to the formation of a number of primary metabolites including phenol, hydroquinone, catechol, benzene dihydrodiol, 1,2,4-trihydroxybenzene, and trans, trans-muconic acid (Guengerich et al., 1991; Schlosser et al., 1993; Seaton et al., 1994; Snyder et al., 1981). However, the

administration of these metabolites to mice has failed to produce the myelotoxicity characteristic of benzene (Eastmond et al., 1987). This failure of known benzene metabolites to exhibit extensive myelotoxicity has led scientist to investigate the role of reactive intermediates formed during ring opening such as trans,trans-muconaldehyde, a reactive dialdehyde that rearranges to form trans,trans-muconic acid (Goldstein et al., 1982b; Witz et al., 1985). The administration of high doses of trans,trans-muconaldehyde to mice resulted in moderate bone-marrow damage. The high reactivity of trans,trans-muconaldehyde and such numerous cellular defenses within the liver as glutathione and aldehyde dehydrogenase make it unlikely that this reactive metabolite would be unable to travel from liver interior to bone marrow in significant quantities without being detoxified or reacting with blood constituents (Kline et al., 1993). Recently Witz and coworkers isolated a metabolite formed from trans,trans-muconaldehyde (6-hydroxy-trans,trans-2,4-hexadienal) that is much less reactive and forms a reversible complex with proteins, allowing it to travel to distant sites within the body (Kline et al., 1993). This metabolite also has been shown to be myelotoxic when administered to mice (Zhang et al., 1995b).

Other investigators have proposed that myelotoxic effects of benzene result from interactive effects of various metabolites. Studies by Eastmond, Smith, and Irons demonstrated that coadministration of phenol and hydroquinone to mice resulted in potent myelotoxic effects (Eastmond et al., 1987). Subsequent studies have shown that this combination, as well as other combinations of metabolites, also exhibits significant myelotoxic and genotoxic effects (Barale et al., 1990; Chen and Eastmond, 1995a; Dimitriadis et al., 1988; Guy et al., 1990; Guy et al., 1991; Hu et al., 1990; Kolachana et al., 1993; Marrazzini et al., 1994; Subrahmanyam et al., 1990). A number of mechanisms have been suggested as being responsible for the synergistic effects of phenol and hydroquinone. Eastmond and coinvestigators proposed that the observed synergism might be due to a phenol-induced stimulation of the conversion of hydroquinone to 1,4-benzoquinone in a reaction mediated by myeloperoxidase, an enzyme found at high concentrations in bone marrow (Eastmond et al., 1987; Smith et al., 1989). Toxicity was believed to be mediated through increased formation of 1,4-benzoquinone, a reactive metabolite previously proposed to be involved in benzene's toxic effects (Dustin, 1950; Irons, 1985; Kracke and Parker, 1934). Due to the metabolite’s highly reactive and toxic nature, attempts to measure directly its toxic and genotoxic effects have been largely unsuccessful (El-Mofty et al., 1992; Guy et al., 1991; Irons and Sawahata, 1985; Kracke and Parker, 1934; Yager et al., 1990). Alternatively, genotoxic effects might be related to a pharmacokinetic interaction resulting in higher levels of hydroquinone reaching the bone marrow (Barale et al., 1990; Legathe et al., 1994). Recent studies by Chen and Eastmond on genotoxicity of the phenol-hydroquinone combination have indicated that the interactive effects may not be due simply to higher concentrations of hydroquinone reaching the bone marrow; rather, they may involve an inhibition of such enzymes involved in DNA replication and repair as topoisomerase enzymes, byphenol, hydroquinone, or their metabolites (Chen and Eastmond, 1995a; Chen and Eastmond, 1995b).

4.6.3. Metabolism Studies in Humans

Although much less is known about benzene metabolism in humans, studies have indicated that humans and laboratory animals metabolize benzene along similar metabolic pathways (Cooper and Snyder, 1988; Inoue et al., 1988a; Inoue et al., 1988b; IPCS, 1993; Sabourin et al., 1989). However, some reaction rates may differ (Henderson et al., 1989; IPCS, 1993; Sabourin et al., 1989).

A recent series of investigations conducted by Smith, Rothman, and coinvestigators on Chinese workers highly exposed to benzene are providing valuable insights into the mechanisms underlying benzene myelotoxicity in humans. These studies also are helping to identify groups of individuals who are at increased risk for benzene toxicity. In one series of studies, individual susceptibility to benzene myelotoxicity was determined by evaluating the ability of benzene-exposed workers to metabolize chlorzoxazone to 6-OH-chlorzoxazone, a reaction catalyzed primarily by the cytochrome P450 2E1 isozyme (Rothman et al., 1995a). After administration of small doses of this muscle relaxant, urine samples were collected in 50 workers with a history of benzene poisoning and compared with 50 age- and sex-matched controls. Overall, individuals with the rapid hydroxylator phenotype exhibited a 2.5-fold increased risk of benzene poisoning as compared to those with the slow hydroxylator phenotype. Among cases with a history of high-level benzene exposure, the rapid metabolizer phenotype was associated with a 22.8-fold increased risk. In the second series of studies, Ross and colleagues utilized DNA collected from the Chinese worker study to investigate the potential role of genetic polymorphisms affecting NAD(P)H:quinone oxidoreductase (NQO1) in the susceptibility of workers to benzene (Rothman et al., 1996b). NQO1 (also known as DT-diaphorase), an enzyme that catalyzes a two-electron reduction of quinones to hydroquinones, has been implicated in several in vitro and animal experiments as protecting against quinones formed during benzene metabolism (Ross et al., 1990; Smart and Zannoni, 1984; Zhu et al., 1995). A point mutation in the *NQO1* gene results in a loss of enzyme activity in homozygous individuals. Analyses performed on 38 workers and 35 controls showed that individuals homozygous for this mutation had a 3.2-fold increased risk of myelotoxicity. These results also are consistent with previous animal and in vitro results and identify groups of individuals with increased susceptibility to benzene's hematopoietic effects (Seaton et al., 1994; Smart and Zannoni, 1984). Recent biochemical studies also have suggested a role for *NQO1* in the differential sensitivity of mice and rats to benzene's myelotoxic effects (Zhu et al., 1995). Metabolic activation by P450 2E1 and detoxication of benzene-derived quinones appear to be important steps in benzene hematotoxicity. Levels of P450 2E1 are known to vary substantially among individuals and ethnic groups and may partially explain response differences seen in different studies (Stephens et al., 1994). Furthermore, P450 2E1 is involved in ethanol metabolism and is readily inducible, indicating that alcohol consumption may affect a person's susceptibility to benzene poisoning (Koop et al., 1989; Stephens et al., 1994).

4.6.4. Genotoxicity Studies Utilizing Animal or In Vitro Systems

Due to its well-known leukemogenic properties, benzene has been the object of a large number of investigations on genotoxicity (Dean, 1978; Dean, 1985; Snyder and Kalf, 1994). As with its myelotoxic

effects, benzene appears to require bioactivation to exert it genotoxic effects (Gad-El-Karim et al., 1984; Plappert et al., 1994; Roh et al., 1987; Tice et al., 1982). Considerable effort has focused on the ability of benzene and its metabolites to interact with DNA. In vitro incubations with benzene metabolites have been used to identify (1) metabolites capable of reacting with DNA and (2) DNA adducts formed in animal experiments (Bleasdale et al., 1993; Bodell et al., 1993; Chenna et al., 1995; Pongracz and Bodell, 1991; Pongracz et al., 1990; Reddy et al., 1989b; Reddy et al., 1990). Most adducts identified to date from in vivo experiments have been formed from hydroquinone/1,4-benzoquinone, although adducts derived from such other benzene metabolites as phenol, catechol, 1,2,4-benzenetriol, or trans,trans-muconaldehyde have been seen in in vitro studies. Studies using radiolabeled benzene that employed a single or daily dosing regimen have detected significant radiolabel binding to both liver and bone-marrow DNA (Arfellini et al., 1985; Gill and Ahmed, 1981; Lutz and Schlatter, 1977; Mazzullo et al., 1989; Snyder et al., 1978). Similar studies using the ^{32}P postlabeling technique have had mixed results, with some investigators detecting DNA adducts and others failing to detect binding (Bauer et al., 1989; Reddy et al., 1989a; Reddy et al., 1989c; Reddy et al., 1990; Reddy et al., 1994). Even in studies where detectable binding to DNA has been detected, the magnitude of covalent binding observed has been low and ranks among the weakest of genotoxic agents, approaching the level of agents that are believed to act though indirect genotoxic mechanisms (Lutz, 1986). Recent studies by Bodell and associates have shown that if benzene is administered twice daily for 1 to 7 days, adduct formation is substantially increased, allowing enhanced detection using the ^{32}P postlabeling technique (Levay et al., 1996; Pathak et al., 1995). The major adduct identified in these studies cochromatographed with an adduct detected in HL-60 cells treated with hydroquinone. A second in vivo adduct corresponded to a DNA adduct formed in HL-60 cells treated with 1,2,4-benzenetriol.

In addition, Smith and colleagues have shown that following the administration of benzene to mice, increased levels of 8-hydroxy-deoxyguanosine were recovered in the bone marrow cells, demonstrating that oxygen radicals were formed in the bone marrow following benzene administration. These results also indicate that reactive oxygen species could contribute to benzene's genotoxic and mutagenic effects (Kolachana et al., 1993). These oxygen radical-derived DNA adducts also were seen following administration of 1,2,4-trihydroxybenzene as well as phenol and hydroquinone in combination. Time-course studies indicated that the adducts disappeared rapidly from bone marrow, presumably due to efficient repair of this type of adduct.

The relatively weak binding of benzene to DNA, combined with its potent clastogenic effects, have led some investigators to investigate the role of protein and enzymes involved in DNA replication and repair as potential targets for benzene's reactive metabolites. In addition, most in vivo studies that have used radiolabeled benzene to investigate binding have recovered substantially more radiolabel bound to proteins than to DNA (Arfellini et al., 1985; Mazzullo et al., 1989).

Other investigators have investigated the inhibitory effects of benzene and its metabolites on specific enzymes involved in DNA replication and repair as well as cell division and homeostasis. These studies have shown that various quinone and quinone-forming metabolites of benzene are capable of inhibiting

topoisomerase II, DNA, and RNA polymerases and interfering with microtubule assembly (Chen and Eastmond, 1995b; Epe et al., 1990; Irons, 1985; Lee et al., 1989; Post et al., 1984; Schwartz et al., 1985).

In vitro mutagenicity testing generally has indicated that benzene and its major metabolites are weakly mutagenic or nonmutagenic in most standard gene-mutation assays (IPCS, 1993; Waters et al., 1988). Interpreting these studies is complicated due to benzene's potentially complex metabolism and the fact that most studies have not employed proper metabolic activation. However, in vivo mutagenicity studies have not detected dose-related increases in mutation frequency in reporter genes in the bone marrow of treated mice (Stratagene, 1994). A significant increase (<twofold) was seen at one dose. Modest 1.5 to 2-fold increases in mutation frequency have been measured in reporter genes from cells isolated from the spleen of transgenic mice (Mullin et al., 1995; Stratagene, 1994). In a 6-week inhalation study in which native CD-1 mice were exposed to benzene, somewhat higher frequencies of 6-thioguanine–resistant splenocytes were observed (Ward et al., 1992). Higher frequencies seen in the latter study may reflect the increased ability of the *HPRT* assay to detect deletions as well as intragenic or point mutations commonly detected in the *lacI* transgenic system. Other in vitro genotoxicity studies focused on chromosome-level alterations have indicated that benzene and its metabolites are capable of inducing chromosome breakage and may interfere with chromosome segregation (IARC, 1982; Waters et al., 1988; Yager et al., 1990). Significant increases in structural chromosomal aberrations, micronuclei, and sister chromatid exchanges (SCEs) also have been detected in the bone marrow and spleen of benzene-treated animals (Chen et al., 1994; IPCS, 1993; Tice et al., 1980; Tice et al., 1981). In addition, significant increases in aneuploidy have been seen in recent studies of benzene-exposed mice and following incubation of cells with benzene metabolites (Chen and Eastmond, 1995a; Chen et al., 1994; Eastmond et al., 1994; Natarajan, 1993; Zhang et al., 1994). These results suggest that large chromosome-level alterations are likely to be major contributors to benzene's genotoxic effects.

4.6.5. Genotoxicity Studies in Benzene-Exposed Workers

Cytogenetic studies of benzene-exposed workers have shown a profile of genotoxicity similar to that seen in laboratory animals. Increased frequencies of structural chromosomal aberrations in lymphocytes of benzene-exposed workers have been reported by numerous investigators (Aksoy, 1988a; Sasiadek, 1992; Sasiadek et al., 1989; Tompa et al., 1994). No obvious breakage pattern has been seen, although some investigators have reported that break distribution occurred in a nonrandom fashion throughout the genome (Li and Ding, 1990; Sasiadek, 1992; Sasiadek et al., 1989). Excess breaks or gaps have been reported to affect chromosomes 1, 2, 4, 6, 7, 9, and 14 (Li and Ding, 1990; Sasiadek, 1992; Sasiadek and Jagielski, 1990; Sasiadek et al., 1989). In one limited cytogenetic study of Chinese patients with chronic benzene poisoning, deletions and gaps in the long arm of chromosome 6 (as well as other aberrations) were seen in peripheral blood lymphocytes of four patients and in a bone-marrow cell of one patient (Li and Ding, 1990). This is particularly interesting in that deletions in 6q- are seen frequently in many types of lymphoid neoplasia, including NHL (Johansson et al., 1993). Elevated frequencies of aneuploidy also have been detected in lymphocytes of exposed workers (Eastmond, 1993). These increases were due to both

chromosome loss and hyperdiploidy and were reported to occur at relatively high frequencies in previously poisoned individuals (Ding et al., 1983; Haberlandt and Mente, 1971). Studies investigating an association between benzene exposure and increased SCE frequencies have yielded both positive and negative results (Karacic et al., 1995; Major et al., 1994; Popp et al., 1992; Sarto et al., 1984; Tompa et al., 1994; Yardley-Jones et al., 1988). No increase in SCE has been seen in several studies where increases in chromosomal aberrations were detected, suggesting that chromosomal aberrations are a more sensitive and consistent endpoint than SCEs for benzene's genotoxic effects (Sarto et al., 1984; Yardley-Jones et al., 1990).

Recent studies of highly exposed Chinese workers are providing a number of significant insights into the genotoxic effects of benzene. Smith and associates have utilized fluorescence in situ hybridization (FISH) with probes specific to centromeric regions of chromosomes 7, 8, and 9 to detect aneuploidy in cultured interphase lymphocytes obtained from 43 benzene-exposed Chinese workers and 44 age- and sex-matched controls (Zhang et al., 1995a). Significant increases in both hyperdiploidy and hypodiploidy were observed using each probe in the cells of workers with the highest benzene exposure (> 31 ppm). Using the chromosome 7 probe, chromosomal alterations also were observed in the lymphocytes of workers exposed at lower levels, suggesting a more frequent involvement of this chromosome in benzene-induced aneuploidy. This observation is noteworthy in that monosomy of chromosome 7 is one of the earliest and most common genetic alterations observed in individuals with chemically induced leukemias (Pedersen-Bjergaard et al., 1995). The 8;21 translocation is another nonrandom alteration commonly seen in ANLL (Pedersen-Bjergaard and Rowley, 1994). As an extension of this previous study, investigators used FISH with painting probes to detect translocations affecting chromosomes 8 and 21 in metaphase cells (Smith et al., 1996a; Smith et al., 1996b). An average of 483 metaphases per person were scored (>77,000 total metaphases). A dose-related 15-fold increase in translocation frequency involving these two chromosomes was seen in exposed workers when compared to controls, suggesting that translocations relevant to leukemia induction can be detected in workers with current benzene exposure.

In addition, these investigators used the glycophorin A (GPA) mutation assay, which measures gene loss, to evaluate the nature of DNA damage produced by benzene in 24 benzene-exposed Chinese workers and 23 matched controls heterozygous for MN alleles (Rothman et al., 1995b). A significant increase in NN variant cell frequency was found in benzene-exposed workers as compared with unexposed control individuals, whereas no significant difference in NØ variant frequency was seen. Increase in NN variants was associated significantly with lifetime cumulative occupational exposure to benzene, suggesting that NN mutations had occurred in longer-lived bone-marrow stem cells. NN variants result from loss of the GPA M allele combined with duplication of the N allele, presumably through mechanisms of recombination or nondisjunction combined with chromosome loss, whereas NØ variants arise from gene inactivation, presumably due to point mutations and deletions. These results originating in the bone marrow of exposed humans suggest that benzene induces chromosome-level mutations rather than producing inactivating mutations within the GPA locus. These findings, which are consistent with previous studies on benzene’s

genetic toxicology and its metabolites, provide further evidence for the hypothesis that chromosome-level genetic alterations contribute to benzene-induced leukemia.

4.6.6. Myelotoxic and Immunotoxic Effects in Humans

The myelotoxic effects of benzene have been recognized for more than a century. Chronic exposure to high benzene concentrations is characterized by bone-marrow toxicity affecting all lineages of blood cells (Aksoy, 1988b; Goldwater and Tewksbury, 1941; IPCS, 1993). Clinical features have been described by Vigliani and Forni (1976) as follows: "Chronic benzene intoxication was described as an aplastic or hypoplastic anemia, either with the clinical feature of a chronic progressive hyporegenerative anemia, or with the clinical pattern of a rapidly fatal hemorrhage, necrotic angina, and septicemia. Many times a progressive hypoplastic anemic was seen to precipitate abruptly with severe granulocytopenia and thrombocytopenia, and to terminate with hemorrhage, septicemia, and death." A number of studies have indicated that, although benzene can affect all major blood elements, decreases in frequency of circulating white blood cells, particularly lymphocytes, is one of the more sensitive indicators of benzene toxicity (Kipen et al., 1988; Moszczynski and Lisievwicz, 1983; Moszczynski and Lisiewicz, 1983; Rothman et al., 1996a). Probably as a consequence of direct myelotoxicity, benzene also has been shown to be immunotoxic, affecting both humoral and cellular acquired immunity. Workers exposed to benzene have been reported to exhibit altered levels of immunoglobulins, autoimmunity, and increased susceptibility to allergies as well as other conditions related to altered lymphocyte function (ATSDR, 1992a; IPCS, 1993; Moszczynski and Lisiewicz, 1983; Zeman et al., 1990).

4.6.7. Hematopoietic and Lymphoid Neoplasia Seen in Humans

Benzene is a widely recognized human leukemogen (IARC, 1982). Initial association between benzene exposure and subsequent leukemia development was proposed in 1928 by Delore and Borgomano, who described a case of acute lymphoblastic leukemia in a worker who had been exposed to benzene for 5 years. Since that time, a large number of leukemia cases in benzene-exposed individuals have been reported (see Aksoy, 1988a; Goldstein, 1977; IARC, 1982 for reviews). These case reports have been followed by a series of epidemiological studies that clearly have linked benzene exposure to hematological neoplasia (Austin et al., 1988; IPCS, 1993; McMichael, 1988; OSHA, 1985). Although many types of hematological malignancy have been reported to be associated with benzene exposure, the most consistent association has been observed with ANLL (Goldstein, 1977; Lamm, 1988; Richardson et al., 1992; Rinsky et al., 1987; Rinsky et al., 1981; Yin et al., 1987).

Within the past few years, updates to these earlier studies, as well as the results of new studies, have become available (Aksoy, 1988a; Anemia, 1992; Paxton et al., 1994a; Paxton et al., 1994b; Richardson et al., 1992; Travis et al., 1994; Vai et al., 1989). In each of these studies, significantly increased risks of ANLL were observed in benzene-exposed workers, consistent with what had been seen in previous studies. However, in the Chinese worker study, probably the most comprehensive epidemiological study on benzene

performed to date, a significant association was seen between benzene exposure and lymphoid and myeloid malignancies (Dosemeci et al., 1994; Travis et al., 1994; Yin et al., 1994). In this case, elevated risks of lymphoma (RR for incidence = 3.5; CI 1.2-14.9) were seen in addition to leukemia (RR for incidence = 2.6; CI 1.3-5.7). Mortality due to lymphoid and hematopoietic cancers also was associated with increasing cumulative benzene exposure, providing additional evidence for the association between benzene exposure and these malignancies (Hayes et al., 1996). Among leukemia subtypes, only AML (RR = 2.6, CI 1.2-10.7) was elevated significantly, although nonsignificant increases also were seen for CML (RR = 2.6, CI 0.7-16.9) and ALL (RR = 2.8, CI 0.5-54.5). In addition, a significantly elevated risk in lymphoid neoplasia was seen when all lymphocytic and histiocytic malignancies were combined (RR = 2.7, CI 1.1-25.7). Increased risks for MDS and aplastic anemia also were seen among exposed workers. This large cohort study, conducted by investigators from several Chinese agencies and the U.S. National Cancer Institute, involved more than 110,000 individuals (74,828 benzene-exposed workers and 35,805 controls) and collected data over a 15-year period (1972 to 1987). Even though the reported increase in lymphoid malignancies was somewhat unexpected, associations between benzene exposure and lymphocytic leukemias and lymphomas have been indicated from other studies (Aksoy, 1988a; Blair et al., 1993; Franco and Fonte, 1984; Vianna and Polan, 1979; Young, 1989; Zorn, 1992). In addition, the general pattern of malignancies seen in the Chinese study is fairly consistent with those observed in the case series of Turkish shoe workers reported by Aksoy (Aksoy, 1988a) and the recent update of the U.S. Pliofilm cohort (Paxton et al., 1994a; Paxton et al., 1994b). A comparison of types of lymphoid and hematopoietic neoplasia reported in these three studies is shown in Table 4, and the pooled results are shown in Figure 6. The frequency of lymphoma (and related diseases) observed in the three studies ranges from 29% in the Chinese study to 14% in the Pliofilm study. The actual incidence of lymphoid malignancies in the Pliofilm study may actually be higher, as one exposed worker committed suicide due to "intolerable itching" resulting from lymphosarcoma (OSHA, 1985). Since suicide was listed as the cause of death on the death certificate without mention of the lymphosarcoma, this case was not included among those dying from benzene-related malignancies.

Additional details about each of these two major types of malignancies associated with benzene exposure are provided below:

4.6.7.1. *Acute Nonlymphocytic Leukemia*

As indicated above, ANLL consistently has been associated with benzene exposure. In about 22% of patients, leukemia was preceded by pancytopenia; in 12%, it was preceded by preleukemia or MDS (Aksoy, 1988a; Goguel et al., 1967). Among the types of frank leukemias, the most common subtypes of ANLL associated with benzene exposure have been reported to be acute myelogenous leukemia (M1 and M2), myelomonocytic leukemia (M4), and erythroleukemia (M6)(Aksoy, 1988a; Crane et al., 1992; Infante, 1995; Jandl, 1987). However, such other subtypes as monocytic (M5) and promyelocytic leukemia (M3) also have been reported (Aksoy, 1988a; Infante, 1995; Mele et al., 1995; Paxton et al., 1994b; Yin et al., 1996). In addition, considerable variability in subtypes has been reported in studies from different geographical

regions. For example, erythroleukemia was very prominent in cases reported from Italy and Turkey but has been seen much less frequently in more recent studies from the United States and China. These differences are believed to be due, at least in part, to changes in hematological classification over time. Based on published descriptions, Cirasino and Invernizzi have proposed that a number of these erythroleukemias would be classified today as MDS (Cirasino and Invernizzi, 1995). Interestingly, the latency period (4 years) reported for cases with acute myelomonocytic leukemia is considerably shorter than that reported for acute myeloblastic leukemia (11.5 years)(Aksoy, 1988a). The difference in latency periods between these two types of leukemia is similar to those seen in therapy-related leukemias induced by other agents (Pedersen-Bjergaard and Rowley, 1994). Latency periods for benzene-induced leukemias have ranged from 1 to more than 40 years with an average of about 11 years being reported by several investigators (Infante, 1995; OSHA, 1985). In the recent update of the Pliofilm study, an average latency period of 24.9 years was observed for leukemias in the benzene-exposed cohort (Infante, 1995).

Table 4. Types of hematopoietic neoplasia observed in three studies of benzene-exposed workers

Type of Leukemia	Case series	Cohort studies		Total	Overall %
	Aksoy 1988a	Paxton et al. 1994b	Yin et al. 1996		
Preleukemia/MDS	7 (9)[a]		7 (10)	14	8.1
ANLL	40 (51)	9 (41)	23[b] (33)	72	42.1
Undifferentiated (M0)	1				
Myelogenous (M1&M2)	23	6	3		
Promyelocytic (M3)	1		4		
Myelomonocytic (M4)	5		1[c]		
Monocytic (M5)	–	2	1[d]		
Erythroleukemia (M6)	10	1			
ALL	5 (6)	1 (5)	5 (7)	11	6.4
CML	3 (4)	3 (14)	9 (13)	15	8.8
CLL	2 (3)			2	1.2
Multiple Myeloma	4[e] (5)	4[f](18)	1 (1)	9	5.3
Lymphoma	17[e] (22)	3[g] (14)	20 (29)	40	23.4
Nonspecified or rare leukemias	1 (1)	2 (9)	5 (7)	8	4.7
TOTALS	79 (100)	22 (100)	70 (100)	171	100

[a]Percentages are in parentheses.
[b]Information was not available for all leukemias by subtype.
[c]May have been M2.
[d]May have been M4.
[e]Tabulated separately.
[f]Includes one plasma-cell sarcoma.
[g]Includes one case of other malignant lymphoid or histicytic neoplasia and one reticulosarcoma.

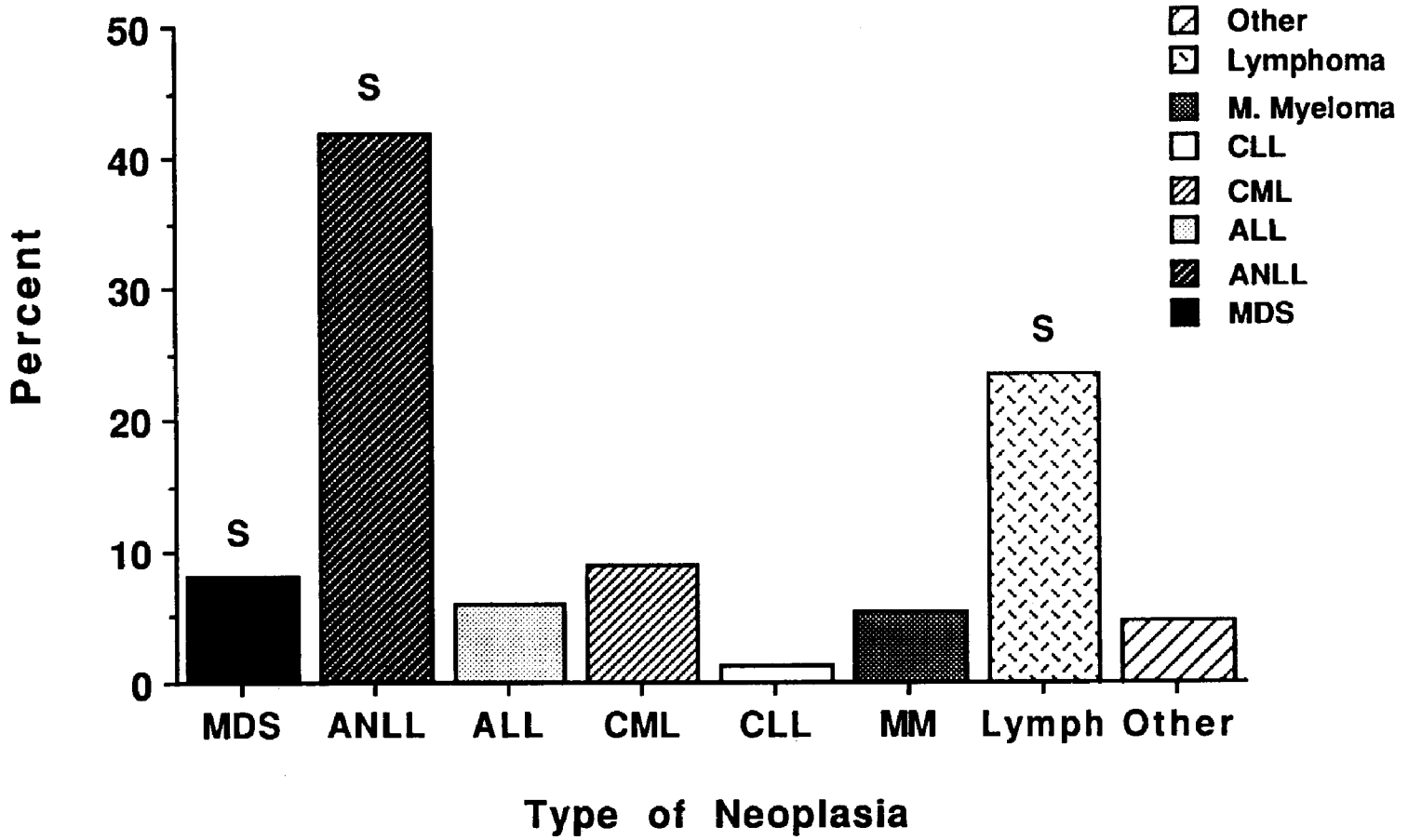

Figure 6. Types of lymphohematopoietic neoplasia observed in benzene-exposed workers. Types of cancer for which evidence is sufficient for association with benzene exposure are labeled with an S. Data are combined from Aksoy, 1988a, Paxton et al., 1994b, and Yin et al., 1996.

TABLE 5. Comparative aspects of human leukemias induced by the six agents

	Disease	FAB[a]	MDS[b]	Chromosome Abnormalities	Latency[c] (yrs)
Ionizing radiation	ANLL	M1-M7	++	-7,-5,7q-,5q-	8-35[d]
	ALL	L1-L2			8
	CML			t(9;22)	5
Alkylating agents	ANLL	M1,M2	++	-7,-5,7q-,5q-	4-5
	[NHL][e]				5-6
Epipodophyllotoxin topo II nhibitors	ANLL	M4, M5	–	11q23 t(8:21)	2-3
Dioxopiperazine topo II inhibitors	ANLL	M2,M3	–	t(15;17) t(8:21)	3
Benzene	ANLL	M1,M2,M4,M6	+	Present[f]	11
	[NHL][e]				
1,3-Butadiene	ANLL[e]	NA[g]	NA	NA	30
	ALL[e]				
	[NHL][e]				

[a]French-American-British classification of leukemia subtypes.
[b]Significant proportion exhibit myelodysplastic phase.
[c]Approximate peak or mode.
[d]Significantly influenced by age at exposure.
[e]Results across studies have been inconsistent.
[f]Clonal chromosomal abnormalities are present. However, the karyotypes observed to date have been inconsistent. Based on clinical descriptions and some case reports, involvement of chromosomes 5 and 7 is likely. In addition, there is some evidence for involvement of translocations similar to those seen with topoisomerase inhibitors.
[g]No information is available.

NA = not available

TABLE 6. Comparison of genetic alterations in human and mouse acute leukemias or lymphomas

	Human				Murine			
	Lineage	***ras*[a]**	***p53*[b]**	**Others**	**Lineage**	***ras***	***p53***	**Others**
Ionizing radiation	Myeloid	++[c]	NA	Unknown[d]	Myeloid	NA	NA	Unknown (del 2D)
	Lymphoid	NA	NA	Unknown	Lymphoid	++	++	Unknown (trisomy 15)
Alkylating agents	Myeloid	+	+	Unknown[d]	Lymphoid	++	+	*Pol β*, Unknown (trisomy 15)
Epipodophyllotoxin-topo II inhibitors	Myeloid	NA	NA	*MLL*	NA	NA	NA	Unknown
Dioxopiperazine-topo II inhibitors	Myeloid	NA	NA	*PML/RARa AML1*	Myeloid	NA	NA	Unknown
Benzene	Myeloid	NA	NA	Unknown	Lymphoid	NA	NA	Unknown
1,3-Butadiene	Myeloid	NA	NA	Unknown	Lymphoid	+[f]	NA[g]	Unknown[e]

[a]Mutations activating one of the *ras* oncogene family detected.
[b]Alterations affecting the p53 tumor-suppressor gene observed.
[c]++, Frequent; +, Infrequent.
[d]A number of hematopoietic growth factors and related genes have been identified in frequently deleted regions.
[e]Involvement of murine leukemia viruses in some lymphomas has been shown.
[f]Activation of *ras* ocogenes has been detected in other tumors from butadiene-exposed mice.
[g]Loss of heterozygosity was detected in other tumors from butadiene-exposed mice.

NA=not available

4.6.7.2. *Lymphoma*

Increases in lymphoma have been observed repeatedly in studies of benzene-exposed workers. This type of malignancy has been seen less consistently than ANLL, and there are inconsistencies in specific types of lymphoma in various reports. Of the 20 lymphomas observed in the Chinese worker study, 17 were NHL (Yin et al., 1995). Relative risks for nodal and extranodal NHL were 4.0 (CI 1.1-25.7) and 1.0 (CI 0.1-22.3), respectively. No cases of Hodgkin's disease were identified. In addition, a case control study conducted in Minnesota and Iowa provided some evidence that benzene exposure was associated with increased risks 17 lymphomas reported by Aksoy in his case series were classified as Hodgkin's disease (Aksoy, 1988a). In addition, an early study by Vianna and Polan indicated that individuals working in occupations with potential benzene exposure had increased risks of lymphosarcoma, reticulum-cell sarcoma, and Hodgkin's disease among older workers (Vianna and Polan, 1979).

4.6.8. Genetic Alterations in Cancer Patients

A significant number of cytogenetic studies have been performed on patients exhibiting benzene-related leukemias and related diseases. Unfortunately, most were conducted before the development of sensitive chromosome-banding techniques. As a result, detailed karyotypic information is available for relatively few individuals. This makes identification of chromosomal regions and genes involved in benzene-induced leukemias very difficult. In general, karyotypes of patients with benzene-induced leukemia exhibit abnormal chromosomal number and structure (Forni, 1979; Wolman, 1977). Beyond this, however, no clear pattern emerges from published karyotypes. Chromosomal alterations similar to those seen following treatment with alkylating chemotherapeutic agents, such as loss of all or part of the long arms of chromosomes 5 and 7, have been reported to occur in leukemia patients previously exposed to of both follicular and diffuse NHL (Blair et al., 1993). Although confidence intervals for each category encompassed 1.0, risk increased with increasing exposure intensity. In contrast, 10 of benzene (Pollini and Colombi, 1964; Van den Berghe et al., 1979). In addition, trisomy or tetrasomy of a C-group chromosome, occasionally identified as chromosome 8 or 9, has been associated with benzene exposure (Antonucci et al., 1989; Erdogan and Aksoy, 1973; Forni and Moreo, 1967). Occasionally, these clonal alterations were observed before the development of frank leukemia (Erdogan and Aksoy, 1973; Forni and Moreo, 1967). Furthermore, translocations have been reported in cells of leukemic patients following exposure to benzene or benzene-containing solvents such as petroleum (Li et al., 1989; Sole et al., 1990; Tasaka et al., 1992; Van den Berghe et al., 1979). Occasionally, these translocations have been identical to those observed in patients following chemotherapy with topoisomerase II inhibitors (Sole et al., 1990; Tasaka et al., 1992).

Due in part to the mixed pattern of karyotypic alterations described above, little is known about genes involved in benzene leukemogenesis. However, genes involved are likely to be similar to those contributing to other types of therapy-related leukemias and some de novo leukemias. Occasional reports have implicated or suggested that a gene might be involved (Snyder and Kalf, 1994; Taylor et al., 1995). For example, a mutation in k-*ras* was seen in DNA isolated from peripheral blood of a worker occupationally

exposed to petrochemicals; the authors postulated that this might be related to benzene exposure (Taylor et al., 1995). However, at this time there is little direct evidence for the involvement of any specific gene or types of genes. Different patterns of aberrations seen in benzene leukemia cases, as well as those seen in genotoxicity studies of exposed workers, suggest that multiple types and mechanisms of genotoxicity may be occurring. One current hypothesis is that both the alkylating-type of chromosomal alterations (aneuploidy and deletions) as well as topoisomerase-type alterations (translocations and deletions) are occurring in benzene-exposed individuals (Frantz et al., 1996). Combination of these types of chromosomal alterations confers increased leukemia risks similar to those reported for cancer patients treated with both alkylating agents and topoisomerase inhibitors (Pedersen-Bjergaard et al., 1992; Sandoval et al., 1993; Smith et al., 1994b).

4.6.9. Hematopoietic and Lymphoid Neoplasia Seen in Rodent Models

A large number of chronic bioassays have been performed in an attempt to identify an animal model for benzene-induced myelogenous leukemia (for a summary see IPCS, 1993). Although minor increases in myelogenous leukemias occasionally have been seen in rodent bioassays (Cronkite et al., 1989; Goldstein et al., 1982), consistent and reproducible increases in these leukemias have not been seen in either mice or rats (Farris et al., 1993; Maltoni et al., 1989; NTP, 1986a). In mouse bioassays, benzene has been reported more consistently to induce increases in lymphoblastic leukemias or lymphomas (Cronkite et al., 1984; Farris et al., 1993; Maltoni et al., 1989; NTP, 1986a; Snyder et al., 1980). In rats, Maltoni and coworkers reported that in some experiments benzene was capable of inducing "reticuloendothelial" tumors on chronic administration of benzene (Maltoni et al., 1983). These tumors were reported to be a combination of hematosarcomas, lymphoblastic leukemias, and one myelogenous leukemia. However, no increase in these tumor types was seen in the rat bioassay conducted by the National Toxicology Program (NTP, 1986a).

Somewhat in contrast to its leukemogenic effects, chronic benzene exposure has been shown to induce solid tumors at a large number of sites in mice and rats, including the Zymbal gland, Harderian gland, mammary glands, oral cavity, skin, lung, preputial gland, and ovary (Haseman et al., 1984). Recent studies have reported on the metabolic capacity and genotoxicity occurring in these target tissues and their proposed relevance in benzene carcinogenicity. All tissues examined had significant levels of peroxidase enzymes, a class of enzymes believed to be involved in metabolic activation of benzene in the bone marrow (Low et al., 1995). In addition, detectable sulfatase levels were observed. The authors suggested that the somewhat unique metabolic capabilities of the target tissues might play a role in the target organ specificity of benzene. Additional studies by Angelosanto and colleagues detected micronuclei in primary Zymbal gland cultures established from benzene-treated animals, demonstrating that genetic damage occurred in these target organs following benzene exposure (Angelosanto, 1995).

Recently, benzene has been tested in two different strains of transgenic mice to determine the sensitivity of mouse models to various types of carcinogenic agents. Benzene was administered for 40 weeks to E mu-pim1 transgenic mice, which overexpress the *pim-1* oncogene in lymphoid tissues and have been

shown to exhibit increased sensitivity to T-cell lymphoma induction by the alkylating agent, N-ethyl-N-nitrosourea (Storer et al., 1995). At the end of a 40-week treatment period, a statistically significant increase in lymphomas was not seen, suggesting the limited usefulness of this transgenic model for benzene-carcinogenicity testing. Other studies have indicated that this transgenic strain was sensitive to benzene's genotoxic effects as measured by micronuclei induction in bone marrow and blood erythrocytes (Armstrong and Galloway, 1993). In a similar study using a different strain of transgenic mouse, administration of benzene by dermal application to the TG.AC transgenic mouse strain, which carries a *v-ras* construct, induced a high frequency of skin papillomas in a dose-related fashion (Tennant et al., 1995). A similar effect was not seen when phenol, one of benzene's primary metabolites, was applied to the mouse skin. Cytogenetic studies were performed for the epidermal sarcomas or squamous cell carcinomas in six benzene-treated TG.AC transgenic animals (French et al., 1994). Two of the six tumors were characterized by trisomy 15, and two exhibited normal karyotypes. The presence of trisomy 15, is somewhat interesting in that this alteration frequently occurs in mouse T-cell lymphomas. No information has been published on the karyotype of the leukemias and lymphomas induced in mice (or rats) by benzene.

4.7. 1,3-BUTADIENE

4.7.1. Background

Butadiene is a widely used industrial chemical and common environmental pollutant. Annual U.S. production in 1990 was estimated to be 3.2 billion pounds, most of which was used for the manufacture of styrene-butadiene rubber and polybutadiene rubber (NTP, 1994). National Institute of Safety and Health (NIOSH) has estimated that 69,555 U.S. workers were potentially exposed to butadiene (NTP, 1994). Currently the permissible exposure level for butadiene is 1000 ppm, but actual occupational exposures are considerably lower, with measured values ranging from 0.005 to 374 ppm (Fajen et al., 1993). Of samples taken in a recent occupation-exposure survey, 85% were below 2 ppm, and only 3.7% exceeded 10 ppm (Fajen et al., 1993). Butadiene also has been detected in cigarette smoke (approx. 0.4 mg/cigarette) and in automobile exhaust (approx. 0.35% of total hydrocarbons in exhaust emissions). Historically, butadiene was considered a safe chemical with no adverse effects seen in mice following acute exposures up to 100,000 ppm (ACGIH, 1993). The permissible exposure level was accordingly set at 1000 ppm (RTECS). However, more recent findings that butadiene is carcinogenic at multiple sites in rats and mice on chronic exposure has raised concerns about the human health risks from occupational and environmental exposures (Huff et al., 1985; Melnick and Huff, 1992). In animal bioassays, significant differences have been seen between mice and rats in the number, types of neoplasia, and concentrations at which tumors have been seen. A significant increase in lung cancers was seen at exposure concentrations as low as 6.25 ppm in B6C3F1 mice, whereas exposures of 1000 and 8000 ppm were necessary to induce mammary gland tumors in rats (Melnick and Huff, 1992). Particularly striking was the increase in thymic leukemias and lymphomas observed in B6C3F1 mice but not rats (Melnick and Huff, 1992). This difference in species sensitivity has been used to provide insights into critical steps involved in butadiene toxicity and carcinogenesis.

In addition to animal data, in recent years an increasing number of epidemiological studies have shown an association between butadiene exposure and the incidence of lymphohematopoietic disease in humans. These studies have raised additional concern about adverse effects of butadiene exposure to humans. Butadiene has been the focus of a number of recent reviews (Bond et al., 1995; IARC, 1992; IARC, 1993; Melnick and Kohn, 1995) to which the reader is referred for additional details. The primary focus of this section will be on mechanisms of toxicity and genotoxicity that are believed to contribute to formation of lymphohematopoietic neoplasms in humans and rodents.

4.7.2. Metabolic Studies Utilizing Animal and In Vitro Systems

The metabolism of butadiene has been shown to be qualitatively similar among the various species studied (Henderson et al., 1993). Butadiene is metabolized via cytochrome P450-dependent monooxygenases initially to butadiene monoepoxide (BDO; 1,2-epoxy-3-butene) and 3-butenal, which tautomerizes to produce crotonaldehyde (Elfarra et al., 1991). The P450 2E1 isozyme appears to be primarily responsible for this reaction, although the 2A6 isozyme can also metabolize butadiene when butadiene is present at high concentrations (Duescher and Elfarra, 1994; Seaton et al., 1995). BDO can be further oxidized to butadiene diepoxide (BDO2; 1,2,3,4-diepoxybutane) in a reaction apparently catalyzed by the hepatic 2E1 isozyme in mice, rats, and humans (Seaton et al., 1995). BDO can be transformed via epoxide hydralase to form butenediol (1,2-dihydroxy-3 butene) in what is believed to be a detoxication process (Henderson et al., 1993; IARC, 1992). This latter product can be metabolized further by P450 enzymes to form 3,4-epoxy-1,2-butanediol. Both BDO and BDO2 also can be metabolized by glutathione-S-transferases to form glutathione conjugates. These are presumed to be detoxication products, although there have been some suggestions that these conjugates might be involved in bioactivation (Guengerich et al., 1995; Thier et al., 1995). BDO and BDO2 are both highly mutagenic and carcinogenic when administered to mice and rats (IARC, 1992). Based on in vivo mutagenicity studies in mouse and human cells, the mutagenic activity of BDO2 appears to be 10 to 100 times higher than the primary BDO metabolite. Other potential reactive metabolites are dihydroxyepoxybutane, crotonaldehyde, and hydroxymethylvinylketone (Waters and Nolan, 1995). However, the most current metabolic schemes seem to favor the epoxide metabolites, BDO and BDO2, as mutagenic and carcinogenic metabolites. The production of BDO2 is higher in female rats than male rats; this has been proposed to play a role in the increased incidence of mammary tumors seen in female rats following butadiene exposure (Thornton-Manning et al., 1995b).

Although bone marrow is one of the key organs for butadiene toxicity in mice, the absence of significant cytochrome P450 2E1 activity in this organ in mouse or human cells indicates that hepatic metabolism or another type of bioactivation is likely to be involved (Genter and Recio, 1994; Maniglier-Poulet et al., 1995). B one marrow contains high levels of peroxidase enzymes, which have been shown to be capable of converting butadiene to BDO (Duescher and Elfarra, 1992; Maniglier-Poulet et al., 1995). However, BDO formation was about 20 times greater in rat and mouse liver microsomes than in mouse bone-

marrow cells and lysates, suggesting the importance of hepatic metabolism in the butidiene bioactivation (Maniglier-Poulet et al., 1995).

Significant metabolism differences in mice and rats are believed to be largely responsible for the toxicity differences between the two species (Bond et al., 1995; Melnick and Kohn, 1995). A number of studies have demonstrated that mice are much more efficient in butadiene uptake and bioactivation and less efficient in detoxication. Studies in liver and lung microsomes have shown that the rate of BDO formation is highest in liver microsomes from B6C3F1 mice. High activation rates were also seen in mouse lung microsomes. Lower reaction rates were seen in the microsomes of Sprague-Dawley rats, humans, and rhesus monkeys (Henderson et al., 1993). Pharmacokinetic studies have indicated that uptake and metabolism of butadiene was greater in the mouse than in the rat or the monkey and that the mouse metabolized much higher percentages to the epoxide metabolites in vivo (Henderson et al., 1993). Recent studies also have shown that BDO, and in most tissues BDO2, can be detected in the blood, bone marrow, heart, lung, fat and thymus following in vivo exposure of B6C3F1 mice to butadiene (Thornton-Manning et al., 1995a). BDO2 was also detected in the heart, lung, fat, spleen, and thymus of Sprague-Dawley rats but at levels 40- to 160-fold lower than those seen in mice. In addition to more efficient bioactivation, the mouse has been shown to have lower epoxide hydrolase activity than the rat, hamster, or monkey; as a result, more glutathione metabolites can be seen in mouse urine. The combination of more efficient activation and lower epoxide hydralase activity results in greater depletion of nonprotein sulfhydryls in the various mouse tissues than in rat tissues, even when rats are exposed to much higher concentrations (Bolt, 1993). Binding of radioactive butadiene equivalents to liver DNA and nucleoproteins could be detected in both rats and mice under these same conditions but was about twofold higher in the mice (Bolt, 1993). Higher levels (about fivefold) of the BDO hemoglobin adduct were also seen in mice than in rats after exposure to butadiene (Albrecht et al., 1993; Osterman-Golkar et al., 1993).

4.7.3. Metabolism Studies in Humans

Evidence indicates that the same metabolites detected in laboratory animals will be formed in humans. Butadiene was reported to be metabolized to BDO by human liver and lung preparations at one-half to one-tenth the rates seen in mouse microsomes (Bond et al., 1993). Studies with human liver microsomes indicated that the rate of hydrolysis by epoxide hydrolase was higher in human liver samples than in mouse or rat samples (Bechtold et al., 1994; Bond et al., 1993). However, lower rates of glutathione conjugation by glutathione-S-transferase were seen in human hepatic microsomes than in mouse or rat (Bechtold et al., 1994). Equal amounts of BDO were formed in human and mouse bone-marrow cells following exposure to butadiene in a reaction that appeared to be catalyzed by myeloperoxidase (Maniglier-Poulet et al., 1995). This indicates that formation of this bioactive metabolite can occur in a target organ for butadiene toxicity. However, the amount of metabolite formed appeared to be significantly lower than seen in liver incubations.

Urinary metabolites resulting from BDO detoxication were identified in samples from rats, mice, hamsters, monkeys, and humans exposed to butadiene (Bechtold et al., 1994). The presence of only the 1,2-

dihydroxy-4-(N-acetylcysteinyl-S-)-butane metabolite in the urine of exposed workers suggests that humans exposed to butadiene in the 3- to 4-ppm range metabolize BDO by hydrolysis to butanediol rather than through direct conjugation with glutathione. This pattern is more closely related to that seen in the monkey than in either rats or mice (Bechtold et al., 1994). The BDO hemoglobin adduct also was detected at low levels in the blood of occupationally exposed workers (Osterman-Golkar et al., 1993). More recent studies have confirmed that blood hemoglobin adduct levels were increased among worker groups with higher potential butadiene exposure than among less-exposed workers (Osterman-Golkar et al., 1996; Sorsa et al., 1996b). However, the measured N-terminal valine hemoglobin adducts were 10 to 20 times lower than those extrapolated for the same exposure dose in mice (Sorsa et al., 1996b). These results indicate that humans can metabolize butadiene to the monoepoxide. However, whether or not humans are able to form the diepoxide in significant quantities at current occupational exposure levels remains uncertain.

4.7.4. Genotoxicity Studies Utilizing Animal or In Vitro Systems

Like benzene, butadiene requires metabolic activation to exert its genotoxic effects (Norppa and Sorsa, 1993). However, in contrast to benzene, bioactivated butadiene binds readily to DNA and is efficient at inducing specific gene mutations. Results of most standard in vitro studies of butadiene mutagenesis and genotoxicity have been negative, probably due to lack of metabolic activation and the difficulty in testing a gas that requires bioactivation (IARC, 1992; Norppa and Sorsa, 1993). Several studies have demonstrated the ability of butadiene to bind to DNA and induce DNA breaks in vivo (Jacobson-Kram and Rosenthal, 1995; Vangala et al., 1993). Following in vivo exposure to butadiene, male B6C3F1 and NIH Swiss mice show macrocytic-megaloblastic anemia (Irons et al., 1986a; Irons et al., 1986b). A leukopenia, related primarily to a decrease in neutrophils, and an increase in bone-marrow erythrocyte micronuclei also has been observed. Consistent treatment-related alterations in bone-marrow cellularity were not seen. In addition to micronuclei, butadiene has been shown consistently to induce chromosomal breakage and sister chromatid exchanges (SCEs) in mouse bone marrow and germ cells (Adler and Anderson, 1994; Adler et al., 1994; IARC, 1992; Irons et al., 1987a; Shelby, 1990; Tice et al., 1987); no increases in micronuclei or aberrations were seen in rat (Autio et al., 1994; IARC, 1992; Norppa and Sorsa, 1993; Sorsa et al., 1996b). A weak induction in SCEs was seen in rat lymphocyte and lung cells (Norppa and Sorsa, 1993). Apparently these differences are due largely to metabolic differences in formation of bioactive metabolites, as both BDO and BDO2 exhibit significant genotoxic effects in both rats and mice (Sharief et al., 1986; Xiao and Tates, 1995). Increases in SCEs were seen following butadiene exposures for 10 days at 6.25 ppm in B6C3F1 mice (Tice et al., 1987). Significant increases in micronuclei were also seen at this concentration but only following exposure for a longer period of Time (13 weeks) and by scoring a large number of cells (10,000 RBC per animal) (Jauhar et al., 1988; Shelby, 1990).

A variety of butadiene-DNA adducts have been detected following the in vitro incubation of butadiene metabolites with isolated nucleotides, DNA, or cell cultures (Leuratti et al., 1994; Leuratti et al., 1993; Neagu et al., 1995; Peltonen et al., 1993). Adducts to the N6 position of adenine and N7 position of

guanine have been detected (Leuratti et al., 1994; Neagu et al., 1995). Recent studies have detected the N6-alkyldeoxyadenosine adduct of BDO in lungs of mice and rats exposed to butadiene by inhalation (Sorsa et al., 1996b). Similar levels were seen in rat and mouse lungs, but the levels were about 10 times lower than those seen in rat liver. In a preliminary report, two BDO-DNA adducts were detected in the urine of a butadiene-exposed worker (Peltonen et al., 1993).

A number of studies have detected increased mutation frequencies in endogenous mouse genes or in reporter genes in transgenic mice, consistent with the ability of bioactive butadiene metabolites to react with DNA. Studies by two groups have demonstrated that three- to fivefold increased frequencies of 6-thioguanine-resistant cells indicative of mutation at the *HPRT* locus can be detected in the splenic T-lymphocytes of B6C3F1 mice following multiple exposures to butadiene at 625-ppm concentrations and higher (Cochrane and Skopek, 1993; Tates et al., 1994). Somewhat higher frequencies also were seen in this study following the intraperitoneal administration of BDO and BDO2 to mice (Cochrane and Skopek, 1993). Follow-up studies by Cochrane and Skopek have shown that, for each of these compounds, about half the mutations induced within *HPRT* in vivo were frameshift mutations (Cochrane and Skopek, 1994b). Transition and transversion mutations also were seen, with a tendency toward substitutions at AT base pairs. The authors commented that the spectrum of mutations induced by butadiene and metabolites was very similar to that seen with ethylene oxide, suggesting that these agents might be working through similar mutagenic mechanisms.

Similar results have been seen with butadiene in studies of transgenic B6C3F1 mice carrying a recoverable *lacI* bacterial gene. Following a 2-week exposure to butadiene at concentrations similar to those seen in the mouse cancer bioassay, the *lacI* mutant frequency in the bone marrow was increased in a concentration-related manner 2- to 3.5-fold over that seen in the controls (Sisk et al., 1994). DNA sequence analysis suggested that the exposed mice had higher frequencies of point mutations at AT sites (Recio and Meyer, 1995).

The three epoxide metabolites of butadiene, BDO, BDO2, and 3,4-epoxy-1,2-butanediol were tested for mutagenicity at *HPRT* and *tk* loci in the TK6 human lymphoblastoid cells (Cochrane and Skopek, 1993; Cochrane and Skopek, 1994a). All three epoxides were mutagenic, but the mutagenicity of BDO2 was much greater than that of BDO, which in turn was greater than 3,4-epoxy-1,2-butanediol. Each compound was also capable of inducing slow growth *tk*-/ mutants, indicating the induction of large-scale mutations by these compounds (Cochrane and Skopek, 1993; Cochrane and Skopek, 1994a). An increase in deletion-type mutants was seen with BDO2 but not BDO, suggesting that the deletions might be related to BDO2's ability to form DNA-DNA and DNA-protein crosslinks (Cochrane and Skopek, 1994a).

Both BDO and BDO2 have been shown to induce SCEs in in vitro incubations with human lymphocytes (IARC, 1992). With exposure to BDO2, blood donors can be classified into BDO2-sensitive or BDO2-resistant (Norppa et al., 1995; Wiencke and Kelsey, 1993; Wiencke et al., 1995). Recent studies have shown that variability in the cytotoxicity and SCE response of different individuals to BDO2 correlates with an individual's genotype for glutathione-S-transferase theta (*GSST1*) (Norppa et al., 1995; Wiencke et al.,

1995). Individuals expressing an active *GSTT1* enzyme were all BDO2-resistant, whereas *GSST1* null (homozygous recessive) individuals were BDO2 sensitive. In similar studies, lymphocytes from blood donors with a homozygous recessive genotype for glutathione-S-transferase M1 exhibited higher frequencies of SCEs upon exposure to BDO (Uuskula et al., 1995). These results indicate that polymorphisms in enzymes involved in butadiene detoxication are likely to influence an individual's in vivo sensitivity to genotoxic effects of this compound.

4.7.5. Genotoxicity Studies in Butadiene-Exposed Workers

A recent assessment of biomonitoring studies for butadiene concluded that they provide no convincing evidence for formation of SCA, micronuclei, or SCEs in lymphocytes of occupationally exposed individuals (Sorsa et al., 1994; Waters and Nolan, 1995). In Sorsa and colleagues' initial studies of butadiene workers in two production facilities, significant increases in aberrations, micronuclei, and SCEs were not seen in exposed workers (Sorsa et al., 1994). However, a more recent study by these same investigators found significantly higher frequencies of chromosomal aberrations in a group of butadiene-exposed workers who lacked the *GSST1* gene, as compared to butadiene workers with the gene. This indicates that a person's genotype may affect his or her susceptibility to this agent's genotoxic effects (Sorsa et al., 1996a). A slightly elevated but not statistically significant increase in chromosomal aberrations was also seen in another group of butadiene workers (Au et al., 1995). In this study by Au and associates, significantly higher frequencies of aberrations were seen in lymphocytes of butadiene-exposed workers following lymphocyte exposure to a challenge dose of ionizing radiation. This suggests that the exposed workers had an altered DNA-repair capacity (Au et al., 1995). Recently, Kelsey and associates investigated a group of workers involved in butadiene monomer production and attempted to correlate butadiene exposure with baseline frequencies of SCE and BDO2-induced SCE (Kelsey et al., 1995). No association was seen between butadiene exposure and the baseline of induced SCE frequencies. The lack of correlation persisted when workers' *GSST1* genotypes were included in the analysis. However, for each of these studies, butadiene-exposure levels for most workers were relatively low (~1 ppm or less).

Ward and colleagues have performed a series of studies of workers at a butadiene monomer production plant to determine whether current exposure was associated with increased variant frequency at *HPRT* (Legator et al., 1993). In the initial study of eight "high" exposed workers, five "low" exposed workers, and six nonexposed controls, a significant increase in *HPRT* variant frequency was seen in the high-exposure group (Legator et al., 1993). Exposures in the high-exposed group averaged about 1 ppm. In addition, a highly significant correlation (r= 0.85) was seen between *HPRT* variant frequency and the urinary concentration of 1,2-dihydroxy-4-(N-acetlycysteinyl-S)butane, a urinary metabolite of butadiene. In this study, only one worker was of the *GSTT1*-null phenotype. Interestingly, this worker had the highest *HPRT* variant frequency (Kelsey et al., 1995). A second study has recently been reported in abstract form in which the *HPRT* variant frequency of workers at high (0.30 ppm), intermediate (0.21 ppm) and low (0.12 ppm)

exposures was determined (Ward et al., 1995b). The authors reported that a significant increase in variant frequency was seen in the high-exposure group.

4.7.6. Hematopoietic and Lymphoid Neoplasia Seen in Humans

Since the initial report of increased lymphomas in butadiene-exposed mice, the carcinogenic effects of butadiene have been the subject of numerous epidemiological studies and considerable controversy. Results of these studies, as well as their consistencies and inconsistencies, have been the focus of a number of recent reviews and commentaries (Bond et al., 1995; Cole et al., 1993; IARC, 1992; IARC, 1993; Landrigan, 1993; Melnick and Kohn, 1995). In general, exposure to butadiene has exhibited some association with increased frequencies of lymphosarcomas, reticulosarcomas, and leukemias. Recently, three studies examined an association between butadiene exposure and lymphohematological malignancies. In a mortality study of 614 workers conducted by Cowles and associates, no deaths due to lymphohematopoietic cancer were observed (Cowles et al., 1994). Industrial hygiene data collected between 1979 and 1992 indicated that most exposures were below 1 ppm. In addition, no differences in hematological results were seen between butadiene-exposed workers and other employees at the manufacturing complex. In contrast, a study of butadiene production workers conducted by Ward and collaborators detected a significant increase in the standardized mortality ratio for lymphosarcomas and reticulosarcomas (Ward et al., 1995a). Three of the four deaths from lymphosarcoma and reticulosarcoma occurred in workers who had been employed for more than 2 years and showed a latency period of more than 30 years. A slightly elevated increase (SMR = 1.23) was also seen for workers developing leukemia (including aleukemia) in this study. There was no monitoring data at these facilities.

In the largest butadiene study to date, Delzell and coworkers conducted a retrospective study on 15,649 men employed at 8 styrene-butadiene rubber manufacturing facilities to determine whether exposure to butadiene was associated with increases of lymphohematopoietic cancers (Delzell et al., 1995). The group encompassed a total of 386,172 person-years of follow-up and averaged 25 years of follow-up per person. In all, 11 excess deaths were from leukemia, based on 48 observed and 37 expected (SMR=131, CI 97-174). More deaths from hematological disorders were seen in individuals who had worked more than 10 years and where more than 30 years had elapsed since hire. Among the 45 hourly workers who died with leukemia, 14 (31%) were lymphocytic in origin, 17 (38%) were myeloid, and 14 (31%) were unspecified. The SMR for these workers by leukemia subtype were 210 (CI 115-352) for lymphocytic, 140 (CI 78-231) for myeloid, and 196 (101-342) for unspecified. Those who died of leukemia had a median period of 28 years from time of hire to death. Mortality was elevated among subjects employed in the polymerization, labor, and laboratories process groups, areas that had the potential for relatively high butadiene monomer exposure. An analysis showed that those dying from leukemia had higher exposures than for all workers. The association with butadiene exposure was stronger for ANLL and unspecified leukemia than for CML or CLL, but the relative risk estimates were imprecise. In summary and as indicated by the authors, the strength and internal consistency of the leukemia findings indicate that employment in the styrene-butadiene industry produced an

excess of this disease. However, the authors emphasize that the risk should be kept in perspective; an excess of 11 deaths was seen in a group that experienced 4000 deaths (with 4500 expected), indicating that at these exposure concentrations butadiene is a relatively weak human leukemogen.

4.7.7. Genetic Alterations in Cancer Patients

No information is available about genetic changes in leukemias or lymphomas associated with butadiene exposure.

4.7.8. Hematopoietic and Lymphoid Neoplasia Seen in Rodent Models

During the course of a 104-week chronic inhalation bioassay of butadiene conducted by the National Toxicology Program in B6C3F1 mice, the study was terminated at 60 to 61 weeks due to reduced survival resulting from fatal tumors (Huff et al., 1985). Significant increases were seen in tumors affecting the heart, lung, forestomach, mammary gland, ovary, and liver. Thymic lymphomas in both males and females comprised the most prominent type of cancer increased by butadiene, with frequencies of 46% and 58% seen at the 625- and 1250-ppm exposure concentrations in male mice. Elevated but lower (20%) frequencies were seen in exposed female mice. These lymphomas were seen as early as week 20, and most of them possessed surface markers indicative of early T-lymphocytes (Miller and Boorman, 1990). Subsequently, experiments were conducted in Sprague-Dawley rats (Owen and Glaister, 1990). Relatively weak but significant carcinogenic effects were observed, with increases in tumors seen in a variety of organs including the pancreas, uterus, Zymbal gland, mammary gland, and testes. Most tumors were increased only at the high 8000-ppm exposure concentration. These results indicated that butadiene was carcinogenic in both mice and rats and that there was a clear difference in response between these two rodent species, particularly in the formation of lymphomas.

Additional studies were performed by the National Toxicology Program (NTP) to provide a better characterization of concentration- and time-dependent responses for butadiene-induced neoplasms (NTP, 1993). B6C3F1 mice were exposed to butadiene at 6.25, 20, 62.5, 200, and 625 ppm for 2 years and for shorter durations at 200, 312, and 625 ppm (male mice). The follow-up studies confirmed butadiene carcinogenicity to B6C3F1 mice, with increased tumor incidences seen for hematopoietic system, heart, lung, forestomach, liver, Harderian gland, preputial gland, ovary, brain, and kidney. An increase in lung tumors was seen at exposure concentrations as low as 6.25 ppm. Significant dose-related increases in both malignant lymphoma and histiocytic sarcoma were seen, with significant increases being detected at exposure concentrations as low as 20 ppm (Melnick et al., 1993; NTP, 1993). Significant increases in frequencies of lymphomas and other tumors were seen in the discontinued- exposure groups. These studies indicated that at similar total exposures, lymphoma insidence was greater in mice exposed to a higher concentration of butadiene for a shorter time than in those exposed to a lower concentration for a longer time (34% incidence at 625 ppm for 13 weeks vs 2% at 200 ppm for 40 weeks; 60% incidence at 625 ppm for 26 weeks vs. 3 % at 312 ppm for 52 weeks). However, a single 2-hour exposure to high concentrations of butadiene (1000, 5000,

and 10,000 ppm) was not sufficient to induce significant neoplasm increases in B6C3F1 mice (Bucher et al., 1993).

4.7.8.1. *Mechanisms Involved in Butadiene-induced Thymic Lymphomas in Mice*

4.7.8.1.1. ***Involvement of murine leukemia viruses*** . Due to the high incidence of thymic lymphomas in these butadiene-exposed mice, an incidence much higher than that seen with other chemicals tested by NTP (Sanders et al., 1995), a series of investigations has been undertaken to understand the mechanisms underlying butadiene-induced lymphomagenesis. As described above, considerable differences in metabolism and pharmacokinetics can be seen in mice and rats following exposure to butadiene. One possible explanation for the high incidence of lymphomas observed in butadiene-exposed mice is Related to the possible activation of a murine retrovirus. Murine retroviruses have been proposed to play an important role in induction of thymic lymphomas by a number of agents, including radiation and urethane. Thymic lymphomas induced by butadiene exhibited increased expression of the murine leukemia virus *env* antigen (Irons et al., 1987b). Chronic exposure to butadiene increased markedly the amount of ecotropic retrovirus recoverable from the bone marrow, thymus, and spleen of B6C3F1 mice (Irons et al., 1987b). Expression of other endogenous retroviruses (such as the xenotropic type) was not elevated. Furthermore, no viruses of any kind were found in similarly treated NIH Swiss mice. This strain only rarely expresses endogenous retroviruses and does not possess intact endogenous ecotropic retrovirus sequences.

To explore further the role of this ecotropic retrovirus in the formation of thymic lymphomas induced by butadiene, B6C3F1 mice and NIH Swiss mice were exposed to 1250-ppm butadiene for up to 1 year (Irons et al., 1989). At the end of the study, the B6C3F1 mice exhibited a fourfold incidence of thymic lymphoma (57%) over that seen in NIH Swiss mice (14%). Tumors detected in the NIH Swiss mouse were morphologically similar to those seen in the B6C3F1 mouse and expressed T-cell but not murine leukemia viral *env* surface antigens (Irons, 1990). As mentioned by Sanders and coauthors, these results were taken as presumptive evidence for involvement of an endogeneous ecotropic retrovirus in the etiology of the lymphomas, although no attempt was made to analyze the tumors for mutations or viral insertions (Sanders et al., 1995). Additional studies by Irons and associates have proposed that BDO exerts its toxic effects on a specific primitive hematopoietic stem cell, resulting in a pattern of hematopoietic pathology similar to that associated with mice bearing mutations at the *W* and *Sl* loci (Colagiovanni et al., 1993; Irons et al., 1993). These results indicate that butadiene, most likely through one or more metabolites, is involved directly in lymphomagenesis and that the expression of an endogenous ecotropic virus significantly increases the incidence of tumors induced by this agent.

4.7.8.1.2. ***Involvement of other cancer-related genes*** **.** Additional studies on selected solid tumors induced by butadiene has provided evidence for the involvement of several cancer-associated genes in the carcinogenesis induced by this agent. In a study of the potential involvement of tumor-suppressor genes in butadiene-induced lung and mammary tumors, losses of heterozygosity were observed at several loci on

mouse chromosome 11 surrounding the *p53* tumor-suppressor gene (Wiseman et al., 1994). These losses were seen in 12 of 17 mammary tumors and 2 of 8 lung tumors. Most losses appeared to result from nondisjunction, but at least two were believed to arise from recombination or deletion. Loss of heterozygosity was also observed at the *Rb-1* tumor-suppressor gene in 7 of 17 mammary tumors and 1 lung tumor. Some evidence also was seen for the loss of an additional putative tumor-suppressor gene on mouse chromosome 4. In two additional studies, activated K- and H-*ras* genes were observed in a portion of lung (6 of 9) and liver (4 of 12) tumors induced by butadiene (Goodrow et al., 1990). Of the 29 Harderian gland tumors examined from butadiene-treated mice, 16 exhibited an H-*ras* mutation at codon 61 (Goodrow et al., 1994). In addition, 11 butadiene-induced lymphomas were examined for transforming genes using the nude mouse tumorigenicity assay (Goodrow et al., 1990). Activated K-*ras* genes were detected in 2 of the 11 tumors assayed. Sequence analysis of the activated K-*ras* genes for various tumors showed that most of these resulted from G-to-C transversions at codon 13. This type of mutation was not seen in tumors isolated from nonexposed animals, providing additional evidence that the tumors were induced by butadiene (Goodrow et al., 1990). Results of these analyses are consistent with in vitro and in vivo mutagenicity studies and indicate that mutagenic activation of oncogenes and inactivation of tumor-suppressor genes is likely to contribute to tumors induced by butadiene.

5. MECHANISMS OF CHEMICALLY OR RADIATION-INDUCED LEUKEMOGENESIS IN HUMANS

Increasing molecular and cytogenetic evidence indicates that human cancers are a consequence of an accumulation of genetic and possibly epigenetic changes that are critical for the transformation of a normal cell into a malignant cell (Fearon and Vogelstein, 1990; Sandberg, 1993; Solomon et al., 1991). Leukemogenesis and, in particular, chemical- and radiation-induced leukemogenesis seems to involve multiple genes and chromosomal alterations. In recent years, a number of models have been described in an attempt to integrate various genetic alterations seen in leukemias and to indicate temporal relationships between specific steps in leukemogenesis (Luna-Fineman et al., 1995; Pedersen-Bjergaard et al., 1995). In the model proposed by Pedersen-Bjergaard and colleagues, treatment-related MDS and ANLL develop by different pathways involving varying numbers of genetic alterations, some of which are primary and some secondary (Pedersen-Bjergaard et al., 1995). In general, genes affected by translocations in leukemic cells appear to be transcription factors and may therefore represent master genes that control the expression of other genes involved in cell proliferation, differentiation, and transformation (Pedersen-Bjergaard et al., 1995). The loss of entire chromosomes or chromosomal regions, as in chromosomes 5 and 7, is believed to play a role in the inactivation of tumor-suppressor genes that influence such important cellular functions as cell cycling, DNA repair, and apoptosis. However, the loss of a single gene copy may be sufficient to cause a significant dysregulation of key steps in hematopoiesis and lymphopoiesis. This condition, known as

haploinsufficiency, where two copies of a gene are needed to maintain a normal phenotype, has been shown to play a role in a variety of genetic disorders (Fisher and Scambler, 1994).

In addition to genetic alterations, it is important to understand the nature of the target cell in which initial alterations occur. The first section will overview what is known about major chromosomal alterations and associated genes involved in secondary ANLL in humans. The second will describe the nature of the target cell in various leukemia types.

5.1. KEY GENES IMPLICATED IN CHEMICALLY INDUCED LEUKEMOGENESIS

5.1.1. Loss of Chromosome 5 or Portions of the Long Arm (-5 and 5q-)

Loss of chromosome 5 or a deletion in the long arm (5q-) has been shown to accompany MDS and ANLL in patients treated with alkylating agents and radiation. Evidence is that these alterations are associated with leukemias induced by solvents, pesticides, and other chemical agents (Cuneo et al., 1992; Fagioli et al., 1992; Mitelman et al., 1981; Zedginidze et al., 1990). Loss of a portion of the long arm occurs somewhat more frequently than loss of the entire chromosome and appears as a middle-to-late step in treatment-related MDS and ANLL (Pedersen-Bjergaard et al., 1995). Deletions of 5q- can encompass a significant portion of the long arm (Rowley and Le Beau, 1989). However, the region believed to be critical for leukemogenesis has been narrowed to 5q31 (Le Beau et al., 1993). Although the distal region of 5q contains a large number of genes involved in hematopoiesis and lymphopoiesis, such as interleukin 3, interleukin 4, interleukin 5, interleukin 9, granulocyte/macrophage colony- stimulating factor, the CD14 myeloid antigen, and a T-cell specific transcription factor, most of these do not appear to be located within the critical region (Le Beau et al., 1993). Only the early growth response 1 gene (*EGR1*), a DNA-binding zinc finger protein that functions as a transcriptional regulator, was deleted in all patients in this particular study. Another putative tumor-suppressor gene, interferon regulatory factor-1 (*IRF-1*), which was localized to 5q31.1, was suggested to be the critically deleted gene in ANLL and MDS (Willman et al., 1993). However, more recent refinements have suggested that both *EGR1* and *IRF-1* lie outside the critical region (Boultwood et al., 1993; Fairman et al., 1995; Kroef et al., 1994). At present identity of the putative tumor-suppressor gene in this region remains unknown but is believed to lie within a small region at band 5q31.1 (Fairman et al., 1995).

Nonrandom breakage affecting the 5q31 region has been seen in otherwise normal lymphocytes from patients with NHL and therapy-related ANLL (Johansson and Mertens, 1988; Mamuris et al., 1990) as well as in individuals with a genetic predisposition for leukemia (Brogger, 1977). In addition, breakage affecting this region has been seen following in vitro exposure of lymphocytes or fibroblasts in normal individuals and cancer patients to variOus chemical agents and radiation (Aurias, 1993; Brogger, 1977; Mertens and Johansson, 1989; Tedeschi et al., 1992; Wu et al., 1995; Yunis et al., 1987). This suggests that the breakage may occur early in the carcinogenic process. Moreover, it suggests that this region is unusually prone to breakage and that cells may be able to tolerate breakage in this region and remain viable.

5.1.2. Loss of Chromosome 7 or Portions of the Long Arm (-7 and 7q-)

Monosomy of chromosome 7 and 7q- are the most common alterations seen in leukemias following treatment with alkylating-type chemotherapy and radiotherapy (Pedersen-Bjergaard and Rowley, 1994). There is also some evidence for the involvement of these alterations in solvent-induced leukemias, including those induced by benzene, and such other agents as pesticides and tobacco (Crane et al., 1989; Cuneo et al., 1992; Fagioli et al., 1992; Mitelman et al., 1981; Sandler et al., 1993; Zedginidze et al., 1990). Loss of the entire chromosome or part of the long arm occurs frequently as an early step in treatment-related MDS (Pedersen-Bjergaard et al., 1995). Among deletions critical regions appear to be within the 7q22-qter area, which is involved in both adult and child leukemias (Luna-Fineman et al., 1995). Within this large chromosomal segment, the critical involvement of 7q22 has been implicated in some studies, whereas others have implicated the 7q32 region (Johnson et al., 1996; Velloso et al., 1996). Three different categories of alterations affecting this chromosome also have been seen: deletions of 7q that have either retained or lost the 7q-specific telomeric sequences, unbalanced translocations in which the telomeric sequences have been lost, and complex rearrangements involving 7q (Tosi et al., 1995).

In addition to myeloid leukemias, alterations affecting 7q are seen infrequently in lymphoid neoplasms (Leukemia-Lymphoma, 1987; Offit et al., 1995). In spite of considerable interest and research, critical genes affecting leukemogenesis located in this region that have been are not known (Pedersen-Bjergaard et al., 1995). A number of genes demonstrated to play a role in hematopoiesis or proposed to play a role in carcinogenesis have been localized to the critical region. For example, a series of homeobox genes has been mapped to the deleted region (Lemieux et al., 1994; Logan et al., 1989; Simeone et al., 1994). In addition, several potential tumor-suppressor genes have been identified or postulated to lie within this chromosomal region (Kumar et al., 1995; Oakahashi et al., 1995; Pedersen and Ellegaard, 1994).

Involvement of other genes that cooperate with monosomy 7 and deletion 7q- has been suggested by several investigators. The presence of *ras* mutations in the bone marrow of some patients with monosomy 7 was suggested by Luna-Fineman and coauthors to indicate that aberrant *Ras*-mediated signaling and alterations of chromosome 7 may cooperate in leukemogenesis, possibly by deregulating different biochemical pathways involved in myeloid growth and differentiation (Luna-Fineman et al., 1995). An association between *ras* activation and monosomy 7 was also reported in patients with de novo MDS (Stephenson et al., 1995). Furthermore, activation of *EVI1*, a developmental gene that is not normally expressed in hematopoietic cells and is involved in the 3;21 translocation, has been reported in the leukemic cells of patients whose karyotypes exhibit monosomy 7 or 7q- (Nucifora and Rowley, 1995).

5.1.3. Translocation Between Chromosomes 8 and 21 and Related Alterations

Translocations affecting the long arms of chromosomes 8 and 21 commonly are seen in de novo leukemias, and recent evidence indicates that leukemias with this translocation also are associated with treatment with topoisomerase inhibitors (Nucifora and Rowley, 1995; Pedersen-Bjergaard and Rowley, 1994). In addition, some evidence points to involvement of this translocation in leukemias associated with

exposure to solvents, including benzene and other agents (Crane et al., 1989; Fourth International Workshop on Chromosomes in Leukemia, 1984; Smith et al., 1996b). This translocation is seen in about 40% of cytOgenetically abnormal cases of acute myelogenous leukemia (M2) and is almost exclusively seen with this leukemia subtype (Nucifora and Rowley, 1995). Based on three-way translocations and other evidence, the critical alteration appears to be a fusion gene formed by the juxtaposition of *AML1*, located at 21q22, and *ETO* (CDR or MTG8) at 8q22 (Nucifora and Rowley, 1995). The *AML1* gene also has been shown to be fused with the *EVI1* gene in the t(3;21), an uncommon aberration seen in topoisomerase-related leukemias, as well as some other rare translocations seen in MDS and AML (Nucifora and Rowley, 1995). *AML1* also has been shown to be involved in the t(12;21), an aberration frequently associated with childhood B-lineage acute lymphoblastic leukemia (Nucifora and Rowley, 1995; Romana et al., 1995).

AML1 shows a 67% homology with *runt*, a segmentation gene in *Drosophila*, with close homology in domains believed to be involved in DNA binding and protein-protein interaction (Miyoshi et al., 1995; Nucifora and Rowley, 1995). Two other human genes exhibiting homology to *runt* and *AML1* (named *AML2* and *AML3)* have been identified. These genes are located at 1p36 and 6p21 (Levanon et al., 1994). The three *AML* genes correspond to the murine *Cbf* (or *PEBP2*) family of genes, which have been shown to be heterodimeric transcription factors that interact with the core enhancer region of the polyoma DNA tumor virus and are involved in viral transcription and replication (Nucifora and Rowley, 1995). These genes are believed to regulate growth and differentiation of hematopoietic cells through transcriptional regulation of genes involved in hematopoiesis (Miyoshi et al., 1995; Nucifora and Rowley, 1995; Satake et al., 1995; Takahashi et al., 1995). Following the fusion of *AML1* to either the *ETO* or *EVI1* gene, the dimerized proteins are postulated to lose the ability to properly activate the promoters that they control and consequently inhibit differentiation of translocation-containing myeloid cells (Nucifora and Rowley, 1995). The major role of fusion proteins would be to act as negative dominant competitors of normal *AML1* (Nucifora and Rowley, 1995). Other models have been proposed in which fusion genes exert effects other than binding to *AML1*, allowing them to act as direct transforming genes (Kurokawa et al., 1995; Nucifora and Rowley, 1995; Tanaka et al., 1995).

5.1.4. Alterations Involving the Long Arm of Chromosome 11 (11q23)

Translocations involving 11q23 are a common characteristic of leukemias associated with epipodophyllotoxin-based chemotherapy. These transformations alter a gene known as *MLL* (also known as *ALL-1*, *HRX* or *Htrx*). This gene has been cloned and bears a strong similarity to the *Drosophila* trithorax gene, which has the characteristics of a homeobox gene (Ross et al., 1994; Yu et al., 1995). Homeobox genes act as master switches that regulate different developmental pathways (Kehrl, 1994). These genes play an important role in regulating pattern formation in embryogenesis and hematopoiesis (Kehrl, 1994; Ross et al., 1994; Yu et al., 1995). Breakpoints involved in the translocation in the leukemic cells are clustered in a 8.3-kb region, and studies of these cells have shown that this gene or chromosomal region is involved in at least 29 different translocations, insertions, and inversions (Rowley, 1993). Translocations result in fusion

genes, all of which have the same region of *MLL* at their 5' ends. However, deletions and trisomy 11 affecting this region also have been described (Canaani et al., 1995). The genes located at the most common translocation partners have been cloned, contain nuclear targeting regions, and at least some probably are transcription factors (Canaani et al., 1995).

As discussed by Canaani and associates (Canaani et al., 1995), the large number of partner genes involved with the 11q23 region indicates that alteration of *MLL* gene function is critical. The observation that, in the six translocation products studied thus far, proteins encode a chimeric protein and not a truncated protein indicates that partner polypeptides have a valuable function. However, the observation of leukemias in which the *MLL* protein has undergone partial duplication with no evidence of partner peptide involvement suggests that the partner is not critical and that *MLL* rearrangements result simply in *MLL* protein inactivation. *MLL* homology to the *Drosophila* trithorax gene makes it very likely that *MLL* controls gene expression in hematopoiesis (Canaani et al., 1995).

As indicated in the section on epipodophyllotoxin-type topoisomerase inhibitors, most therapy-related leukemias exhibiting 11q23 alterations are diagnosed as myelomonocytic or monocytic leukemias, although other types of ANLL or ALL are seen occasionally. In addition to involvement in therapy-related leukemias, rearrangements of this chromosomal region and the *MLL* gene have been seen in a large proportion (up to 70%) of de novo infant ALL cases as well as lymphomas (primarily NHL), CML at blast crisis, and MDS (Leukemia-Lymphoma, 1987; Rowley, 1993). Nonrandom breakage affecting the 11q23 region also has been observed in lymphocytes isolated from patients with therapy-related ANLL (Mamuris et al., 1990).

The murine homologue of *MLL* recently has been cloned and sequenced (Ma et al., 1993). It exhibits greater than 93% homology to the human *MLL* gene in four domains and 66 to 69% homology in three shorter regions (Canaani et al., 1995). The murine gene is located in the proximal region of mouse chromosome 9, a region not reported to be commonly associated with murine leukemias or lymphomas (Ma et al., 1993). Recently, the mouse homologue (*Mll* or *All-1*) was targeted by homologous recombination in embryonic stem cells to allow gene's function in pattern development to be assessed in the resulting mutant mice (Yu et al., 1995). Complete deficiency of the *Mll* gene was lethal, with homozygous mice dying during embryogenesis. *Mll* heterozygous mice exhibited retarded growth, axial and sternal malformations, and some hematopoietic abnormalities including anemia, decreased platelet counts, and reduced numbers of B-cells. These results demonstrate that *MLL* positively regulates homeobox gene expression, displays haploinsufficiency, and influences normal hematopoiesis.

5.1.5. Translocation Between Chromosome 15 and 17

A translocation between chromosome 15 and 17 [t(15;17)(q22;q12-21)] is present in nearly 100% of cases with acute promyelocytic leukemia (M3) and is considered diagnostic for this leukemia subtype (Borrow et al., 1990; Nichols and Nimer, 1992). In addition to de novo leukemias, leukemias exhibiting this translocation have been shown to be induced by topoisomerase inhibitors, particularly such dioxopiperazine

types as bimolane and razoxane (Hoffmann et al., 1995; Zhang et al., 1993). Acute promyelocytic leukemias are rare and have not been commonly associated with occupational or environmental exposures. However, two recent studies have implicated benzene exposure as a risk factor for this type of leukemia. Acute promyelocytic leukemia (M3) was the most common subtype (of the limited number that could be classified) seen in recent Chinese studies on benzene, suggesting a possible involvement of this translocation type in benzene leukemogenesis (Yin et al., 1996). In addition, a significant association between employment as a shoemaker and acute promyelocytic leukemia was reported recently in an Italian case-control study (Mele et al., 1995). Exposure to high levels of benzene occurred commonly among Italian shoemakers. In this translocation, the retinoic acid receptor alpha (*RARa*) gene located on chromosome 17 is fused with the *PML* (for promyelocytic leukemia) gene on chromosome 15, forming a chimeric *PML-RARA* gene. Following treatment with retinoic acid, APL cells undergo terminal differentiation in vitro, and the use of retinoic acid in vivo has resulted in a 85 to 90% remission rate among APL patients (Chen and Chen, 1992). *RARa* gene breakpoints are localized consistently within intron 2, whereas those of the PML gene are clustered primarily in introns 3 and 6 (Yoshida et al., 1995). The critical role of the *RARa* gene has been demonstrated in a small group of patients with APL by its rearrangement and fusion to a zinc finger gene on chromosome 11q (Hogge, 1994; Love and Gudas, 1994). The *PML/RARa* fusion protein is believed to lead to a block in promyelocytes maturation and differentiation, but the mechanism by which this occurs is not known (Love and Gudas, 1994). Recent evidence indicates that normal *RARa* protein is involved in regulating the *Hoxb1* gene located within the b chromosomal cluster of homeobox genes in the mouse (Boylan et al., 1995; Love and Gudas, 1994). One model proposes that the *PML/RARa* fusion protein exerts a dominant negative effect by diverting the retinoid X receptor and other nuclear antigens into aberrant structures that are tightly bound to chromatin (Weis et al., 1994). This aberrant nuclear arrangement is believed to result in altered myeloid differentiation (Dyck et al., 1994). Treatment with retinoic acid causes a relocalization of these proteins to restore normal subnuclear structure (Dyck et al., 1994; Weis et al., 1994).

5.1.6. Additional Alterations

A variety of such chromosomal alterations as trisomy 8, trisomy 21, inv (16), del (17p), del (12p), monosomy 18, del (20q) and dup (1q) have been reported to occur in chemically induced leukemias (Pedersen-Bjergaard et al., 1995). For some of these, genes likely to be involved in preleukemic cell transformation, such as the p53 gene at 17p13 or the *MCL1* gene at 1q21 have been identified; for others, such as trisomy 8, little is known about the genes involved (Craig et al., 1994; Pedersen-Bjergaard et al., 1995). In addition, activation of N-*ras* has been reported to occur in patients in radiation-induced leukemias. (The study included patients with AML, CML, and ALL). Involvement of the t(9;22) forming the *abl-bcr* fusion protein has been implicated in radiation-induced CML. For additional information about these genes and p53, the reader is recommended to the following sources: Bishop, 1991; Bos, 1989; Hesketh, 1994.

5.2. THE TARGET CELL IN DE NOVO AND CHEMICALLY INDUCED HEMATOPOIETIC NEOPLASIA

Generally, diagnosis of acute myelogenous leukemia is based on the presence of excess blast cells in the blood or bone marrow (Mehrotra et al., 1995). Histochemical, immunological, and cytogenetic studies are often used to provide confirmatory evidence for the diagnosis. In spite of the observation that AML is a disease of blast cells, it remains controversial whether genetic changes underlying clonal expansion occur in a primitive pluripotent hematopoietic stem cell or whether transformation occurs later in progenitor cells committed to a specific lineage (or, occasionally, two lineages in the case of biphenotypic leukemias) (Mehrotra et al., 1995). Through the use of cytogenetic analyses, molecular probes, and studies on patients heterozygous for the X-chromosome–linked glucose-6-phosphate dehydrogenase gene, a number of investigators have shown that myeloid leukemias and other chronic myeloproliferative disorders are clonal diseases (Fialkow, 1990). These studies also have indicated that chronic myelogenous leukemia, polycythemia vera, and certain other myeloproliferative disorders originate in stem cells capable of developing into myeloid, erythroid, and lymphoid lineages (Fialkow, 1990). This is perhaps most evident with CML in which, following the blast crisis, many leukemic cells show lymphoid characteristics and surface antigens and are positive for lymphocyte-specific gene expression such as expression of terminal deoxynucleotidyltransferase (Erslev and Gabuzda, 1985).

With respect to acute myeloid leukemias, Fialkow and colleagues have also provided evidence that two different types of leukemia exist, one in which the transforming event occurs in the primitive pluripotential compartment and another in which the transforming event occurs in a more mature progenitor compartment (Fialkow, 1990; Fialkow et al., 1987). In general, aberrant cells involved in AML have been identified as occurring in progenitor cells restricted to myeloid and erythroid lineages, with occasional involvement of the megakaryocyte lineage (see Mehrotra et al., 1995 for discussion). One study indicated that in one-third of leukemias, the leukemic clone expressed the surface markers of the multipotent myeloid progenitor cell (CFU-GEMM) and that two-thirds exhibited the markers of a more restricted lineage (CFU-GM) (Sullivan, 1993). Occasionally, alterations have been seen in lymphoid cells, indicating that the alteration occurred at the more primitive pluripotent stem-cell stage. However, others have argued that the primitive multipotential stem cells are more commonly involved in AML (Greaves, 1993).

Recently, a number of studies have provided additional evidence for involvement of early hematopoietic stem cells in malignant transformation. Studies by Pallavicini and colleagues using FISH and immunofluorescent sorting of hematopoietic subpopulations have demonstrated that high frequencies of aberrant (aneuploid) cells were present in the primitive stem-cell compartment ($CD34^+$) of patients with AML, even in cases in which the leukemic cells did not express the CD34 antigen ($CD34^-$) (Mehrotra et al., 1995). Aberrations were also seen in lymphoid and erythroid cells in cases where the predominant leukemic cells lacked lymphoid or erythroid differentiation markers. Based on an analysis of aberrant-cell frequency and compartment size, the authors proposed that these cytogenetically abnormal primitive stem cells represent preleukemic cells requiring the expression of aberrant genes or additional transforming events (i.e.,

clonal expansion, decreased apoptosis, etc.) for the leukemic phenotype to be expressed. Similar results were seen by Haase and coinvestigators indicated that malignant transformation could occur at the level of the early hematopoietic stem cell [CD34+/CD38-] (Haase et al., 1995). Other studies have indicated that, in some samples obtained from MDS or AML patients, monosomy 7 and trisomy 8 seen in leukemic cells may be present in single or multiple lineages, including the lymphoid lineage (Knuutila et al., 1994; van Lom et al., 1995). Interestingly, in studies by Knuutila and associates, leukemias in which the clonal karyotypic abnormality was a translocation or other structural chromosome abnormality seemed to be more restricted in their lineage.

Most of the above studies were performed on patients with de novo leukemias. Much less is known about the origin of alterations and transforming events in leukemic cells resulting from exposure to chemicals and radiation. The induction of CML, ALL, and ANLL by ionizing radiation clearly indicates that the pluripotent stem cell is the target in radiation-induced leukemias (Preston et al., 1994). For alkylating agents, ANLL is the predominant type of lymphohematopoietic neoplasm induced, indicating that the critical transforming event most likely occurs at the level of the committed myeloid progenitor cell. Most treatment-related leukemias show multiple lineage involvement, consistent with an origin at the multipotent myeloid progenitor cell (CFU-GEMM) (Keinanen et al., 1988). The critical event possibly occurs in the more primitive pluripotential stem cell but, as part of the leukemic process, the cells become lineage-restricted and differentiate within the myeloid lineage (Irons and Stillman, 1993).

In addition to ANLL, ALL is infrequently but consistently observed following treatment with alkylating agents (Hunger et al., 1992). This indicates either that the pluripotent stem cell is affected or that the multipotent lymphoid progenitor cells also are targeted by these agents. In a similar fashion, a large majority of leukemias induced by topoisomerase inhibitors are myeloid in nature, suggesting that critical alterations are occurring at the myeloid stem-cell level. However, secondary ALL also has been seen in a small proportion of patients treated with topoisomerase II inhibitors (Felix et al., 1995a; Hawkins et al., 1992; Hunger et al., 1993; Narayanan et al., 1994; Zhang et al., 1993). In several cases of ALL, translocations or deletions affecting 11q23 and confirmed rearrangements within the *MLL* gene have provided additional evidence that these cases were related to treatment with topoisomerase II inhibitors (Felix et al., 1995a; Hunger et al., 1993; Zhang et al., 1996). In one case of a pro-B-ALL, the leukemic cell was also CD34 positive (Zhang et al., 1996). Collectively, these data indicate that, at least part of the time, the transforming event occurred at the level of the early progenitor cell or pluripotent stem cell.

Other evidence lends some support for involvement of the pluripotent stem cell in chemical leukemogenesis. The relatively long latency periods of 5 to 6 years for alkylating agents and over 10 years for benzene provide some evidence for involvement of an early stem cell. Observations of long-term hematopoiesis derived from retrovirally marked transplanted cells in mouse models have indicated that a succession of clones occurs during hematopoiesis; new clones emerging about every 4 to 6 months are responsible for virtually 100% of steady-state hematopoiesis (Bagby, 1994). However, other mouse experiments have indicated that an individual stem cell can reconstitute hematopoiesis in a lethally irradiated

mouse and give rise to a significant fraction of blood cells for periods up to 15 months—a period representing over one-half the animal's life (Keller and Snodgrass, 1990; Lemischka, 1992; Ogata et al., 1995). Similar results recently have been reported in hematopoiesis experiments in larger animals (safari cats), indicating that hematopoiesis involving one or a few cells is not restricted to the mouse model (Abkowitz et al., 1995). These studies indicate that one (or a few) stem cells have a vast self-renewal capacity and can contribute to hematopoiesis for long periods of time. Note that these experiments were conducted in animals in which the normal bone marrow had been destroyed by lethal irradiation. The relevance of these observations to hematopoiesis in normal animals or humans, or under conditions where less severe toxicity occurs, remains to be determined. However, recent studies of an atomic-bomb survivor may provide some insight into the involvement of individual stem cells in humans following radiation exposure. In this report by Kusunoki and associates, a clonal translocation was detected in the T- and B-lymphocytes as well as the myeloid cells of one atomic bomb survivor (Kusunoki et al., 1995). Results from this individual imply that a single altered stem cell from an adult can generate long-lived myeloid and lymphoid progeny that can amount to several percent of the total population of circulating lymphocytes and hematopoietic precursors.

Involvement of an early myeloid progenitor cell or pluripotent stem cell in chemically induced leukemias is supported by studies of Cuneo and associates. These studies found that 20 of 25 AML patients (80%) with reported exposure to pesticides and solvents were positive for the CD34 stem-cell marker, whereas only 22% of the nonexposed patients showed positive staining for this marker (Cuneo et al., 1992). More frequent involvement of the CD34 marker in leukemic clones of exposed patients, when compared to nonexposed individuals, indicates that chemical exposure was associated with a leukemic transforming event within a more primitive cell. Additionally, stem-cell involvement has been associated with bone-marrow toxicity induced by alkylating agents (Gale, 1988). With agents such as busulfan, mitomycin C, or the nitrosoureas, a considerable length of time between dosing and the onset of toxicity suggests that early hematopoietic stem cells are being targeted (Gale, 1988). Moreover, the observation that aplastic anemia following exposure to alkylating agents and some times to benzene frequently precedes ANLL development provides evidence for involvement of the committed myeloid stem cell (CFU-GEMM) as all four myeloid lineages, (granulocytes, erythrocytes, monocytes, and megakaryocytes) are affected by aplastic anemia (Jandl, 1987).

Involvement of bone-marrow–derived stem or progenitor cells also has been implicated in leukemogenesis and lymphomagenesis in mouse models. Due to the myeloid lineage involvement, critical transforming event in radiation-induced myeloid leukemogenesis almost certainly involve bone-marrow myeloid progenitor or pluripotent stem cells. However, the stage at which critical events occur has not been established. Studies of radiation-induced myeloid leukemias have indicated that they were monoclonal in origin (Wright, 1995). In some mice, the leukemias appeared to originate in a cell that had been committed to a specific myeloid lineage whereas in other mice, the target cell appeared to be capable of differentiating into both myelomonocytic and erythroid cells. In radiation and probably in chemically induced lymphomagenesis,

recent evidence indicates that the initial effect occurs in the bone marrow (Kotler et al., 1994). Following irradiation, the preleukemic thymocyte precursors then migrate from bone marrow to the thymus (detectable 6 weeks after irradiation), remain relatively constant in number during most of the premalignant latency period, and emerge as a monoclonal lymphoma (Kotler et al., 1994). In both chemical- and radiation-induced models, depletion of bone-marrow cells capable of populating the thymus appears to be an obligatory step in lymphomagenesis (Irons et al., 1993). Following irradiation and exposure to agents such as butadiene, the capacity of the bone marrow to repopulate the thymus is dramatically impaired (Irons et al., 1993). Although this scenario appears to be relatively simple, cellular events leading to transformation T-cell in the thymus following radiation or chemical treatment appear to be complex, interactions among the thymic cells, thymic microenvironment, and bone marrow (Sen-Majumdar et al., 1994a).

The role of stem cells in murine leukemogenesis also is suggested by involvement of these cells in toxic effects induced by leukemogenic agents (Botnick et al., 1979; Cronkite et al., 1982). For example, a number of studies have demonstrated adverse effects of benzene exposure on committed and noncommitted stem cells of mice (Cronkite et al., 1982; Gill et al., 1980). In some cases, defects in the proliferative potential of stem cells appeared to persist throughout the life of the animal (Botnick et al., 1979).

Evidence for NHL induction following exposure to radiation, alkylating agents, and benzene also suggests that lymphoid cells can be targeted by chemical agents and radiation (Henry-Amar and Somers, 1990; Koletsky et al., 1986; van Leeuwen et al., 1994; van Leeuwen et al., 1989; Yin et al., 1996). These trends, while still somewhat tentative, are important in that NHL generally is thought to originate within mature lymphoid cells (Greaves, 1993). NHL, which is seen as a secondary cancer in patients with Hodgkin's disease, appears to be related to a complex interaction between chemotherapy with alkylating agents and extended field radiation (Sont et al., 1992; van Leeuwen et al., 1989). More recent studies have provided additional evidence for the contribution of chemotherapy in the etiology of NHL in patients treated for Hodgkin's disease (Boivin et al., 1995; van Leeuwen et al., 1994). In these studies, significantly elevated risks were seen in patients treated only with chemotherapy (van Leeuwen et al., 1994), and significant correlations were seen between specific chemotherapeutic agents and NHL risk (Boivin et al., 1995). Increased risks were seen for previous treatment with mechlorethamine and a combined group of "other rare drugs" (Boivin et al., 1995). However, NHL is consistently observed in immunosuppressed individuals, and immunosuppression may be involved in the induction of chemical- or radiation-induced NHL because radiation, alkylating agents, and benzene all have immunosuppressive effects (Tucker, 1993; Weisenburger, 1994).

6. MECHANISMS OF CHEMICAL- OR RADIATION-INDUCED LYMPHOHEMATOPOIETIC NEOPLASIA IN MICE

Two major types of lymphohematopoietic neoplasia, thymic lymphomas and myeloid leukemias, are induced in mice by radiation and chemical agents. As in human cancers, lymphomagenesis and leukemogenesis in the mouse appear to involve multiple pathways involving genetic and, possibly, epigenetic events. In contrast with secondary leukemias in humans, much less is known about the genes involved, particularly in murine myeloid leukemias. This is particularly true for topoisomerase inhibitors, where very little is known about types of induced neoplasia and the genes involved. An overview of mechanisms involved in thymic lymphomas and myeloid leukemias in the mouse following exposure to radiation and chemical agent is presented below.

6.1. THYMIC LYMPHOMA OR T-CELL LEUKEMIA

Thymic lymphoma (or T-cell leukemia) is generally the most common type of hematopoietic neoplasm to develop in the mouse, either spontaneously or following exposure to ionizing radiation, chemical agents, or viruses (Furmanski and Rich, 1982). Some strains of mice, such as the AKR strain, exhibit a very high spontaneous incidence (90% by 12 months) of thymic lymphoma, whereas this neoplasm occurs infrequently or rarely in other strains (Krueger, 1990). The biology and pathogenesis of thymic lymphoma, including the role of murine leukemia viruses in its induction and progression, has been the object of extensive investigation. Although murine leukemia viruses undoubtedly are capable of inducing lymphomas in sensitive mice, their role in lymphomas observed following treatment with radiation or chemical agents is less certain. Current evidence indicates that these leukemia viruses may influence the final incidence of leukemia but do not appear to play a significant role in their initiation (Irons et al., 1993). Fundamental differences have been seen between lymphomas induced by radiation and most chemical agents and those induced by viruses (Kotler et al., 1994; Richie et al., 1985; Sen-Majumdar et al., 1994a). As a result, this discussion will focus on known mechanisms of lymphomas induced by radiation or chemicals and not on virally induced lymphomas. For additional information on the general biology of rodent lymphomas or the action of murine leukemia viruses, recommended sources are Krueger, 1990; Pattengale, 1990; Pattengale, 1994; and Pattengale and Taylor, 1983 for rodent lymphomas; and Kaplan, 1967; and Yefenof and Kotler, 1995 for murine leukemia viruses.

Development of thymic lymphoma induced by radiation or chemicals is currently believed to be nearly identical in most mouse models with a requirement for a preleukemia event in bone marrow, accompanied by macrocytic anemia, thymic hypoplasia, and subsequent development of lymphoma in the thymus (Irons et al., 1995).

A number of different models describing early and late stages of murine lymphomagenesis have been proposed. Models by Irons and Hsiao have described epigenetic or phenotypic changes, whereas those of Newcomb and Hsiao (to some extent) have focused on genetic changes. Based on their recent studies,

Irons and coworkers have suggested that the critical initial event or preleukemic lesion is suppression of a subpopulation of primitive hematopoietic progenitor cells that is common to the radiation, chemical, genetic, and spontaneous models of mouse leukemogenesis (Colagiovanni et al., 1993; Irons et al., 1995). Hsiao and coworkers have argued that early nondisseminated leukemic cells differ from normal thymic pre-T cells in their ability to differentiate in a normal fashion (Hsiao et al., 1995). They have proposed that inhibition of differentiation coupled with unscheduled self renewal is the mechanism underlying the induction of murine thymic lymphomas and leukemias (Hsiao et al., 1995). Their recent results have indicated that loss of wild-type p53 plays a significant role in the conversion of a thymus-confined lymphoma into a widely dispersed T-cell leukemia. In their study, 10 of 17 murine thymic leukemias induced by radiation possessed mutations in the p53 gene. These ten were widely disseminated throughout the body, whereas the seven with wild-type p53 were confined to the thymus (Hsiao et al., 1995).

Newcomb and associates have studied genetic events occurring in C57Bl/6J mice following administration of N-methylnitrosourea (Newcomb et al., 1995). In these studies, lymphomas have been classified as stage I, II, or III representing lymphomas at early, middle, and late stages of development. Using this approach, the investigators found that mutations the *ras*, primarily Ki-*ras*, oncogene appear as an early event and eventually can be detected in 90% of the lymphomas. Trisomy of chromosome 15 was an early involved alteration that was seen in 90% of stage III lymphomas. In contrast, p53 mutations occurred less frequently and appeared to be late events in lymphomagenesis. However, the observation that some lymphomas lacked mutations in *ras* and manifested trisomy 15 showed that additional oncogenes could substitute for mutations in Ki-*ras*. These results indicate that chemically induced murine lymphoma can result from multiple genetic alterations affecting multiple pathways.

6.2. MYELOID LEUKEMIA

Myelogenous leukemias are uncommon in mice but can be induced in some strains by viruses, radiation, and some chemicals (Storer et al., 1982). For a general description of murine myelogenous leukemias, recommended sources are Perkins, 1989; and Seki and Inoue, 1990. The spontaneous incidence of myeloid leukemias ranges from 0 in most strains of mice to 2 to 6% in the RFM strain (Storer et al., 1982; Wright, 1995). Following exposure to 3 Gy of X irradiation, the incidence of myeloid leukemia in the RFM strain was increased from 2 to 6% to 30 to 40% (Wright, 1995). In the CBA/H, SJL/j, or C3H strains, around 20 to 25% of the mice exposed to whole-body radiation eventually develop myeloid leukemia. At higher doses, the incidence of leukemia decreases and returns to near-control levels (Riches, 1995). These leukemias have a latency of 6 to 30 months, with a peak incidence at 18 months (Wright, 1995). Using FAB criteria, these myeloid leukemias have been classified (Wright, 1995): M2 and M4 were the most common subtypes; M5 were less common; and a few examples of M6, M0, and M1 were seen. No examples of M3 or M7 leukemias were observed.

Studies of radiation-induced myeloid leukemias have indicated that they were monoclonal in origin (Wright, 1995). In some mice, leukemia appeared to originate in a single cell that already had been

committed to specific lineage, whereas in other animals the target cell appeared to be capable of differentiating into both myelomonocytic and erythroid cells. These results indicate that there may be different target cells in murine myelogenous leukemia, in a fashion similar to that seen in human myeloid leukemias (Fialkow, 1990; Fialkow et al., 1987).

Irons and associates have conducted a series of mechanistic studies on the origin of myelogenous leukemias induced by chemicals in mice (Irons et al., 1995; Irons and Stillman, 1993). Their research has focused on alterations in cell proliferation and differentiation that may contribute to early events in leukemogenesis. This is based on the premise that in rapidly dividing tissues such as bone marrow, proliferation and differentiation must be tightly regulated by various growth factors and cytokines. Agents altering the rates of proliferation or differentiation can change the size of the bone-marrow target cell population and restrict its lineage pathway, thereby increasing their likelihood of subsequent leukemia development. In these studies, agents known or likely to be associated with leukemia in humans were all able to act synergistically with recombinant GM-CSF in vitro to alter the recruitment of a resting population of hematopoietic progenitor cells that are normally unresponsive to this cytokine (Irons and Stillman, 1993). This resulted in a twofold increase in the size of the proliferating cell compartment generally regarded to be at risk of transformation in leukemogenesis. These effects were seen in in vitro experiments with busulfan, melphalan, lomustine, doxorubicin and etoposide as well as the 4-hydroxycyclophosphamide and hydroquinone (metabolites of cyclophosphamide and benzene respectively) that are believed to be involved in leukemogenesis (Irons and Stillman, 1993; Irons et al., 1992). Interestingly, the coadministration of the benzene metabolite phenol with hydroquinone did affect the magnitude of the hydroquinone-induced enhancement of CFU-GM but rather decreased the optimal concentration of hydroquinone from 10^{-6}M to 10^{-10}M (Irons et al., 1992). In contrast to results seen with leukemogenic chemicals or their metabolites, an enhancement of CFU-GM was not seen with hydroxyurea, vincristine, and—colchicine, myelotoxic agents not associated with leukemogenesis. As a continuation of these studies, the authors have demonstrated that this phenomenon can be observed in bone-marrow cells isolated from animals administered cyclophosphamide and busulfan in vivo (Irons and Stillman, 1993). In addition, alterations in the number of granulocytic precursors and bone-marrow cell kinetics have been seen in vivo following benzene exposure (Dempster and Snyder, 1991; Irons et al., 1979; Niculescu and Kalf, 1995). These intriguing results indicate that a common property among leukemogenic agents, regardless of their mechanisms of cytotoxicity or genotoxicity, is their ability to alter the dynamics of resting hematopoietic progenitor cells in response to recombinant GM-CSF.

Genetic studies of mouse leukemogenesis have indicated that deletion of a portion of mouse chromosome 2 is a frequent event occurring in murine myelogenous leukemias. Interstitial deletions reportedly affect the C2-E5/F1 regions, whereas terminal deletion break points are generally clustered in the C2 and C3/D regions (Wright, 1995). Cells containing these aberrations, which can be detected shortly after a leukemogenic dose of radiation, increase in proportion with time. Chromosome 2 aberrations were seen in

bone-marrow cultures established 4 months after irradiation in 80 to100% of treated animals (Wright, 1995). In addition, loss of the Y chromosome frequently occurs as a late event in leukemogenesis (Wright, 1995).

As indicated in the radiation section, little is known about genes involved in myeloid leukemogenesis in the mouse. Due to its location on mouse chromosome 2, the homeobox gene *Hox 4.1* has been suggested to have a potential role in myeloid transformation (Janowski et al., 1990; Rithidech et al., 1995). Silver and colleagues have reported that a deregulation of *IL-1*β was seen in three murine AMLs involving a rearrangement of mouse chromosome 2 (Janowski et al., 1990; Silver et al., 1989). A similar effect was not seen for the closely linked *IL-1 alpha* gene, the *beta* 2 *microglobulin* gene, or the *c-abl* oncogene located on chromosome 2 (Janowski et al., 1990; Silver et al., 1988). Based on the genetic homology between the D and E regions of mouse chromosome 2 and the 2q24-32 and 11p11-13 regions of human chromosomes, possible involvement of the *WT1* tumor-suppressor gene located at 11p13 also has been proposed (Rithidech et al., 1995). In addition, homology between the distal portion of mouse chromosome 2 and the long arm of human chromosome 20 has been seen, suggesting a possible relationship; deletion of 20q13 is seen occasionally in de novo and therapy-related human leukemias (Pedersen-Bjergaard and Rowley, 1994; Williamson et al., 1995).

7. RELEVANCE OF RODENT MODELS OF INDUCED LEUKEMIA OR LYMPHOMA

As indicated above, rodent models have proven to be valuable models for understanding hematopoiesis and xenobiotic metabolism, for identifying myelotoxic and genotoxic effects of carcinogenic agents, and for identifying critical genes involved in leukemogenesis (Kociba and Kociba, 1990). For the most common human lymphohematopoietic cancers, tumors affecting the same cell type and exhibiting similar characteristics have been identified in rodents (Pattengale, 1994; Pattengale and Taylor, 1983; Perkins, 1989; Ward et al., 1990). Pattengale and associates have argued that close parallels can be seen between certain human B- and T-cell lymphomas and lymphoid neoplasms seen in murine systems and that the majority of murine lymphomas and related leukemias have close clinicopathologic similarities corresponding to analogous human NHL and related leukemias (Pattengale, 1994; Pattengale and Taylor, 1983). Parallels also can be seen between human myeloid leukemias and those seen in mice (Perkins, 1989). Statements can be made that the majority of rat leukemias and lymphomas have similar counterparts in humans (Ward et al., 1990).

In spite of these similarities, a few notable concerns have been raised about rodent models. One is the dissimilarity in the B-T ratio of lymphocytes and in the lymphoma-leukemia incidence in mice compared to humans. Humans have been reported to have more B-cell neoplasms than T-cell lymphomas, whereas mice have more T-cell lymphomas than B. However, as Pattengale has argued, the differences are not great if one compares the B-T ratio in older mice with that of the adult human population (Pattengale, 1994). Additionally, some strains such as the AKR mouse or Fischer 344 rat have very high spontaneous incidences of lymphomas or leukemias, which raises questions about the relevance of these models for estimating human risk (Furmanski and Rich, 1982; Ward et al., 1990). Certainly as recommended by IARC, caution should be used in interpreting of results for species that exhibit high spontaneous frequeNcies for a particular tumor (IARC, 1980). High spontaneous incidences of lymphohematopoietic neoplasia are not unique to selected strains of rodents; high incidences of leukemia can be seen in such genetically susceptible humans as those with Fanconi's anemia or Down's syndrome (Butturini et al., 1994; Zipursky et al., 1992).

One notable concern related to chemically induced leukemias involves the difference in lineage observed in induced lymphohematopoietic neoplasia in rodents as compared to humans. As shown in Table 3, for most agents associated with lymphohematopoietic cancers in humans including radiation, benzene, alkylating agents, and the topoisomerase inhibitors, ANLL is the predominant type of cancer seen. In contrast, lymphoid neoplasms, usually thymic lymphomas or T-cell leukemias, generally are seen for these same agents in mice and to a lesser extent in rats. This indicates that rodent models are less-than-perfect models for leukemias induced in humans by chemicals or radiation. The reason for this lineage difference is unknown but may be related to fundamental differences in hematopoiesis, DNA repair, or possibly apoptosis between the two species (Dumenco et al., 1993; Erexson et al., 1991; Kligerman et al., 1992). As presented in Table 2, lymphocytes comprise a much larger proportion of nucleated cells in mouse blood and bone

marrow than is seen in comparable human blood and bone-marrow samples. This suggests either that these cells have divided more than similar human cells or they do not undergo apoptosis as readily. Since both active cell division and inefficient ability to undergo apoptosis often confer increased susceptibility to genetic lesions and carcinogenesis, the increased proportion of lymphoid cells in rodent blood and bone marrow may be relevant to the frequent involvement of lymphoid neoplasms seen in rodents. In addition, significant differences in the extent of DNA damage and subsequent repair have been seen in studies of rodent and human cells (Erexson et al., 1991).

However, in spite of its limitations, the mouse model has been highly effective at detecting the known human leukemia-inducing agents. For example, of the 12 agents for which data was available in Table 3, 11 induced lymphomas and leukemias in mice; vinyl chloride was the exception, and it was clearly carcinogenic in other tissues. These studies indicate that the mouse model is useful in identifying human leukemogens and can be an important contributor to the hazard-identification step of the risk-assessment paradigm.

A related concern about the rodent model is that magnitude of response seen in animal bioassays is sometimes much higher than that seen in humans. For most human carcinogens tested, a fairly good quantitative correlation is seen between estimates of lymphohematopoietic cancers in animal models and those in humans (Allen et al., 1988; Dedrick and Morrison, 1992; Kaldor et al., 1988; Perera et al., 1989). For example, Dedrick and Morrison saw a good correlation between carcinogenic potency for leukemias in humans induced by melphalan, chlorambucil, and cyclophosphamide as compared to lymphosarcomas in mice and rats when potency was based on total lifetime exposure to the bioactive compounds (Dedrick and Morrison, 1992). However, for some agents such as butadiene, the response seen in animals appears to be much greater than that seen in studies of occupationally exposed human populations (Bond et al., 1995). Several factors, including metabolism, pharmacokinetics, and genetic susceptibility, appear to contribute to the difference in response seen in mice, rats, and humans. However, there is evidence that the involvement of a murine leukemia virus in butadiene-induced lymphomas in the B6C3F1 mouse is largely responsible for high incidences of lymphoma observed (Irons et al., 1989). Murine leukemia viruses also have been implicated in T-cell lymphomas induced by radiation and chemicals in mice (Janowski et al., 1990; Kaplan, 1967). As explained by Irons and associates, little or no evidence suggests that murine leukemia is viruses play a role in the ***initiation*** of radiation and chemical-induced leukemia, but evidence substantial that they have a major influence on the final ***incidence*** of lymphoma and leukemia in this mouse model (Irons et al., 1993). Butadiene exposure to NIH Swiss mice, a strain which does not possess intact endogenous ecotropic viruses, results in T-cell lymphomas, albeit at a lower incidence than seen in the B6C3F1 experiments (Irons et al., 1989). These results indicate that, even in cases where murine leukemia viruses are involved in tumorigenesis, mouse experiments can be used to identify carcinogenic agents. However, incidence data in such experiments are unlikely to allow accurate dose-response estimates to be made. In these cases, it would seem wiser to model tumor incidences based on the incidences seen in bioassays where murine leukemia viruses were not involved. Alternatively and preferably, mechanism-based risk assessment should

incorporate information on metabolism and pharmacokinetics as well as such other biologically relevant endpoints as altered differentiation, genetic alterations, and DNA adducts should be performed.

The above discussion highlights the need for mechanistic information providing an accurate understanding of the relevance of chemically induced tumors in rodents to cancers seen in humans, particularly in relation to an assessment of dose response. As described above, rapid progress is being made in identifying genes and chromosomal regions involved in chemically induced leukemias in humans. In addition, some basic research has been conducted showing parallels for genes and chromosomal regions involved in leukemogenesis and lymphomagenesis in rodents and humans (Pattengale, 1994; Spira, 1983). However, for the most part these parallels are seen for *de novo* leukemias and not chemically induced leukemias. With the exception of the *ras* oncogene, very little is known about genes involved in chemically induced leukemias and lymphomas in rodents. Trisomy 15 has been seen frequently in chemically or radiation-induced lymphomas, and at least three oncogenes, *myc*, *sis* and *ghr*, are located on this mouse chromosome (French et al., 1994). The role, if any, that these play in chemically induced lymphomagenesis is unclear. A series of studies has been conducted in an attempt to identify the critical region and genes involved in lymphomagenesis (Spira, 1983; Uno et al., 1989; Wirschubsky et al., 1986). However, critical genes located on the long arm of this chromosome remain to be identified.

Homeobox genes have been shown to play an important role in hematopoiesis and in the formation of lymphohematopoietic cancers in humans and mice (Kehrl, 1994; Yu et al., 1995). Recent confirmation that the *MLL* gene located at 11q23 on human chromosomes is a homeobox gene as well as the appearance of hematopoietic abnormalities in mice heterozygous for the murine homologue of this gene clearly indicate a role for homeobox genes in hematopoiesis and in leukemias induced by the epipodophyllotoxin-type of topoisomerase II inhibitors in humans (Pedersen-Bjergaard and Rowley, 1994; Yu et al., 1995). In addition, recent evidence that *RARa* protein is involved in regulation of a homeobox gene points to an involvement of homeobox genes in human leukemias induced by dioxopiperazine-type topoisomerase inhibitors that exhibit the t(15;17) (Love and Gudas, 1994). However, there is no information is available on the role that these specific genes play in chemically induced murine leukemias or lymphomas.

The homeobox gene *Hox 4.1* has been mapped to the D region of mouse chromosome 2, which is consistently deleted in radiation-induced myelogenous leukemias in the mouse (Rithidech et al., 1995). In addition, a cluster of homeobox genes has been mapped to the long arm of chromosome 7, which is deleted in ANLL in humans following treatment with alkylating agents (Lemieux et al., 1994; Logan et al., 1989; Simeone et al., 1994). Although this is interesting observation, a direct relationship has not been demonstrated between specific human and mouse homeobox genes in these cases. The human homologue for *Hox4.1* lies on human chromosome 2, whereas murine homologues for human homeobox genes on chromosome 7 have been mapped to mouse chromosome 6 (Kehrl, 1994). Structural and numerical aberrations affecting mouse chromosome 6 were the second most common type of alteration seen in at least one study of mouse myeloid leukemias (Hayata, 1983). Based on these observations, homeobox genes appear likely to play an important role in human and murine leukemias. This will continue to be a promising

area for research on mechanisms of leukemogenesis and may provide a bridge between the human experience and the mouse model.

As indicated above, a fairly good correlation in carcinogenic potency also was seen in comparisons of various carcinogenic agents in rodents and humans, with the exception that carcinogenicity was seen for actinomycin D in the rat but not in humans (Kaldor et al., 1988). Reports of compounds yielding positive results in animal bioassays for which there is no evidence of carcinogenicity in humans are problematic and difficult to address, usually due to limitations inherent in human epidemiological studies and the limited amount of mechanistic information available for most agents. In some cases, these results may be truly false positives (due to type-1 error) related to unique species-specific metabolic pathways or mechanisms of carcinogenesis. For example, Irons and associates suggested by that, in some cases, thymic lymphomas observed in mice may not be relevant for estimating human risk due to species-specific mechanisms of toxicity (Irons et al., 1995). In addition to these mechanistic differences, discrepancies in outcome may be related to differences in exposure patterns, exposure concentrations, or sample sizes. Given this situation, the recommendation presented in the preamble to the IARC monographs would appear to be relevant (IARC, 1994b). It states, "All known human carcinogens that have been studied adequately in experimental animals have produced positive results in one or more animal species. . . . Although this association cannot establish that all agents and mixtures that cause cancer in experimental animals also cause cancer in humans, nevertheless, in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents and mixtures for which there is sufficient evidence of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans. The possibility that a given agent may cause cancer through a species-specific mechanism which does not operate in humans should also be taken into consideration."

8. SUMMARY AND CONCLUSIONS

Chemical- and radiation-induced lymphomagenesis and leukemogenesis are complex processes involving multiple genes, chromosomal alterations, and, probably altered differentiation. In addition, such other factors as metabolic capabilities, DNA repair, and genetic susceptibilities can influence cancer incidence. Given the complexity and multiplicity of steps, animal models are unlikely to reproduce precisely all the critical stages involved in development of chemical-induced leukemias or lymphomas in humans. However, in spite of these limitations, rodent models have proved valuable for identifying and understanding almost all stages in leukemogenesis. Mouse models have been important for identifying metabolic pathways, immunotoxic and myelotoxic effects, and the processes and genes involved in hematopoiesis and leukemogenesis. In addition, chronic animal bioassays using mouse models appear to be effective in identifying human leukemia–inducing agents.

By evaluating the characteristics of known leukemia-inducing agents, a number of generalizations appear to be warranted. (1) The primary type of lymphohematopoietic cancer induced by chemicals and

radiation in humans is myeloid leukemia (ANLL), with the exception of immunosuppressive agents associated almost exclusively with lymphoma development. (2) Potent human leukemia–inducing agents induce significant myelotoxicity and structural chromosomal aberrations in exposed humans. Similar effects are seen when these agents are administered to animal models. (3) Administration of human leukemia–inducing agents to mice results in increases in lymphohematopoietic tumors. However, in contrast to the human, these tumors are primarily lymphoid in origin. (4) The rat is considerably less responsive than the mouse for induction of lymphohematopoietic neoplasia following administration of human leukemogens. However, the resulting neoplasms in the rat also are primarily lymphoid in origin.

A more detailed examination of the six different types of leukemia-inducing agents revealed additional insights. For ease of comparison, many key characteristics of each class of agent is presented in Tables 5 and 6. Although there were similarities among a number of agents, classes of agents appeared to exert their carcinogenic effects through different basic mechanisms. For example, radiation and alkylating agents exhibited similarities in types of nonrandom chromosomal aberrations observed in leukemic clones and in the frequent appearance of MDS prior to ANLL. However, they differed in the frequency involvement of *ras* oncogene and in the more common occurrence of ALL and CML induced by radiation. The epipodophyllotoxin- and dioxopiperazine-type topoisomerase inhibitors exhibited some similarities to each other—the frequent involvement of translocations, short latency periods, and absence of MDS preceding ANLL. However, they differed from each other by the types of translocations induced and subtypes of ANLL developing in patients. Leukemias induced by topoisomerase inhibitors were considerably different from those induced by either radiation or alkylating agents. Benzene exhibits some similarities to the radiation and alkylating agents (occurrence of MDS, longer latency period, etc.) but enough differences exist, at least compared to the alkylating agents (absence of DNA adducts, lack of specific gene mutations), to indicate that it acts through an additional or different mechanism.

Butadiene is quite different from the other specific agents examined. Although detailed information is lacking on types of induced leukemia and related genetic changes, butadiene appears to have a significant number of similarities to ethylene oxide and vinyl chloride (see Table 3). This is not particularly surprising because all three are small-molecular-weight compounds that appear to act through the formation of DNA adducts by epoxide intermediates. The reaction of these agents to form N-alkyl DNA adducts may be related to their relatively weak leukemogenicity; efficient repair of these types of adducts has been proposed (Vogel and Natarajan, 1995). Similarities among these agents in their metabolism, DNA adducts, mutational spectra, and induced rodent tumors has been mentioned by a number of investigators (Cochrane and Skopek, 1994b; Miller and Boorman, 1990; Vogel and Natarajan, 1995).

Using available information on toxicity, genotoxicity, and types of leukemia, scientist may be able to identify classes of leukemia-inducing agents and derive risk estimates for each class and agents within each class. However, due to significant differences among classes of leukemia-inducing agents, that more than one model probably will be needed as biologically based models are incorporated into future risk-assessment approaches. Current rapid progress in the field of cancer research undoubtedly will increase our

understanding of leukemogenesis in humans and rodents and allow more accurate use of rodent models in the risk-assessment process.

9. RECOMMENDATIONS FOR FUTURE RESEARCH

In reviewing information on the six different classes of leukemia-inducing agents, a large number of areas for research are apparent. A number of areas that I consider to be of high priority are listed below.

1. In recent years, great progress has been made in identifying a number of genes involved in human leukemogenesis, particularly those involved in leukemias induced by the topoisomerase inhibitors. However, almost nothing is known about the genotoxic effects of these agents in humans or about the carcinogenic effects of the topoisomerase inhibitors in rodents. Cancer bioassays in the mouse should be performed for etoposide, teniposide, bimolane, and related compounds that have been widely used clinically or currently are being used. Cytogenetic studies and molecular genetic studies should be performed at various times during the bioassay to identify chromosomal regions and genes involved in murine leukemogenesis and the stages at which they are involved. These studies should focus on the involvement of the homeobox or homeobox-regulating genes that have been implicated in human leukemias induced by these agents. Similar cytogenetic and molecular genetic studies should be performed at various time intervals in patients using these drugs therapeutically.

2. Two recently published prospective studies have shown a significant association between frequency of chromosomal alterations in the peripheral blood lymphocytes and an increased risk of cancer (Bonassi et al., 1995; Hagmar et al., 1994). Indications are that myelotoxicity, genetic susceptibility, and metabolic capabilities, as well as age and gender, can play critical roles in carcinogenesis. One important area for future research would be to conduct prospective studies for groups exposed to human leukemogens at high, medium, and low concentrations to determine the predictive value of chromosomal aberrations in general; specific aberrations affecting chromosomes 5, 7, 11q23, etc.; activation of *ras;* immunotoxicity, or myelotoxicity for subsequent development of leukemia. DNA and urine samples also should be obtained from these individuals to allow genetic and metabolic polymorphisms to be correlated with toxicity, genotoxicity, and the eventual onset of leukemia.

3. Most genes identified as involved in leukemogenesis or lymphomagenesis have been identified by cytogenetic studies of nonrandom chromosomal alterations in the cells of leukemia patients. Although information of this type has been obtained for leukemias induced by radiation,

alkylating agents, and topoisomerase inhibitors, very little cytogenetic information is available from leukemias or lymphomas induced by benzene and butadiene. Obtaining this information will be critical for identifying the mechanisms underlying lymphohematopoietic cancers induced by these agents. In addition, permanent cell lines and DNA samples of both normal cells and leukemic cells should be obtained from these patients to allow additional analyses to be performed. As a practical comment, for highly exposed individuals this will almost certainly involve obtaining samples and conducting studies in less-developed countries where exposure to benzene and butadiene is still high. In addition, an international registry for benzene- and butadiene-induced leukemias may need to be established, similar to that of uncommon leukemias such as those associated with Fanconi's anemia (Auerbach and Allen, 1991; Butturini et al., 1994).

4. Metabolic activation plays a critical role in toxic and genotoxic effects of benzene and butadiene and probably in the induction of lymphohematopoietic cancers by these agents. Detailed comparative studies on metabolic capabilities of humans, mice, and rats should be performed, and factors influencing metabolism should be identified. A particular emphasis should be placed on metabolic activation by the cytochrome P450 2E1 isozyme and other genes (*NQO1*, *GSST1*, etc.) likely to influence the effects of these agents.

5. Cancer bioassays employing mouse strains that exhibit myelogenous leukemia following radiation exposure (CBA, RFM, etc.) should be performed for the alkylating agents and butadiene to see if myelogenous leukemia can be induced by these agents in these strains. This approach has been attempted for benzene using the CBA strain without clear results (Cronkite, 1987; Farris et al., 1993).

6. Fundamental information on normal hematopoiesis, and particularly hematopoiesis following exposure to leukemogenic agents, will need to be obtained for both humans and rodents. Some questions that should be addressed include: Can the initial transforming steps occur in resting cells or do they require cell replication? What is the contribution on average and in extreme cases of an individual stem cell to the total formation of blood cells? For how long does an individual cell participate in active hematopoiesis? Following the recruitment of quiescent pluripotent stem cells into an active proliferating state, can these dividing stem cells return to a quiescent state and, if so, in what proportion? Which genes and processes are involved in apoptosis of myeloid cells, and how is this process altered by exposure to leukemic agents? Why and in what specific ways does hematopoiesis in humans and rodent differ? Answers to these questions undoubtedly will contribute to our understanding of the processes contributing to development of lymphohematopoietic neoplasia and allow more accurate estimates of human risks to be made from rodent models.

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