The Secondary Leukemias: Challenges and Research Directions

Malcolm A. Smith, Ronald P. McCaffrey, Judith E. Karp*

Acute myelogenous leukemia (AML) arising following exposure to genotoxic agents has been recognized as a distinctive entity for more than 40 years. Secondary, or therapy-related, AML accounts for 10%-20% of all AML cases. This review addresses four overarching areas of investigation focused on secondary AMLs: 1) dissection of the molecular structure of the induced genetic lesions and identification of the functional consequences of these changes, thereby providing clues to the pathogenesis of secondary AML and potentially serving as a basis for innovative therapeutic interventions; 2) identification and characterization of mechanisms of DNA damage and the orderly repair of such damage; 3) identification and application of accurate biomarkers of leukemogenesis for the purpose of risk prediction and quantification, potentially allowing recognition of patients especially susceptible to the leukemogenic effects of chemotherapy (for genetic or acquired reasons) and allowing their treatment for cancer to be modified on the basis of this susceptibility; and 4) design and implementation of longitudinal clinical and genetic monitoring of high-risk populations (i.e., individuals undergoing cytotoxic therapies for primary cancers). This review of the literature relating to these areas builds upon these seemingly disparate areas of research so that they can be more effectively utilized together to address the problem of secondary AML. Ultimately, the evaluation of these areas will improve our understanding of de novo leukemia and will serve as a springboard for the development of new concepts of therapy and prevention. [J Natl Cancer Inst 1996;88:407-18]

Acute myelogenous leukemia (AML) arising following exposure to genotoxic agents has been recognized as a distinctive entity for more than 40 years. The first descriptions of these “induced” or environmental–occupational leukemias involved exposures to radiation, followed by recognition of AML cases related to exposures to benzene and arsenicals (1,2).

Secondary, or therapy-related, AML accounts for 10%-20% of all AML cases and can be viewed as a subset of environmental–occupational leukemias (3,4). AML cases occurring following cytotoxic therapy were first described 25-30 years ago, concomitantly with the achievement of prolonged survival and cures in Hodgkin’s disease patients (5,6). The leukemias evolved from a background of multiagent cytotoxic therapies that included alkylating agents, often in combination with radiation therapy (6,7). The AML cases arising after DNA-damaging chemotherapeutic agents and the AML cases caused by diverse environmental factors (e.g., benzene, petroleum, organic solvents, and arsenical pesticides) are genotypically similar; the majority are characterized by deletions of all or part of chromosomes 5 and/or 7 (3,4,8,9). More recently, AML cases related to prior treatment with epipodophyllotoxins (etoposide and teniposide) have been identified, often having translocations involving chromosome band 11q23 (8,10-12).

Secondary AML is of growing concern because of the increasingly armamentarium of genotoxic drugs that damage DNA in a variety of ways, because of the use of these genotoxic agents in combination and at higher dose intensities, and because of the lengthening survival in many patients. To address some of the complex issues surrounding these devastating leukemias, the National Cancer Institute (NCI) and the Leukemia Society of America (LSA) jointly hosted the Workshop on Secondary Leukemias (held January 29-30, 1995, in Bethesda, MD) to define research opportunities for elucidating mechanisms underlying the etiology and pathogenesis, the treatment, and ultimately the prevention of secondary AML. The workshop addressed four overarching areas for investigation: 1) dissection of the molecular structure of the induced genetic lesions and identification of the functional consequences of these changes, thereby providing clues to the pathogenesis of secondary AML and potentially serving as a basis for innovative therapeutic interventions; 2) identification and characterization of mechanisms of DNA damage and the orderly repair of such damage; 3) identification and application of accurate biomarkers

*Affiliations of authors: M. A. Smith (Clinical Trials Evaluation Program, Division of Cancer Treatment, Diagnosis, and Centers), J. E. Karp (Chemoprevention Branch, Division of Cancer Prevention and Control), National Cancer Institute, Bethesda, MD; R. P. McCaffrey, Section of Medical Oncology, Evans Department of Clinical Research, Boston University Medical Center, MA. Correspondence to: Judith E. Karp, M.D., National Institutes of Health, Executive Plaza North, Rm. 212C, Bethesda, MD 20892. See "Notes" section following "References."
of leukemogenesis for the purpose of risk prediction and quantification, potentially allowing recognition of patients especially susceptible to the leukemogenic effects of chemotherapy (for genetic or acquired reasons) and allowing their treatment for cancer to be modified on the basis of this susceptibility; and 4) design and implementation of longitudinal clinical and genetic monitoring of high-risk populations (i.e., individuals undergoing cytotoxic therapies for primary cancers). This review of the literature relating to these areas builds upon these themes and attempts to synthesize these seemingly disparate areas of research so that they can be more effectively utilized together to address the problem of secondary AML.

Secondary AML Following Treatment With Alkylating Agents

Secondary AML that follows treatment with alkylating agents has a characteristic morphologic and clinical phenotype (Table 1) (8,13). Typically, these cases present with a myelodysplastic syndrome that culminates in an acute leukemia with a less common phenotype (M6 or M7 with concomitant myelofibrosis). Their peak incidence is 4-6 years following the initiation of cytotoxic therapy for the first malignancy, although they can occur with a latent period as short as 12 months or as long as 15-20 years. Chromosomes 5 and/or 7 are preferentially involved in secondary AML cases following alkylator therapy.

Myelodysplasia and AML with losses or deletions in all or parts of chromosomes 5 and/or 7 have been detected following autologous bone marrow transplantation for various cancers, including lymphomas (14). Although alkylating agents are one mainstay of myeloablative regimens, the hematopoietic stem cell damage that causes these cases may occur during the cytotoxic therapies given prior to bone marrow harvest and myeloablation, rather than as a consequence of the bone marrow transplantation preparative regimen (15).

Chromosome 5 encodes a number of key genes that direct hematopoiesis and that may play a role in leukemia initiation and progression and in full leukemia transformation (16-18). This entire gene segment may be intrinsically unstable (16,17) and is lost to varying extents in de novo AML as well as in environmentally induced and alkylator-induced AML (3,4,6,8,16). The 5q31-33 region of the long arm of the chromosome contains at least nine genes that influence hematopoietic cell growth and differentiation (e.g., granulocyte–macrophage colony-stimulating factor [GMCSF] and interleukins 3, 4, 5, and 9). Ablation of these growth factors could play a role in the promotion or progression of a transformed clone. Other interesting genes in the 5q31-33 region include the interferon regulatory factor 1 (IRF1) gene and the early growth response 1 (EGR1) gene, both of which are deleted in some cases of myelodysplasia and certain AML cases (17,18). IRF1 is a transcriptional activator of the interferon genes, and EGR1 is a zinc-finger protein that is required for differentiation along the monocyte–macrophage lineage. EGR1 is the only gene found to be consistently deleted in both de novo and secondary AML cases that have chromosome 5 deletions (17). Other genes at 5q31-33 include the FMS oncogene (encoding the receptor for macrophage colony-stimulating factor 1, or CSF1) and the CDC2SC gene (encoding a phosphatase that activates the cyclin-dependent kinase, CDC2).

While the majority of patients with 5q deletions exhibit losses at the 5q31 locus, a small subgroup of patients (in particular those with refractory anemia) exhibits deletion of 5q33 sequences without accompanying 5q31 gene losses (16). Furthermore, no consistent mutations have been detected in the many candidate genes located on 5q31-33 in preleukemia or leukemia (16,17); however, the deletion of one or more of these genes in itself may be sufficient to induce cancer. Such an important role for gene dosage has been demonstrated (or hypothesized) for a number of genetic diseases, in which deletion of critical genes creating a “haploinsufficient” state appears to be sufficient to result in a clinical syndrome (e.g., aniridia [PAX6], Greig’s syndrome [GL13], and the CATCH-22 syndrome [TUPLE1]) (19-22). “Haploinsufficiency” for critical genes involved in hematopoiesis could disrupt the balance between growth and differentiation leading to dysmyelopoiesis and “differentiation arrest.”

**Topoisomerase-Associated Secondary AML**

The DNA-intercalating anthracyclines and the topoisomerase II (topo-II)-directed epipodophyllotoxins (e.g., etoposide and teniposide) entered the oncologists’ therapeutic armamentarium in the 1970s and 1980s, respectively. In the late 1980s, a new form of therapy-related AML associated with prior epipodophyllotoxin therapy was recognized (23-25). In contrast to alkylating agent-associated secondary AML, epipodophyllotoxin-associated secondary AML exhibits a shorter latent period (median of 24-30 months), monocytic phenotypes (French–American–British [FAB] M4 or M5 classification), and an acute presentation with substantial blast counts (rather than gradual pancytopenia and fibrosis) (Table 1) (8,26).

The epipodophyllotoxins cause DNA damage via the intranuclear enzyme topo-II and stabilize the DNA–topo-II covalent linkage (27,28). In addition to their other biochemical activities, the anthracyclines inhibit topo-II in a similar manner (29,30). Exposure of cells to topo-II-active agents increases the frequency of illegitimate recombination events (31), a physiologic activity that may be related to both the cytotoxicity and the leukemogenicity of the topo-II inhibitors (26,32).

The leukemogenic translocations induced by the topo-II-active agents often involve the MLL gene (mixed-lineage or myeloid–lymphoid leukemia; also known as ALL1 and HRX) located at chromosome band 11q23 (12,33-36). The MLL gene is the human homologue of the *Drosophila* trithorax gene that

---

**Table 1. Clinical characteristics of secondary AMLs**

<table>
<thead>
<tr>
<th>DNA-damaging agents</th>
<th>Topoisomerase-directed agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency, y</td>
<td>Genotype Losses, deletions (−5, −7)</td>
</tr>
<tr>
<td>Presentation</td>
<td>M6 and M7 (with myelofibrosis)</td>
</tr>
<tr>
<td>Antecedent myelodysplastic syndrome</td>
<td>Abrupt, no prodrome</td>
</tr>
</tbody>
</table>
regulates embryonic differentiation in *Drosophila* and which appears to play a role in controlling differentiation in primitive hematopoietic stem cells (12,35). At least 21 different translocations involving chromosome band 11q23 have been identified, suggesting that the MLL gene has numerous translocation partners with which it can combine to produce fusion proteins that cause marked growth dysregulation and leukemic transformation (36,37).

The molecular characteristics of MLL-associated acute leukemias are germane to their pathogenesis, to their diagnosis, and to a diversity of treatment issues (e.g., monitoring of minimal residual disease during therapy and novel treatments directed against the leukemia-specific fusion messenger RNAs and proteins). Molecular dissection of the breakpoints on 11q23 is providing insight into the pathogenesis of topo-II-associated secondary leukemias (8,10,38-40). Most of the breakpoints occur in a 9-kilobase (kb) region of the genomic DNA that includes exons 5-11 of the MLL gene. This genomic region contains a number of interesting DNA sequences that could be involved in illegitimate recombination. They include the following: Alu sequences, topo-II consensus-binding sequences, matrix-associated sites, VDJ recombinase recognition sites, and sequences with homology to the chi recombinatorial element in *Escherichia coli* (35,41-44). Identification of the DNA sequences at the breakpoint sites (both in the MLL gene and in its translocation partners) will suggest which nuclear enzymatic activities are involved in the translocation events. Preliminary data suggest that the breakpoint sites for secondary AML translocations occur in a different segment of the 9-kb breakpoint region than those associated with de novo AML (Rowley J; personal communication). Should this observation be verified for a larger number of cases, it will provide evidence for distinctive pathogenetic mechanisms for the de novo and secondary AML cases that have translocations involving the MLL gene.

The dioxypiperazine derivatives (e.g., RazoXane, ICRF-187, and bimolane) are another class of topo-II inhibitors that have been associated with secondary AML. The interaction of the dioxypiperazines with the topo-II enzyme results in inhibition of formation of DNA–topo-II complexes, rather than stabilization of DNA–topo-II complexes (45-48). Members of this family of agents have been used to treat psoriasis in the U.K. (RazoXane) and in China (bimolane). After prolonged daily treatment with these agents to high cumulative doses, cases of AML have been observed (49-52). In those cases with informative cytogenetics, t(8;21) and t(15;17) (associated with FAB M2 and M3 subtypes, respectively) have been most commonly observed (49,53).

The camptothecins, another class of antitumor agents, target the topoisomerase I (topo-I) enzyme (54,55). Like topo-II, topo-I forms a covalent linkage with DNA and is involved in DNA replication and recombination. The camptothecins (e.g., topotecan, irinotecan, and 9-aminocamptothecin) stabilize the DNA–topo-I covalent complex, causing cytotoxic events when a DNA replication fork meets a DNA–topo-I complex (56). Like topo-II inhibitors such as etoposide, topo-I inhibitors induce sister chromatid exchanges and are mutagenic, primarily by induction of gene deletions and rearrangements (57,58). As the topo-I-active agents are more widely used in clinical trials, it will be important to carefully monitor their propensity to contribute to the development of secondary leukemias.

The molecular phenotype of the 11q23-associated acute leukemias is clearly linked to the pathogenesis and diagnosis of these leukemias but may also be relevant to treatment issues. For example, it may be possible to monitor response to anti-leukemia treatment by detection of minimal residual disease using probes for the aberrant chromosome band 11q23 region. New approaches to treatment of these secondary AMLs are needed, since current leukemia treatment strategies are rarely successful at achieving long-term survival. Specific treatments may be designed to exploit the unique molecular aberrations associated with leukemogenesis. One strategy under investigation is the use of antisense constructs and ribozymes directed against the fusion gene products encoded by chimeric genes. At least in theory, these antisense constructs and ribozymes could target in a highly selective way the leukemia-related fusion gene or gene products. Newly developed models in the severe combined immunodeficiency (SCID) mouse that utilize engraftment of human leukemia cells provide a preclinical model for testing these and other novel treatment strategies against cells with specific translocations (39,60).

**DNA Damage and Repair: a Focal Point for Leukemogenesis**

The repair of DNA damage is critical to the maintenance of genomic integrity. In order to maintain effective "genomic surveillance" of both endogenous replication errors and exogenous-induced genetic lesions, there are at least three distinctive repair mechanisms: 1) nucleotide excision repair, 2) base excision repair, and 3) DNA mismatch repair (61-64). Nucleotide excision repair is capable of repairing the widest variety of induced DNA lesions, whereas base excision repair is critical for correction of lesions induced by alkylation as well as by reactive oxygen species (61). Newly highlighted in human disease, DNA mismatch repair targets endogenous errors in DNA replication (62). Each pathway requires a distinct cascade of genes and proteins that are recruited into action when specific types of DNA damage are "sensed" (Table 2). Given the involvement of exogenous mutagens in the pathogenesis of secondary leukemias, it is plausible that a defect in the nucleotide excision repair pathway could play a contributory role. Whatever the specific mechanism(s) involved, the consequences of defective DNA repair are evinced on the genomic level by mutations and aberrant recombination events that can lead to cell cycle dysregulation, genomic instability, uncontrolled proliferation, blockage of differentiation, and abrogation of apoptosis (with net drug resistance).

A number of familial syndromes are associated with defective DNA repair and/or DNA damage hypersensitivity and include ataxia-telangiectasia (AT), Bloom syndrome, Fanconi’s anemia, xeroderma pigmentosum (XP), and Cockayne’s syndrome (CS). These genetic conditions have allowed identification of critical components of the various DNA repair pathways (Table 2). Studies of XP, a disorder characterized by the inability to excise UV radiation-induced DNA damage, have been especially valuable in elucidating individual components of nucleotide excision
repair (65). XP is composed of multiple complementation groups, each of which represents a defect in a specific component of the nucleotide excision repair process (66). The XPA gene product, for instance, recognizes and complexes with the UV radiation-damaged DNA (67,68). The XPG gene product is a nuclease that makes an incision at a site 3' to the damaged DNA (65,69), whereas the XPF product functions as part of the DNA damage recognition complex and as a nuclease making an incision in the damaged DNA strand 5' to the lesion (70). The gene products for XP complementation groups XPB and XPD have DNA helicase activity (71-73) and are components of the transcription factor complex TFIHH (74).

The multicomponent nature of the TFIHH complex may provide a molecular basis for the observed coupling of transcription and DNA repair (74,75). RNA polymerase, the enzyme involved in transcription, is a highly processive enzyme that remains tightly bound to DNA at the site of a DNA lesion (e.g., a cyclobutane pyrimidine dimer) (76). In one model for the coupling of transcription and DNA repair, the RNA polymerase enzyme that is stalled at a DNA lesion undergoes a conformational change that leads to recruitment of TFIHH and other factors required for nucleotide excision repair (74). Further support for the role of the TFIHH complex in coupling transcription and DNA repair comes from studies identifying the genes responsible for CS (a syndrome associated with defective strand-specific repair of transcriptionally active genes): The CSA and CSB (ERCC6) gene products associate with subunits of TFIHH, and CSB contains consensus helicase motifs (77-79).

The coupling of transcription and DNA repair may explain the variability in DNA repair rates between expressed and unexpressed genes (80), between the transcribed and nontranscribed strands of individual genes (81), and between specific gene segments within a single gene (82). The faster repair of lesions on the transcribed strand compared with the nontranscribed strand suggests that lesions on the nontranscribed strand may be protected from the repair process, permitting them to persist and become incorporated permanently into the genome. It is provocative that the characteristics of persisting mutations induced by certain carcinogens (e.g., polycyclic aromatic hydrocarbons [PAHs] from tobacco smoke and UV light) in the tumor suppressor gene p53 suggest that the causal lesion occurred on the nontranscribed strand of DNA (83-88).

The tumor suppressor gene p53 plays an integral role in the repair of DNA damage induced by ionizing radiation and other types of genotoxic stress (89-92). Given the role of p53 in the cell's response to DNA damage, it is plausible that abnormal p53 activity could result in reduced ability to repair DNA damage (93-95), leading to genomic instability and increased susceptibility to leukemogenesis (91,94,96,97). In this light, the role of p53 in the cellular response to DNA damage is summarized below.

The study of AT, an inherited multisystem disorder caused by mutations in a recessive gene on chromosome bands 11q22-23, has helped to elucidate the critical role of p53 in the cell's response to genomic damage (98). AT is characterized at both the molecular and clinical levels by hypersensitivity to radiation-induced DNA damage (99). The AT gene (ATM) has been identified (100) and shows sequence similarity to a family of signal transduction mediators with homology to phosphatidylinositol 3-kinase (101,102) that control progression through the G1 phase of the cell cycle [tor1 and tor2 in yeast (103,104), mTOR in rodents (105), and FRAP in humans (106)]. The ATM gene also shows homology to the yeast rad3 gene product (which has helicase activity), is required for the nucleotide excision DNA repair pathway, and has homology to human ERCC2/XPD) (107-109). Available data support a model in which specific types of DNA damage cause activation of the AT gene product, leading to p53 induction, which causes transcriptional activation of specific growth-arrest–DNA-damage-inducible (GADD) gene products (98,110,111). With the identification of the ATM gene, characterization of the individual steps in this pathway should be forthcoming.

One mechanism by which p53 induces G1 arrest following DNA damage is by transcriptional activation of the gene encoding a 21-kd cyclin-dependent protein kinase (CDK) inhibitor known as p21WAF1/CIP1 (wild-type p53-activated fragment 1 and CDK-interacting protein 1) (112-114). p21WAF1/CIP1 binds to multiple G1 CDKs and blocks the activation of cyclin–CDK complexes, thus limiting their ability to phosphorylate target substrates, notably the retinoblastoma proteins (pRbs) (112,115,116). In turn, the maintenance of pRb in the hypophosphorylated state impedes movement across the G1/S boundary.

p53 may play a more direct role in modulating DNA repair than simply delaying progression through G1 and allowing more time for DNA repair to occur. One mechanism by which p53 may more directly affect DNA repair is by recognizing and binding to damaged DNA and then subsequently interacting with and modifying the activity of components of the nucleotide excision repair pathway. Wild-type p53 is able to form stable complexes with DNA lesions, including insertion–deletion mismatches and lesions induced by ionizing radiation (117,118). The C-terminal domain of p53 that is involved in binding to damaged DNA also binds to transcription-repair factors (XPB, XPD, and CSB) involved in the nucleotide excision repair pathway (95,119). XPB is a component of the TFIHH transcription factor (75) and has helicase activity (71,72). When DNA is
damaged, p53 interactions with XPB and other transcription-repair factors may stall transcription either at the initiation stage or at the elongation stage, thereby modulating preferential repair of DNA damage within actively transcribed genes (119).

p53 may also modulate the balance between DNA repair and DNA replication through p21\(^{WAF-1/CIP-1}\) inhibition of proliferating cell nuclear antigen (PCNA). PCNA is an essential DNA replication protein and both facilitates loading of polymerase d (in cooperation with replication factor C) and acts as a processivity factor for DNA polymerase d (120-122). The p21\(^{WAF-1/CIP-1}\) directly binds to PCNA and inhibits DNA replication catalyzed by the DNA polymerases d and e (123-125). Thus, p21\(^{WAF-1/CIP-1}\) that is induced by p53 following DNA damage not only inhibits cell cycle progression through its interactions with CDKs, but also directly inhibits DNA synthesis by binding to PCNA. However, inhibition of PCNA following DNA damage could be problematic, since PCNA also plays a pivotal role in nucleotide excision repair (126-128), and inhibition of DNA repair following DNA damage would be counterproductive. The answer to this apparent paradox is that p21\(^{WAF-1/CIP-1}\)-mediated inhibition of PCNA differentially blocks synthesis of long (i.e., kilobase) DNA fragments but not short (e.g., 10-base) DNA fragments (124,125) and that PCNA-dependent nucleotide excision repair is not blocked by p21\(^{WAF-1/CIP-1}\) (124).

Another mechanism by which p53 may affect the balance between DNA replication and repair is via interactions between p53-induced GADD45 and PCNA. Following a genotoxic stress, p53 functions as a transcription factor to transactivate GADD45 (98,129). GADD45 causes growth suppression (130) and suppresses entry into S phase when it is overexpressed (131). At the molecular level, GADD45 associates with PCNA and stimulates PCNA-mediated nucleotide excision repair (131). The combined effect of the p53-induced p21\(^{WAF-1/CIP-1}\) and GADD45 following a genotoxic injury is inhibition of entry into the S phase (through inhibition of CDKs), slowing of ongoing DNA replication (through interactions with PCNA), and stimulation of DNA repair. Thus, p53 plays an important role in the cell’s response to DNA damage, and alterations in p53 function can lead to genomic instability and to an increased risk of neoplastic transformation (91,94,96,97).

Studies in the BALB/c mouse plasmacytoma model provide additional data supporting a coupling of tumorigenesis and DNA repair efficiency. Following UV radiation-induced DNA damage in splenic B lymphoblasts, the nucleotide excision repair of the c-myc 5' and regulatory regions (but not the repair of the 3' flank of c-myc or of dihydrofolate reductase gene segments) proceeds at a markedly higher rate in cells from mice resistant to plasmacytoma development than in cells from mice susceptible to plasmacytoma development (82). The 5' and regulatory regions of c-myc are the major sites of chromosomal breaks and translocations in B-cell lymphomas and leukemias bearing c-myc translocations and rearrangements. The mouse plasmacytoma system validates the notion that alterations in DNA repair efficiency, especially the differential repair of gene segments involved in recombination or transcriptional regulation, may contribute to neoplasia.

### Molecular Epidemiology: Foundation for Clinically Relevant Risk Prediction and Quantitation

The discipline of molecular epidemiology incorporates a number of interdependent components that, when analyzed singly or in combination, may allow prediction of an individual’s risk for tumorigenesis following exposure to a potential tumor initiator or tumor promoter (132,133). Molecular genetic and biochemical processes that contribute to the leukemogenic susceptibility of an individual may be numerous, ranging from the efficiency of DNA repair processes to polymorphisms in the metabolism of mutagenic agents (such as chemotherapy).

One initial step in identifying a susceptible population is to identify whether there is heterogeneity within the population in the mutagenic burden incurred following cancer treatments. Two tests that have been applied to measuring somatic cell genetic damage following cancer therapy are the HPRT mutant frequency assay and the glycophorin A somatic mutation assay (134-137). For both tests, increases in mutant frequency are observed in cancer patients following treatment with cytotoxic agents (138-144). One factor that has been associated with increased mutant frequency following cytotoxic therapy is serum folate (145). Among a small series of breast cancer patients receiving diverse chemotherapy regimens that included cyclophosphamide, higher mutant frequencies were observed among patients with lower pretreatment serum folate levels than among patients with higher levels. It will be of interest to attempt to identify additional patient factors associated with higher mutation frequencies following cytotoxic therapy.

The HPRT mutation assay has an advantage over the glycophorin A assay in that it can be performed on all patients and does not depend on heterozygosity for measurement. In addition, use of the HPRT assay allows identification of specific molecular changes resulting from the mutagenic challenge (134,135,146). The HPRT mutation assay and molecular genetic analysis of mutant clones have demonstrated that spontaneously occurring mutations are most often point mutations (134), that ionizing radiation tends to cause large deletions (146,147), and that mutations occurring in infants typically involve translocations at VDJ recombinase recognition sites (148).

An important question is whether the HPRT gene can serve as a surrogate marker for susceptibility to secondary AML, even though the target cell of the assay (i.e., a lymphocyte) is different from the target cell for leukemogenesis (an early hematopoietic cell). This question can be tested only if populations with higher mutation frequencies following cytotoxic therapy can be identified and if the risk of secondary AML in these patients can be reliably compared with the risk of secondary AML in patients with lower mutation frequencies. If indeed HPRT (or perhaps another gene) is a reliable surrogate marker, then hypersensitivity to mutagen exposure may be useful as a biomarker of genetic susceptibility for leukemogenesis, in much the same way as microsatellite instability linked to a defect in DNA mismatch repair or chromosomal fragility in AT can be used to identify populations at risk for certain cancers (149).

Accepting that variations in mutagenic burden following treatment with cytotoxic agents likely exist, then it becomes im-
important to identify pathogenetic mechanisms for the variation in mutagenic burden. A reasonable hypothesis is that higher levels of carcinogen activation (or lower levels of carcinogen detoxification) lead to higher levels of DNA adduct formation, higher levels of persistent mutations, and increased risk of cancer development following exposure to environmental carcinogens (or to increased risk of leukemia following exposure to cancer therapies). Numerous examples from the genetic epidemiology literature suggest that this is the case, and several examples are briefly described.

The glutathione S-transferases (GSTs) are a supergene family capable of detoxifying a number of carcinogenic electrophiles by catalyzing their conjugation to glutathione (150). For example, PAHs are detoxified by conjugation with glutathione, preventing the formation of PAH–DNA adducts (151). Two of the members of this class, glutathione S-transferase M1 (GSTM1) and glutathione S-transferase theta (GSTT1), are homozygously absent (the null phenotype) in a significant percentage of the population (approximately 50% for GSTM1 and 20% for GSTT1) (152,153). As would be predicted by the role of GSTM1 in carcinogen detoxification, persons with the null phenotype for GSTM1 show higher levels of cytogenetic damage (e.g., as measured by sister chromatid exchange) (154), higher levels of PAH–DNA adducts (155,156), and higher levels of mutagenic activity in their urine (157) than do persons with the GSTM1 gene present. Molecular epidemiologic studies suggest that persons with GSTM1 deficiency may have modest increases in susceptibility to certain cancers, including some types of lung cancer (158-163), bladder cancer (164-168), colon cancer (169), melanoma (170), and malignant gliomas (171,172). Of relevance to secondary AML etiology, a preliminary report (173) noted elevated frequencies for both GSTM1 and GSTT1 null genotypes among patients with myelodysplastic syndrome. This observation requires confirmation in larger epidemiologic studies.

Genetic variations in cytochrome P450 enzymes, another class of enzymes involved in carcinogen metabolism, have been associated with increased risk of certain cancers (132,174). The cytochrome P450 enzymes are involved in metabolic activation of precarcinogens to reactive metabolites, as well as in the detoxification of natural products. Thus, a plausible hypothesis is that individuals with higher levels of carcinogen-activating enzymes will have higher levels of DNA adduct formation (156) and higher risks of subsequent cancer development, whereas individuals with lower levels will be relatively protected from cancer risks (132).

One member of the CYP-450 family, aromatic hydrocarbon hydroxylase (AHH), is encoded by the CYP1A1 gene and is induced in the presence of PAHs. Alleles of the CYP1A1 gene have been identified that have higher levels of activity and higher levels of inducibility than the wild-type form (174,175). Smokers with a genetic polymorphism of CYP1A1 that is associated with higher AHH activity have higher levels of DNA–PAH adducts than smokers lacking this allele (176). In a molecular epidemiologic study (177) of a Japanese population, persons homozygous for the higher activity allele appeared to be at increased risk of developing lung cancer (174,177), although this association has not been observed in most studies of non-Oriental populations (178-181).

Another member of the CYP-450 family showing genetic polymorphism is CYP2D6. Tobacco smoke nitrosoamines can be activated by CYP2D6 (182,183), and individuals with genotypes consistent with higher levels of CYP2D6 activity were found to have higher levels of 7-methyl-deoxyguanosine monophosphate (a DNA adduct associated with tobacco-specific nitrosoamines) than individuals with genotypes predictive of low CYP2D6 activity (156). The CYP2D6 enzyme has traditionally been assayed by measuring the extent of metabolism of debrisoquine (an antihypertensive medication), and genetic polymorphisms have been identified that are predictive of the debrisoquine metabolizer phenotype (184,185). Approximately 90%-95% of the population are classified as extensive metabolizers of debrisoquine, and these persons are reported in some studies to be at increased risk of developing lung cancer in comparison to the 5%-10% of the population who are poor metabolizers (186-189). Other studies, however, have not found increased risk of lung cancer for the extensive debrisoquine metabolizer phenotype (190) or for genotypes associated with extensive metabolism (191), illustrating how difficult it can be to define and understand the contributions of genetic susceptibility to cancer risk (192). An increased risk of liver cancer has also been reported for persons who are homozygous for functional CYP2D6 genes, compared with persons with fewer than two functional CYP2D6 genes (193).

Studies evaluating both CYP1A1 and GSTM1 polymorphisms (161,174,177,194) illustrate another important concept—that persons with high levels of carcinogen-activating enzyme activity (e.g., alleles of CYP1A1 with higher activity) and low levels of detoxifying enzyme activity (e.g., the GSTM1 null genotype) may be at higher risk of developing specific types of cancers. In a similar manner, persons with high levels of two carcinogen-activating enzymes that are in the same metabolic pathway would be predicted to be at increased risk of developing cancer. Such a relationship is suggested for colon cancer in which heterocyclic aromatic amines activated by N-acetyltransferase 2 (NAT2) and CYP1A2 are thought to play an etiologic role (195): Individuals who are both rapid acetylators (mediated by NAT2) and rapid N-oxidizers (mediated by CYP1A2) of heterocyclic amines appear to be overrepresented among colon cancer cases (196,197).

Another method that may be useful in identifying cancer patients who may be at risk for subsequently developing secondary AML is identification and quantitation of known leukemogenic translocations in hematopoietic cells following chemotherapy. It is technically feasible to use polymerase chain reaction assays to detect translocations involving the MLL gene in peripheral blood or bone marrow cells (detection limit of approximately one abnormal cell among 10^5 normal cells). Evaluations of large numbers of patients treated with topo-II-active agents will be necessary to define a relationship between the level of MLL translocation events during and after chemotherapy and subsequent risk of developing AML. If a relationship can be demonstrated, then detection of these molecular lesions could identify individuals at particular risk for leukemogenesis from topo-II-active agents, and alternative chemo-
therapy agents could be considered for these patients. It may also be feasible to use in vitro assays to evaluate the sensitivity of normal hematopoietic precursors to induced DNA damage at the critical 11q23 gene loci.

Longitudinal Monitoring of High-Risk Populations

Concerns about secondary AML were heightened recently with the detection of six cases of AML arising in the National Surgical Adjuvant Breast and Bowel Project B-25 trial among approximately 2550 women treated for stage II breast cancer with one of three dose schedules of adjuvant therapy combining cyclophosphamide, doxorubicin, granulocyte colony-stimulating factor, and tamoxifen (for postmenopausal women) (198). The leukemias occurred in women aged 50-69 years at 10-18 months after they began adjuvant therapy. All of the AML case subjects had either FAB M4 or FAB M5 morphology, and two of the case subjects on whom cytogenetic analysis was performed had 11q23 abnormalities.

Available data from large cooperative group clinical trials suggest that secondary AML occurring so quickly after the initiation of therapy is unusual for adjuvant therapy using doxorubicin and cyclophosphamide at standard doses. Thus, concerns were raised that the dose-intensive use of cyclophosphamide in combination with doxorubicin may increase the risk of secondary AML. To address these concerns, the Cancer Therapy Evaluation Program (CTEP) of the NCI has established a monitoring plan to quantify the risk of secondary AML following dose-intensive treatment strategies. The monitoring plan to accomplish this objective builds upon the CTEP/NCI experience with the epipodophyllotoxin-monitoring plan (199). The underlying assumption of the epipodophyllotoxin-monitoring plan is that estimates of risk can be obtained most quickly and reliably by pooling data from multiple protocols that all administer epipodophyllotoxins using similar schedules and cumulative doses. The CTEP/NCI plan provides for complete ascertainment of secondary AML/myelodysplastic syndrome cases by using existing cooperative group mechanisms for patient follow-up and for reporting serious adverse events. The plan identifies the target population prospectively (on the basis of their entry into the protocols of the monitoring plan), so that both secondary AML/myelodysplastic syndrome cases and total patient-years of follow-up for the entire patient population could be accurately determined. In a similar manner, NCI cooperative group protocols that prescribed similar doses and dose intensities of alkylators and anthracyclines were grouped together so that the case subjects on whom cytogenetic analysis was performed had 11q23 abnormalities.

The secondary leukemias are an ironic and tragic consequence of progress in the treatment of other cancers and are likely to increase as success with cytotoxic therapies for solid tumors and for other hematologic cancers also increases. As outlined in Table 3, molecular studies of the “induced” leukemias will inform issues surrounding genomic instability and the emergence of particular genomic lesions as a consequence of specific toxin-DNA interactions. Longitudinal studies that quantify dose-risk relationships for leukemogenic agents should provide an ability to define individual risk for leukemia induced by a particular toxin. Serial monitoring of individuals at risk for the earliest genetic changes could lead to intervention before cumulative genetic damage and irreversible transformation ensue. And finally, for those individuals who are defined as being susceptible to the leukemogenic effects of a drug, it may be possible to avoid the offending agent altogether (if alternative options exist). In this regard, the study of therapy-related AML

### Table 3. Selected key areas of investigation of secondary leukemias

- Dissect the structure and function of induced genetic lesions as a basis for innovative therapeutic interventions.
- Identify and characterize mechanisms of DNA damage and repair.
- Develop and apply accurate biomarkers of leukemogenesis for the purpose of risk prediction and quantification and as the first step toward prevention.
- Design and implement longitudinal clinical and genetic monitoring of high-risk populations.
provides an excellent template for all environmental–occupational cancers with respect to dissecting the molecular epidemiology, quantitating individual risk, and characterizing formative "premalignant" genetic changes as a molecular foundation for developing, implementing, and measuring clinical prevention interventions. Given their present resistance to current therapeutic strategies, these challenging leukemias also provide an important testing ground for mechanistically novel approaches. Ultimately, all of these studies will inform our understanding of so-called "de novo" leukemia and serve as a springboard for the development of new concepts of therapy and prevention.

References


416 REVIEW

Journal of the National Cancer Institute, Vol. 88, No. 7, April 3, 1996

Downloaded from https://academic.oup.com/jnci/article-abstract/88/7/407/1011820 by guest on 21 March 2019
**Notes**

Participants at the National Cancer Institute (NCI)-Leukemia Society of America (LSA) Workshop on Secondary Leukemias: J. Abrams (NCI, Bethesda, MD), J. Ajani (The University of Texas M. D. Anderson Cancer Center, Houston), R. Albertini (University of Vermont, Burlington), M. Amylon (Stanford University Medical Center, CA), P. Aplin (Roswell Park Cancer Institute, Buffalo, NY), D. Bell (National Institute of Environmental Health Sciences, Research Triangle Park, NC), W. Bigbee (University of Pittsburgh, PA), C. Bloomfield (Roswell Park Cancer Institute), V. Bohr (National Institute on Aging, Baltimore, MD), J. Boice (NCI), M. Bookman (Fox Chase Cancer Center, Philadelphia, PA), N. Breslow (University of Washington, Seattle), P. Burke (Johns Hopkins Oncology Center, Baltimore), M. Caligiuri (Roswell Park Cancer Institute), B. Cheson (NCI), R. Curtis (NCI), A. DeCillis (National Surgical Adjuvant Breast and Bowel Project, Philadelphia), J. Deeg (Fred Hutchinson Cancer Center, Seattle, WA), P. Donner (Northwestern University, Chicago, IL), M. Doukas (VA Medical Center, Lexington, KY), M. Evans (National Institute on Aging), C. Feinberg (Children’s Hospital of Philadelphia), J. Finerty (NCI), S. Forman (City of Hope Medical Center, Duarte, CA), A. Fornace (NCI), M. Friedman (Food and Drug Administration, Rockville, MD), R. Geller (Emory University, Atlanta, GA), R. Gelman (Dana-Farber Cancer Institute, Boston, MA), H. Gill-Super (University of Chicago), S. Gore (Johns Hopkins Hospital, Baltimore), B. Grant (University of Vermont), M. Grever (Johns Hopkins Oncology Center), K. Hays (University of Pittsburgh), P. Ho (NCI), J. Jacobson (NCI), J. Karp (NCI), S. Kaufmann (Mayo Clinic, Rochester, MN), P. Keegan (Food and Drug Administration), B. Kimes (NCI), J. Kisch (NCI), V. Land (Pediatric Oncology Group, Chicago), R. Larson (University of Chicago Medical Center), M. LeBeau (University of Chicago), R. Lemons (Primary Children's Medical Center, Salt Lake City, UT), E. Levine (Roswell Park Cancer Institute), M. Lizizow (Mayo Clinic, R. McCaffrey (Boston University Medical Center), K. McCarty (University of Pittsburgh), K. Miller (New York University School of Medicine), S. Mitra (University of Texas Medical Branch—Galveston), S. Murphy (Children’s Memorial Hospital, Chicago), E. Paeta (Monefaire Hospital and Medical Center, New York, NY), J. Pederson-Bjergaard (Rigshospitalet, Copenhagen, Denmark), Y. Pommier (NCI), J. Radich (University of Texas, San Antonio), B. Raney (The University of Texas M. D. Anderson Cancer Center), P. Ravdin (University of Texas Health Science Center, San Antonio), R. Rednor (University of Pittsburgh), E. Reed (NCI), M. Reiling (St. Jude Children’s Research Hospital, Memphis, TN), J. Rowe (University of Rochester Medical Center, NY), J. Rowley (University of Chicago Medical Center), E. Saulvilli (NCI), P. Schulman (North Shore University Hospital, Manhasset, NY), K. Shannon (University of California, San Francisco), D. Shirmerring (NCI), M. Smith (NCI), J. Soggin (NCI), W. Stoll (Loyola University Medical Center, Chicago), M. Tallman (Northwestern University), P. Therese (European Organization for Research and Treatment of Cancer, Brussels, Belgium), L. Travis (NCI), W. Velasquez (St. Louis University Medical Center, MO), G. Ventrice (Harvard University, Cambridge, MA), R. Wu (NCI), and C.-H. Yang (NCI). The Secondary Leukemia Workshop was supported by the NCI and the LSA.

We gratefully acknowledge the thoughtful review of the manuscript and comments provided by Drs. Janet Rowley, Michele Evans, Douglas Bell, and Richard Albertini. We thank Dr. Rowley for communicating data prior to publication.

Manuscript received August 2, 1995; revised October 30, 1995; accepted December 14, 1995.