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Biomarkers of Leukemia Risk: Benzene as a Model

Martyn T. Smith and Luoping Zhang

Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, California

Although relatively rare, leukemias place a considerable financial burden on society and cause psychologic trauma to many families. Leukemia is the most common cancer in children. The causes of leukemia in adults and children are largely unknown, but occupational and environmental factors are strongly suspected. Genetic predisposition may also play a major role. Our aim is to use molecular epidemiology and toxicology to find the causes of leukemia and develop biomarkers of leukemia risk. We have studied benzene as a model chemical leukemogen, and we have identified risk factors for susceptibility to benzene toxicity. Numerous studies have associated exposure to benzene with increased levels of chromosome aberrations in circulating lymphocytes of exposed workers. Increased levels of chromosome aberrations have, in turn, been correlated with a heightened risk of cancer, especially for hematologic malignancy, in two recent cohort studies in Europe. Conventional chromosome analysis is laborious, however, and requires highly trained personnel. Further, it lacks statistical power, as only a small number of cells can be examined. The recently developed fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR)-based technologies have allowed the detection of specific chromosome aberrations. These techniques are far less time consuming and are more sensitive than classical chromosomal analysis. Because leukemias commonly show a variety of specific chromosome aberrations, detection of these aberrations by FISH and PCR in peripheral blood may provide improved biomarkers of leukemia risk. — *Environ Health Perspect* 106(Suppl 4):937–946 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-4/937-946smith/abstract.html

Key words: genetic susceptibility, chemical exposure, molecular epidemiology, chromosome aberrations, fluorescence in situ hybridization, polymerase chain reaction, children

Classification of Leukemia

Leukemia is a cancer of the blood-forming system and has been defined as the uncontrolled proliferation of hematopoietic cells that have lost the capacity to differentiate normally to mature blood cells (1). Leukemias are generally classified into lymphocytic and myeloid categories, according to the cell lineage affected, and can be further designated as acute or chronic leukemias. Acute leukemia is characterized by aggressively proliferating cells that rapidly colonize the bone marrow and prevent normal blood cell maturation; chronic leukemia progresses much more slowly. Thus, there are four general categories of leukemias: acute lymphocytic (ALL), chronic lymphocytic (CLL), acute myeloid (AML), and chronic myeloid (CML). These classifications are not exhaustive because a small number of acute leukemias have features characteristic of both the myeloid and lymphoid lineages, and are thus designated acute biphenotypic leukemias (2). In addition, another minor form of chronic leukemia, hairy cell, accounts for less than 2% of all cases (3).

The frequencies of these four major types of leukemia in children differ from those in adults (Table 1). Based on a research report from the National Cancer Institute (NCI), AML is the most common form among adults, followed by CLL (3). On the other hand, ALL, which occurs relatively rarely among adults (approximately 6%), accounts for most childhood leukemia cases (4). The acute leukemias are further classified into subtypes under the French–American–British (FAB) system. The subtypes and frequencies of acute leukemias are summarized in Table 2. In the United States, myeloblastic and/or monoblastic (M1, M2, M4, M5) leukemias are the most common AMLs in both children and adults (4,5), with the M4 category being the most common form in newborns (6). L1 is the most common subtype of ALL in children, whereas L2 is more frequently seen among adults (Table 2) (7). However, the FAB classifications have not proved useful in the clinical management of ALL; therefore, ALL is more often subclassified according to immunophenotype in the clinical setting (4). Under this system, early pre-B cell and pre-B cell are the most common forms of childhood ALL (Table 2).

Incidence of Leukemia

The global incidence of leukemias is about 8 to 9 per 100,000 people each year. Approximately 250,000 new cases occur annually worldwide, about 28,000 of those in the United States (8,9). Leukemia accounts for 2.5% of overall cancer

Table 1. Classification of leukemia and frequencies in children and adults.

Cell lineage	Frequency, %	
	Childhood	Adult
Lymphocytic		
ALL	75	6
CLL	Rare	25
Myeloid		
AML	20	54
CML	5	15

Data from the National Institutes of Health (3).

incidence and 3.5% of cancer mortality in the United States. However, its incidence among children demonstrates its significance. Although childhood cases (through 14 years of age) account for about 12% of all leukemias, childhood cancer is the second biggest killer of children (after accidents) and leukemia is the most common form of childhood cancer (10). The incidence of childhood ALL in the United States has increased approximately 20% over the past two decades, mostly in the 0- to 4-year-old age group (10). Over the course of this century, leukemia rates have also generally increased. The incidence of leukemia grew steeply between 1900 and 1940 (11), and in Denmark increased 3-fold between 1943 and 1977, primarily because of increases in AML (12). Between 1969 and 1977, AML also increased 20% in the United States. Other studies indicate a rise in myeloid leukemias in other industrialized countries during the same period (13). Although overall leukemia rates have remained relatively stable over the last 20 years, the incidence of AML, which accounts for about 80 to 90% of acute leukemias in adults (3,5), has increased substantially among men over 40 years of age (14). The increased incidence of AML among older males and the fact that the highest rates of acute leukemia occur in industrial areas both suggest the importance of occupational and environmental risk factors. In addition, the incidence of certain forms of preleukemia, known as myelodysplastic syndromes (MDS), may be increasing, but this could actually reflect increased awareness on the part of physicians and extended use of diagnostic procedures in elderly patients and may not be due to changes in etiologic factors (15). However, the incidence of MDS in Danish children has been reported higher than generally assumed and approximates the incidence of childhood AML (16). MDS are life threatening, as illustrated by the recent death of the famous astrophysicist Dr. Carl Sagan.

Established Causes of Leukemia

Heredity, radiation, chemical exposures, and treatment with chemotherapeutic agents have been implicated in the development of leukemia. Viral infection by at least one known virus, human T-cell leukemia/lymphotropic virus type I (HTLV-1), is a well-understood cause of adult T-cell leukemia (17). The current etiology of leukemia was extensively reviewed last year by Sandler and Ross (10) and Greaves (18). The risk factors thought to be involved in leukemias are summarized in Table 3.

Genetic predisposition may play a major role in both adult and childhood leukemia (Table 3). Although the Leukemia Society of America emphasizes the fact that anyone may develop the disease, an increased risk exists among Eastern European Jews, and a decreased risk exists among Asians (10) (differences in diet and lifestyle may play a role, however). Individuals with a family history of leukemia or lymphoma have a 5.6-fold increased risk for AML (10). Parents of children with Down syndrome also have an increased risk of leukemia, and individuals with Down syndrome have a 10- to 20-fold increased risk and a greatly increased incidence of a particular subtype of leukemia, AML-M7 (10). This association may involve a potential leukemia gene called

Table 2. Subtypes and frequencies of acute leukemias.

Subtypes	Description	Frequency, % Children	Frequency, % Adults[a]
AML			
M0	Minimal myeloid differentiation	2	?
M1	Poorly differentiated myeloblasts	13	10
M2	Myeloblastic with differentiation	28	40
M3	Promyelocytic	6	10
M4	Myeloblastic and monoblastic	19	15
M5	Monoblastic	21	10
M6	Erythroleukemic	1	5
M7	Megakaryoblastic	10	>5
ALL			
According to morphology (FAB)			
L1	Small and homogeneous	85	31
L2	Larger and heterogeneous	14	60
L3	Larger and homogeneous	1	9
According to immunophenotype			
Early pre-B cell	—	57	—
Pre-B cell	—	25	—
Transitional pre-B cell	—	1	—
B cell	—	2	—
T cell	—	15	—

[a]AML in adult data includes children and adults; however, as childhood AML accounts for a small fraction of all AML cases, these figures may represent adult percentages. Data modified from Pui (4), Lichtman (5), and Mauer (7).

Table 3. Established and potential risk factors of adult and childhood leukemia.

Risk factors	Leukemias Adult	Leukemias Childhood
Genetic factors	Family history	Concordance of infant leukemia in twins
Genetic syndromes		Down syndrome, Bloom syndrome, ataxia telangiectasia, Fanconi anemia, Familial monosomy 7, etc.
Ionizing radiation	Atomic bombing Nuclear accidents/testing Occupational exposure Radiotherapy Residential radon	In utero exposure to diagnostic X-rays Paternal preconception exposure
Chemical exposure	Benzene Petrochemicals Organic solvents Pesticides Chemotherapeutic drugs	Parental exposure to solvents/pesticides Maternal exposure to topoisomerase II inhibitors
Others	Viral infection (HTLV-1) Diet Smoking	Common infections (?) Diet (maternal and child) Parental smoking Previous maternal fetal loss Maternal age and alcohol consumption High birth weight

Assembled from Pui (4), Sandler and Ross (10), and Greaves (18).

AML1 at 21q22 (*19*). Another common genetic abnormality is the rearrangement of the *MLL* gene at 11q23, which is found among 80% of infants with leukemia (*10*). A familial form of monosomy 7 has also been recognized, in which two or more siblings develop myeloid leukemia before the age of 20 (*20*). This may involve a tumor suppressor gene on chromosome 7. As yet, however, no leukemia-specific suppressor genes have been identified, and these inherited genetic defects can explain the causes for only a small but significant proportion of acute leukemias (up to 5%) (*18*).

Another group of risk factors includes occupational and environmental exposure to radiation or chemicals (Table 3). The best established cause of leukemia among children is *in utero* exposure to diagnostic X-rays (*10*). Leukemia in adults is strongly associated with occupational exposure to ionizing radiation (*18*). Marie Curie and her daughter Irene both probably died of leukemia, and one of the greatest risks to astronauts in traveling to Mars or beyond may be leukemia from cosmic radiation exposure. There is little evidence, however, that nonionizing radiation such as electromagnetic fields (EMF) induces leukemia. Indeed, two recent studies have shown that EMF exposure is not a major risk factor for leukemia in children (*21*) or in adults (*22*).

Occupational exposure to chemicals, especially solvents containing benzene, has been associated with leukemia (*23*). Workers exposed to benzene with exposures greater than 200 ppm-year have an additional risk of developing AML, which is more than 20 times greater than that of the general population (*24*). The chemotherapeutic treatment of cancer induces secondary myeloid diseases, including AML and MDS. This induction is a major clinical problem and accounts for up to 10 to 20% of all AML and MDS cases diagnosed (*25*). Drugs presenting the most risk are alkylating agents, such as melphalan and busulfan, and epipodophyllotoxin topoisomerase II inhibitors. About 8% of patients treated with alkylating agents developed AML within 5 years after beginning treatment (*3*). Children with ALL treated with epipodophyllotoxins had a 5 to 12% cumulative risk of AML (*26*).

Because most people in the general population are not exposed to chemotherapeutic drugs or occupationally exposed to radiation or chemical solvents, exposure to these agents cannot explain the causes of the majority of leukemia and MDS cases diagnosed each year. We conservatively estimate that the causes of at least 20,000 (approximately 70%) of the 28,000 new leukemia cases that develop annually in the United States are unexplained. Thus, the causes of leukemia remain largely unknown. Although some success has been achieved in treating leukemias, especially in children, mortality rates have remained relatively high (approximately 75% in the United States) (*9*). Further, treatment may cause long-term damage and increased morbidity. Leukemias, therefore, place an enormous financial burden on society and cause serious psychologic trauma for many families (*27*). Identifying the causes of leukemia is therefore an important public health concern, as it could lead to the eventual prevention of this disease. Traditional epidemiologic studies have largely failed to identify the causes of leukemias in the general population. We have taken a molecular epidemiologic approach, in which traditional epidemiologic methods are combined with the latest tools of molecular biology and cytogenetics, in investigating the causes of leukemia. In recent years, our laboratory has been searching for potential biomarkers of benzene exposure that may be used to find the causes of leukemia in the general population. Benzene has served as a model environmental leukemogen in these studies.

Benzene as a Model Chemical Leukemogen

Benzene's toxic effects on the marrow were first described in 1897 (*28,29*) and the first case report of leukemia from benzene appeared in 1928 (*30*). The ability of benzene to cause AML was first fully established in the 1970s following epidemiologic studies in Italy and Turkey (*23,31–33*). There have been numerous reports of smoldering leukemias and preleukemias produced by benzene (*23*). These would likely be classified as MDS today. Recent studies in China, led by Hayes and Yin (*34,35*) and jointly sponsored by the NCI and the Chinese Academy of Preventive Medicine (CAPM), have established that benzene causes AML and MDS in humans and have also suggested that benzene exposure may be associated with non-Hodgkin's lymphoma, lymphocytic leukemia, lung cancer, and nasopharyngeal cancer.

Benzene is an important commercial product, with approximately 2 billion gal produced annually in the United States. It is used mainly as a starting material in the synthesis of numerous chemicals. The main public health issue concerning benzene in the United States and other developed countries is its use as a component of gasoline and the fact that the shift to unleaded gasoline has tended to increase its benzene content (*36–42*). In the United States, the current benzene content of gasoline is generally below 1%, but in other countries super unleaded gasoline can contain greater than 5% benzene (*43*). Another major source of public exposure to benzene is cigarette smoking. A pack-a-day smoker inhales approximately 2 mg/day, and nonsmokers who live, travel, or work with smokers are exposed to benzene through side-stream or second-hand smoke (*44*). Because benzene is also present in many foodstuffs, the background level of benzene intake for nonsmokers has been estimated at 0.5 mg/day (*45*). It is therefore difficult, if not impossible, to avoid exposure to benzene. Furthermore, benzene and solvents containing more than 1% benzene continue to be used in many countries, including China, former members of the Soviet Bloc, South America (*46–49*), and even Spain, where a case of benzene-induced aplastic anemia was recently described (*50*).

Biomarkers in the Molecular Epidemiology of Benzene-Exposed Workers

Biomarkers are indicators of molecular and cellular events in biologic systems and may allow epidemiologists to better examine relationships between environmental hazards and human health effects. Biomarkers can be classified into three categories: biomarkers of exposure, biomarkers of susceptibility, and biomarkers of early effect. Along with colleagues from the the the CAPM in Beijing, the Shanghai Hygiene Anti-Epidemic Center, the NCI, and other institutions in the United States, we have applied various biomarker methods to samples obtained from workers exposed to high levels of benzene. The goal of these studies is to develop and validate *a*) biomarkers of exposure to benzene, which include urinary levels of benzene metabolites, DNA adducts, protein adducts (such as albumin or hemoglobin adducts), etc.; *b*) molecular markers of susceptibility to benzene, such as inherited genetic factors or defects and polymorphisms of enzymes involved in the metabolism of benzene, including cytochrome P4502E1 (CYP2E1), myeloperoxidase (MPO), NAD(P)H:quinone oxidoreductase (NQO1), glutathione *S*-transferase (GST), etc.; and *c*) biomarkers of the early effects of benzene, including hematotoxicity (complete blood cell counts), gene mutations (glycophorin A [GPA] and *ras*, etc.), and chromosome

aberrations detected by fluorescence *in situ* hybridization (FISH), G-banding, and a micronucleus assay. An overview of the studies has been presented previously (51), and only those findings pertaining to susceptibility and identification of early effects will be discussed here, along with the generalizability of the findings to date.

Biomarkers of Susceptibility to Benzene Hematoxicity

As described previously, individuals with genetic defects or syndromes are highly susceptible to leukemias, though only a small proportion of leukemia cases involve such inherited susceptibility. It is possible that in a much larger percentage of cases, inherited polymorphisms in genes that encode carcinogen activation and detoxification enzymes, such as the cytochrome P450s and GST, could contribute indirectly to the leukemia risk. Multiple clinical reports suggest that people vary greatly in their susceptibility to health risks from benzene exposure. One possible reason might be interindividual variation in metabolic activation and detoxification of benzene and its primary metabolites.

Several enzymes that are involved in benzene metabolism and clearance have been identified. Benzene is metabolized by the hepatic enzyme CYP2E1 to benzene oxide, which spontaneously forms phenol. Phenol, in turn, is further metabolized by CYP2E1 to di- and trihydroxybenzenes such as hydroquinone (HQ), catechol

(CAT), and 1,2,4-benzenetriol (BT) (52) (Figure 1). CYP2E1 therefore plays an essential role in benzene toxicity by activating it to potentially toxic metabolites (53,54). On the other hand, GST can detoxify benzene oxide by converting it to a less toxic or nontoxic derivative, phenylmercapturic acid (55). The polyhydroxy metabolites HQ, CAT, and BT are further converted in the bone marrow by MPO to benzoquinones, which are potent hematotoxic and genotoxic compounds (Figure 1). Benzoquinones can, in turn, be converted back to less toxic hydroxybenzenes by NQO1 (53,56) (Figure 1).

Between 5 and 20% of people in a given population may lack significant NQO1 activity (57–59), potentially making them susceptible to benzene toxicity. This variation is caused by a homozygous mutation (^{609}C → T) at position 609 in the *NQO1* gene, which occurs among 5 to 6% of Caucasians and African Americans and as many as 18 to 20% of Chinese and other Asians (59-61). To test the hypothesis that individuals who were homozygous for the *NQO1*^{609} mutation and possessed high CYP2E1 activity would be susceptible to benzene hematotoxicity, a case–control study of occupational benzene poisoning was conducted (low white blood cell count ≤4000/mm³) in Shanghai (51,58). CYP2E1 activity was estimated by the fractional excretion of chlorzoxazone in 50 cases of benzene poisoning and 50 controls. Subjects with both a rapid fractional excretion of

chlorozoxazone and homozygous *NQO1* mutant alleles were at a 7.6-fold increased risk of benzene poisoning (58). We are also currently investigating the role of the *NQO1* ^{609}C → T mutation in acute leukemia in general, including therapy-related leukemias. Preliminary evidence suggests that the *NQO1* polymorphism is a risk factor for some types of therapy-related leukemia.

MPO activates all the phenolic metabolites of benzene to highly toxic free radicals and quinones (62–64). MPO is an enzyme found primarily in neutrophils and their precursors. An inherited polymorphism in the *MPO* gene has recently been described (65). The polymorphism is a single base substitution (G to A) in an *Alu* repeat in the promoter region of the *MPO* gene. The presence of an A rather than a G at this site decreases expression by about two-thirds in homozygous mutant individuals (65). Theoretically, then, people who have mutant homozygous alleles in *MPO* should be at lower risk of benzene hematotoxicity. This hypothesis is being tested in our laboratory using a new restriction fragment length polymorphism/polymerase chain reaction (PCR) method for detecting the mutant allele (66). Interestingly, this new method has recently been used to show that individuals with homozygous mutant alleles in the *MPO* gene are at significantly decreased risk of lung cancer. Further, earlier studies using sequencing showed that cases of AML-M3 and AML-M4 have a decreased incidence of the mutant allele, also suggesting that homozygous mutant individuals would be resistant to these subtypes of AML (65). However, this analysis was based on only eight cases of AML-M3 and -M4 and requires confirmation.

The potential role of GST polymorphisms in benzene hematotoxicity is currently unclear. The GST-μ (GSTM1) and GST-θ (GSTT1) subclasses are especially effective at detoxifying epoxides, including benzene oxide that is converted to nontoxic phenylmercapturic acid (Figure 1) (55). Recent data, however, suggest that GSTs will not provide protection against benzoquinone metabolites of benzene because the glutathione conjugates of these metabolites are also hematotoxic (67). Although one study reported that the *GSTT1* null genotype (homozygous gene deletion) was associated with an increased risk of MDS (68), a larger, more recent study did not find such an association (69). Clearly, the role of GSTs in susceptibility to benzene hematotoxicity and to acute leukemia and MDS deserves further study.
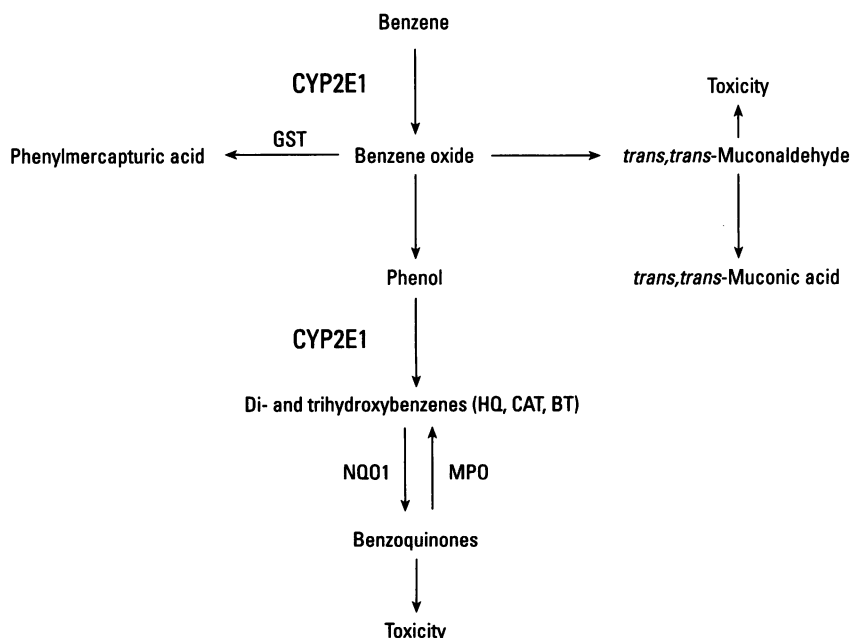

Figure 1. Pathways of benzene metabolism leading to toxicity and detoxification.

A summary of the potential role of different genetic polymorphisms and metabolizing enzyme activities in susceptibility to benzene toxicity is provided in Table 4. In theory, individuals with high activities of CYP2E1 and MPO and homozygous mutations in the *NQO1* and *GSTT1/GSTM1* genes would have the highest risk for benzene hematotoxicity.

Biomarkers of Early Effect from Benzene Exposure

Another potential method of predicting who is most at risk for benzene-induced leukemia is to determine the extent of the genetic damage it produces in exposed individuals, using biomarkers of early effect. One means of assessing genetic damage is to measure mutations in specific genes such as *GPA* (*70,71*). An increased *GPA* mutation frequency has been found in children with leukemia and in people exposed to radiation and leukemogenic anticancer drugs (*72*). An increased level of gene-duplicating mutations in *GPA* has also been found in benzene-exposed workers (*73*). Interestingly, this increased mutation frequency was correlated with cumulative exposure to benzene. Because cumulative exposure to benzene may correlate best with leukemia risk, the *GPA* assay appears to have potential as a biomarker of early biologic effect for benzene and other leukemogens. The *GPA* assay has drawbacks, however. First, it is relatively insensitive: High benzene exposure (mean time-weighted average [TWA] at 72 ppm) only elevated the combined mutant frequency from 16.3 to 23.0 per million, a 41% increase (*73*). Second, it can only be performed on *GPA* heterozygous (type MN) individuals, who statistically constitute only 50% of any given population under study. Thus, although the *GPA* assay can provide important mechanistic information, it may not be an ideal biomarker of early effect.

The most common means of detecting genetic damage has traditionally been conventional cytogenetics. Numerous publications, including the classic early studies of

Tough and co-workers (*74,75*) and Forni and colleagues (*76,77*), have demonstrated the clear association between benzene exposure and increased levels of chromosome aberrations in peripheral blood cells. More recent studies have suggested that benzene may induce aberrations at TWA concentrations below 10 ppm (*78–83*) and have selective effects on certain chromosomes (*84–87*). We are currently investigating the utility of these data in improving the risk assessment for benzene. Because chromosome aberrations in peripheral blood lymphocytes are associated with increased risk for overall cancer incidence (*88*), especially for increased mortality from hematologic malignancies (*89*), it is possible that specific chromosome aberrations may provide even better markers of future leukemia risk.

Specific Chromosome Aberrations as Biomarkers of Leukemia Risk

Specific chromosome aberrations are the hallmark of human leukemia (*90–92*). Aneuploidy, the loss or gain of specific chromosomes in AML and MDS (such as trisomy 8 and monosomy 5 and monosomy 7), is commonly observed, as are specific chromosome translocations, inversions, and deletions [e.g., t(8;21), t(9;22), inv(16), and long-arm deletion of chromosome 5] (*91*). Up to 65% of acute leukemias contain nonrandom somatically acquired chromosomal translocations or inversions (*93*). These numerical aberrations and structural rearrangements affect gene expression in ways that subvert normal cell proliferation, differentiation, and survival.

The loss of chromosomes 5 and 7 and their long-arm deletions are the two most common changes in therapy-related AML (t-AML) and MDS, especially among patients previously treated with alkylating agents (*94*). Treatment with topoisomerase II inhibitors is associated with balanced chromosome aberrations, such as t(4;11), t(6;11), and t(11;19), in t-AML (*94,95*). These specific chromosome aberrations are

also more common among leukemia patients with previous exposure to chemical solvents (including chronic exposure to benzene, insecticides, petroleum, etc.) (*96,97*). For example, one recent study found an association between monosomy 7/long-arm deletion of chromosome 7 (del[7q]) and previous exposure to paints (odds ratio 7.5) (*97*). In addition, trisomy and monosomy of the C-group chromosomes (6-12, X) were present in the bone marrow and blood of several benzene-induced AML patients (*98–101*). Among these cases, clonal expansion of trisomy C, identified as trisomy 9 (*98*), and of trisomy D (*100*) were observed in all leukemic cells examined. Monosomy 7 was also found in 100% of the bone marrow cells of one of the benzene-induced MDS cases (*102*). Interestingly, the Philadelphia chromosome was observed by classical cytogenetics in a case of preleukemia (leukopenia) resulting from chronic exposure to benzene for 4 years without the signs of leukemia; after 4 years without exposure, the aberration disappeared (*101*).

Thus, specific chromosome aberrations have been observed in both leukemia and preleukemia patients previously exposed to benzene. However, our studies have addressed an additional question: whether benzene exposure induces these specific chromosome aberrations, which might lead to the development of leukemia in exposed but nondiseased individuals. In answering this question, we believe that measuring disease-specific chromosome aberrations in exposed workers would be more significant than measuring general nonspecific aberrations, not only because disease-specific chromosome aberrations probably have better predictive value, but because recent studies in our laboratory suggest that chemicals cause aneuploidy of specific chromosomes or produce greater damage to some chromosomes than to others (*103*). Most previous studies measured only general chromosome aberrations in benzene-exposed workers by conventional cytogenetic analysis (*78–80*). The classic assay, however, allows few cells to be examined, requires highly trained personnel, and does not readily detect specific chromosome aberrations.

Detection of Specific Chromosome Aberrations by FISH

Specific chromosome aberrations can now be detected by FISH (*104–106*). FISH offers several major advantages (*107*) over

Table 4. Susceptibility to benzene hematotoxicity: hypotheses on polymorphisms of enzymes involved in metabolic activation of benzene and its detoxification.

Susceptibility to benzene hematotoxicity	Activation		Detoxification	
	CYP2E1	MPO	NQO1	GSTT1/GSTM1
High	High	High	Homozygous	Homozygous
Medium	High	?	Wild-type/heterozygous	?
	Low	?	Homozygous	?
Low	Low	Low	Wild-type/heterozygous	Wild-type/heterozygous

?, uncertain. Data from Ross (*53*), Rothman et al. (*58*), and our current hypotheses.

conventional chromosome aberration analysis: FISH requires less-highly trained personnel; FISH is easier to perform, allowing analyses to be performed in less time; FISH analysis of metaphase cells is simpler, making it possible to analyze 10 times more metaphases; and FISH can detect very specific events identical to those found in leukemia cells. These types of events may be more strongly associated with subsequently developing leukemia than overall estimates of damage. FISH can therefore be used to detect leukemia-specific aberrations in a timely, sensitive, and cost-effective manner.

However, like conventional cytogenetic methods, metaphase analysis by FISH can only be performed on dividing cells. In peripheral blood, the cells that can most readily be stimulated to divide are the T lymphocytes; therefore, the most common technique used is cytogenetic analysis of metaphases from these cells. Peripheral T lymphocytes, though a target of the hematotoxic effects of benzene (108,109), are clearly not the target of genotoxic damage responsible for the development of AML and MDS. Thus, the validity of measuring AML-specific chromosome aberrations in peripheral T cells might be questioned. However, T cells might be considered a useful surrogate target because at least a portion are relatively long lived (> 1 year) and accumulate aberrations. Further, chromosome aberrations that confer selective advantages on cells of the myeloid lineage [e.g., del(7q), t(8,21)] should have no effect on T lymphocytes. Hence, detection of specific aberrations in T-cell metaphases is a measure of the number of cumulative critical hits that have occurred in the blood, and presumably the bone marrow, of control and exposed individuals on a per-cell basis. Specific chromosome aberrations in circulating T lymphocytes, which act as long-lived surrogates for stem cells in the marrow, may therefore serve as useful biomarkers of leukemia risk for benzene.

We applied FISH to determine the presence of specific chromosome aberrations in the lymphocytes of workers exposed to benzene and matched controls. Initially, we studied hyperdiploidy levels of chromosome 9 in interphase cells because trisomy 9 had been observed in benzene-poisoned patients (98,110) and benzene metabolites induce hyperdiploidy of this chromosome in cultured lymphocytes in vitro (111,112). High benzene exposure increased hyperdiploidy of chromosome 9 in the lymphocytes of otherwise healthy workers, with trisomy 9 being the most prevalent form (113). We

have used interphase cytogenetics to study the hyperdiploidy of chromosomes 7 and 8. The findings were briefly reported in abstract form (114) and will be published elsewhere. Interphase cytogenetics cannot be used, however, to confidently detect monosomy or rare translocations because of artifacts related to probe overlap (104). Monosomy 5 and 7 and translocations (8;21) are among the most common aberrations observed in AML (90,91). We have therefore begun to use chromosome painting and region-specific fluorescent probes to examine AML-specific aberrations, including monosomy 5, monosomy 7, del(5)(q31), del(7)(q22q34), and t(8;21), in metaphase spreads prepared from the lymphocytes of workers exposed to benzene and matched controls. Increased frequencies of t(8;21) and trisomy 8 and 21 have been detected among workers exposed to benzene (115). Monosomy 5 and 7 and their long-arm deletions also increased in the exposed workers (116). Table 5 briefly summarizes the specific chromosome aberrations observed in AML and MDS detected in benzene-exposed workers by FISH.

Detection of Specific Chromosome Aberrations by PCR-Based Technology

Specific chromosomal aberrations can also be detected by PCR and reverse transcriptase (RT)–PCR (117–120). These methods hold a number of advantages over FISH, including the ability to detect very rare events (1 copy/10^7 cells vs 1/10^4 cells by FISH) and the ability to study large numbers of people easily and at low cost. These seemingly potent advantages are offset, however, by two major disadvantages. First, the high sensitivity of PCR makes it prone to false-positive results caused by sample contamination. Second, quantitation is difficult, especially for RT–PCR. The former drawback can be overcome with extremely rigorous lab procedures, but the latter is mainly restricted to a qualitative value such as number of individuals giving positive results. Because chromosomal translocations involve the formation of

novel messenger RNAs and fused DNA sequences, these aberrations have been those mainly detected by PCR-based procedures capable of identifying the novel but rare sequences in millions of normal sequences. Liu et al. (118) demonstrated that the BCL2 translocation [t(14;18)], commonly found in patients with non-Hodgkin's lymphoma, could be detected in the blood of healthy individuals. Biernaux et al. (121) observed similar results in 117 normal subjects tested by RT–PCR for the presence of BCR-ABL fusion in RNA from the t(9;22) (q34; q11) translocation. In both studies the translocation could be detected in up to 40% of normal healthy subjects, and its presence increased in frequency with age. PCR-based procedures therefore hold great promise for detecting specific chromosome aberrations, especially when used in combination with FISH. We are currently attempting to detect translocations t(14;18), t(9;22), t(8;21), and t(11q23) by both PCR and FISH in the peripheral blood of workers highly exposed to benzene and matched controls.

Benzene and Childhood Leukemia

Clusters of childhood leukemias have occurred around Superfund sites (122), and in Britain, Knox (123) reported that cases of childhood leukemia commonly occur closer to industrial installations. He concluded: "The common patterns of close association of clustered and nonclustered cases imply a common etiological component arising from a common environmental hazard—namely the use of fossil fuels, especially petroleum" (123). This work implicates benzene and petroleum products in the development of childhood leukemia, but the findings are highly controversial and were challenged in the literature (124). Recently, Knox and co-workers (125) expanded on their original findings and examined relationships between addresses at birth and death of children dying from leukemia and other cancers in Britain and the sites of potential environmental hazards. They studied all 22,458 children 0 to 15

Table 5. Specific chromosome aberrations observed in AML/MDS are detected in benzene-exposed workers.

Chromosome aberration	AML/MDS	Benzene-exposed
Aneuploidy	Trisomy 8 and 21	+7, +8, +9, +21
	Monosomy 5 and 7	–5, –7
Long-arm deletion	5q–, 7q–	5q–, 7q–
Translocation	t(8;21), t(9;22)	t(8;21)
Inversion	inv(16)	Not done

Data from Le Beau (90), Hagemeijer and Grosveld (91), Zhang et al. (113,114,116), and Smith et al. (115).

years of age who died from leukemia or cancer in England, Wales, and Scotland between 1953 and 1980. They found that childhood cancers were geographically associated with two main types of industrial atmospheric effluent, namely: petroleum-derived volatiles and kiln and furnace smoke and gases, as well as effluents from internal combustion engines. These findings support their earlier conclusion that benzene exposure is responsible for at least a portion of childhood cancers. There seems to be no positive association, however, between car ownership and childhood ALL (126).

The findings of Knox and co-workers are consistent with earlier reports from Holland (127), China (128), the United States (129), Britain (130), and Japan (131) of an association between parental exposure to solvents containing benzene and increased risk of childhood leukemia. The study in Britain (130) utilized face-to-face interviews for exposure assessment and found an odds ratio for parental benzene exposure as high as 5.81 (95% confidence interval [CI] 1.67–26.44). These studies imply that benzene or its metabolites cause genetic damage in female or male germ cells, which is then passed on to the offspring or causes direct genetic damage in the fetus following maternal exposure. They also imply that key changes related to the development of childhood leukemia occur before birth. This idea is strongly supported by the work of Ford, Greaves, and co-workers, which has clearly shown that genetic changes related to leukemia development occur before birth in

a number of cases (132), including twins who developed T-cell leukemia at 9 years of age (133). Benzene crosses the placenta, and reproductive studies in both humans and rodents have shown that benzene exposure of either the male or the female can have harmful effects on the fetus (134,135). The idea that exposure of the male can lead to leukemia in the offspring is supported by the recent, quite startling finding that paternal preconception smoking was related to a significantly elevated risk of childhood cancers, particularly acute leukemia and lymphoma (136). The risks rose with increasing pack-years of paternal preconception smoking for ALL (p for trend = 0.01) and total cancer (p for trend = 0.006). Compared with children whose fathers had never smoked cigarettes, children whose fathers smoked more than 5 pack-years prior to their conception had adjusted odds ratios of 3.8 (95% CI = 1.3–12.3) for ALL. Clearly, more studies are needed of the relationship between parental benzene exposure and childhood leukemia, but evidence is mounting that parental genotoxic exposure is important and that key changes involved in the subsequent development of childhood leukemia can occur before birth.

Biomarkers of Childhood Leukemia Risk

We are studying a large number of cases of childhood leukemia in Northern California using molecular approaches and sub-classification. FISH and PCR are being used as tools to subclassify leukemias into

cytogenetic or molecular subtypes and help determine etiology, as first suggested many years ago by Kessler and Lilienfeld (137) and expanded upon by Sandler and Collman (11). Further, we aim to examine whether certain cytogenetic changes are present at birth, as is suggested by the research findings described previously. If key genetic changes occur in utero or are inherited from one or both parents, we may be able to detect these changes at birth using analysis of neonatal blood spots (Guthrie cards) from leukemia cases by PCR (138). If specific changes are detectable, it may be possible in the future to predict which children are most at risk of subsequently developing leukemia. Recently, Gale et al. (138) have reported that t(4;11) MLL-AF4 gene fusion sequences can be detected in neonatal blood spots of all patients 0.5 to 2 years of age.

Conclusion

Biomarkers of susceptibility to benzene-induced hematotoxicity have been developed and more will surely be forthcoming. We and others are testing the utility of these biomarkers in predicting who is at risk for hematotoxicity and leukemia from occupational and other environmental exposures. FISH and PCR-based procedures, which measure the early effects of benzene and specific chromosome aberrations, also hold promise in predicting who is most at risk from exposure to benzene and other potential leukemogens. This endeavor deserves long-term study and is a future goal of our laboratory.

REFERENCES AND NOTES

1. Sawyers CL, Denny CT, Witte ON. Leukemia and the disruption of normal hematopoiesis. Cell 64:337–350 (1991).
2. Matutes E, Morilla R, Farahat N, Carbonell F, Swansbury J, Dyer M, Catovsky D. Definition of acute biphenotypic leukemia. Haematologica 82:64–66 (1997).
3. U.S. National Institutes of Health. Leukemia. Rpt no 94-329. Bethesda, MD:National Cancer Institute, 1993.
4. Pui CH. Childhood leukemias. N Engl J Med 332:1618–1630 (1995).
5. Lichtman MA. Acute myelogenous leukemia. In: Williams Hematology (Beutler E, Lichtman MA, Coller BS, Kipps TJ, eds). New York:McGraw-Hill, 1995;272–298.
6. Jandl JH, ed. Acute myelogenous leukemia. In: Blood: Textbook of Hematology, 2nd Ed. Boston:Little, Brown and Company, 1996;853–901.
7. Mauer AL. Acute lymphocytic leukemia. In: Williams Hematology (Beutler E, Lichtman MA, Coller BS, Kipps TJ, eds). New York:McGraw-Hill, 1995;1004–1016.
8. Schiffer CA, Schimpff SC. Acute leukemia. In: Comprehensive Textbook of Oncology. Vol 2 (Moosa AR, Schimpff SC, Robson MC, eds). Baltimore:Williams & Wilkins, 1991;1203–1211.

9. LSA. Facts about Leukemia, Lymphoma, Hodgkin's Disease and Myeloma. Report. New York:Leukemia Society of America, 1996-1997.
10. Sandler DP, Ross JA. Epidemiology of acute leukemia in children and adults. Semin Oncol 24:3–16 (1997).
11. Sandler DP, Collman GW. Cytogenetic and environmental factors in the etiology of the acute leukemias in adults. Am J Epidemiol 126:1017–1032 (1987).
12. Hansen NE, Karle H, Jensen OM. Trends in the incidence of leukemia in Denmark, 1943-77: an epidemiologic study of 14,000 patients. J Natl Cancer Inst 71:697–701 (1983).
13. Selvin S, Levin LI, Merrill DW, Winkelstein W Jr. Selected epidemiologic observations of cell-specific leukemia mortality in the United States, 1969-1977. Am J Epidemiol 117:140–152 (1983).
14. Bloomfield CD, Foon KA, Levine EG. Leukemias. In: Medical Oncology (Calabresi P, Schein PS, eds). New York:McGraw-Hill, 1993;459–501.
15. Aul C, Gattermann N, Schneider W. Age-related incidence and other epidemiological aspects of myelodysplastic syndromes. Br J Haematol 82:358–367 (1992).
16. Hasle H, Kerndrup G, Jacobsen BB. Childhood myelodysplastic

syndrome in Denmark: incidence and predisposing conditions. Leukemia 9:1569–1572 (1995).

17. Franchini G. Molecular mechanisms of human T-cell leukemia/lymphotropic virus type I infection. Blood 86:3619–3639 (1995).

18. Greaves MF. Aetiology of acute leukaemia. Lancet 349:344–349 (1997).

19. Sawyers CL. Molecular genetics of acute leukaemia. Lancet 349:196–200 (1997).

20. Shannon KM, Turhan AG, Rogers PC, Kan YW. Evidence implicating heterozygous deletion of chromosome 7 in the pathogenesis of familial leukemia associated with monosomy 7. Genomics 14:121–125 (1992).

21. Linet MS, Hatch EE, Kleinerman RA, Robison LL, Kaune WT, Friedman DR, Severson RK, Haines CM, Hartsock CT, Niwa S, et al. Residential exposure to magnetic fields and acute lymphoblastic leukemia in children. N Engl J Med 337:1–7 (1997).

22. Verkasalo PK. Magnetic fields and leukemia—risk for adults living close to power lines. Scand J Work Environ Health 22(Suppl 2):1–56 (1996).

23. Aksoy M. Benzene Carcinogenicity. Boca Raton, FL:CRC Press, 1988.

24. Wong O. Risk of acute myeloid leukaemia and multiple myeloma in workers exposed to benzene. Occup Environ Med 52:380–384 (1995).

25. Pedersen-Bjergaard J, Philip P. Two different classes of therapy-related and *de-novo* acute myeloid leukemia? Cancer Genet Cytogenet 55:119–124 (1991).

26. Poplack DG. Acute lymphoblastic leukemia. In: Principles and Practice of Pediatric Oncology (Pizzo PA, Poplack DG, eds). Philadelphia:J.B. Lippincott, 1993;431–481.

27. Kazak AE, Barakat LP, Meeske K, Christakis D, Meadows AT, Casey R, Penati B, Stuber ML. Posttraumatic stress, family functioning, and social support in survivors of childhood leukemia and their mothers and fathers. J Consult Clin Psychol 65:120–129 (1997).

28. Santesson GG. Uber chronische Vergiftungen mit Steinkohlentheerbenzin; vier Todesfalle. Arch Hyg 31:336–376 (1897).

29. Le Noir A, Claude J. Sur un case de purpura attribute a l'intoxication par le benzene. Bull Mem Soc Med Hop Paris 3:1251–1261 (1897).

30. Delore P, Borgomano C. Leucemie aique au cours de l'intoxication benzenique. Sur l'origine toxique de certaines leucemies aigues et leurs relations avec les anemies graves. J Med Lyon 9:227–233 (1928).

31. Vigliani EC, Saita G. Benzene and leukemia. N Eng J Med 271:872–876 (1964).

32. Vigliani EC, Forni A. Benzene and leukemia. Environ Res 11:122–127 (1976).

33. Aksoy M, Dincol K, Akgun T, Erdem S. Haemotological effects of chronic benzene poisoning in 217 workers. Br J Ind Med 28:296–301 (1971).

34. Hayes RB, Yin SN, Dosemeci M, Li GL, Wacholder S, Chow WH, Rothman N, Wang YZ, Dai TR, Chao XJ, et al. Mortality among benzene-exposed workers in China. Environ Health Perspect 104:1349–1352 (1996).

35. Yin SY, Hayes RB, Linet MS, Li GL, Dosemeci M, Travis LB, Li CY, Zhang ZN, Li DG, Chow WH. A cohort study of cancer among benzene-exposed workers in China: overall results. Am J Ind Med 29:227–235 (1996).

36. Chan C, Spengler JD, Ozkaynak H, Lefkopoulou M. Commuter exposures to VOCs in Boston, Massachusetts. J Air Waste Manage Assoc 41:1594–1600 (1991).

37. Fleming AF. Benzene in petrol: a continuing hazard. Lancet 336:1076–1077 (1990).

38. Hartle R. Exposure to methyl *tert*-butyl ether and benzene among service station attendants and operators. Environ Health Perspect 101(Suppl 6):23–26 (1993).

39. Infante P, Schwartz E, Cahill R. Benzene in petrol: a continuing hazard. Lancet 336:814–815 (1990).

40. Jakobsson R, Ahlbom A, Bellander T, Lundberg I. Acute myeloid leukemia among petrol station attendants. Arch Environ Health 48:255–259 (1993).

41. Kawai T, Yamaoka K, Uchida Y, Ikeda M. Benzene exposure in a Japanese petroleum refinery. Toxicol Lett 52:135–139 (1990).

42. Rushton L. Benzene exposure in the petroleum distribution industry associated with leukemia in the United Kingdom: overview of the methodology of a case-control study. Environ Health Perspect 104(Suppl 6):1371–1374 (1996).

43. Knott D. U.K. unleaded gasoline draws fire in Parliament. Oil Gas J 92:40–41 (1994).

44. Wallace L. Major sources of exposure to benzene and other volatile organic chemicals. Risk Anal 10:59–64 (1990).

45. Wallace LA. The Exposure of the General Population to Benzene. Princeton, NJ:Princeton Scientific Publishing, 1989.

46. Midzenski MA, McDiarmid MA, Rothman N, Kolodner K. Acute high dose exposure to benzene in shipyard workers. Am J Ind Med 22:553–565 (1992).

47. Yin SN, Li Q, Liu Y, Tian F, Du C, Jin C. Occupational exposure to benzene in China. Br J Ind Med 44:192–195 (1987).

48. Karacic V, Skender L, Prpic-Majic D. Occupational exposure to benzene in the shoe industry. Am J Ind Med 12:531–536 (1987).

49. Fishbein L. Benzene: Uses, Occurrence and Exposure. IARC Monograph on Benzene and Alkylated Benzenes. Vol 10. Lyon:International Agency for Research on Cancer, 1988;67–96.

50. Milla F, Ribera JM, Navarro JT, Granada I, Xandri M, Batlle M, Flores A, Junca J, Feliu E. Benzene induced aplastic anemia followed by RAEB after re-exposure. In: Fourth International Symposium on Myeloplastic Syndromes, 24-27 April 1997, Barcelona, Spain. Abstract.

51. Rothman N, Smith MT, Hayes RB, Li G-L, Irons RD, Dosimeci M, Haas R, Stillman WS, Linet M, Xi L-Q. An epidemiologic study of early biological effects of benzene in Chinese workers. Environ Health Perspect 105(Suppl 6):1365–1370 (1996).

52. Koop DR, Laethem CL, Schnier GG. Identification of ethanol-inducible P450 isozyme 3a (P450IIE1) as a benzene and phenol hydroxylase. Toxicol Appl Pharmacol 98:278–288 (1989).

53. Ross D. Metabolic basis of benzene toxicity. Eur J Haemotol 57(Suppl):111–118 (1996).

54. Valentine JL, Lee SS, Seaton MJ, Asgharian B, Farris G, Corton JC, Gonzalez FJ, Medinsky MA. Reduction of benzene metabolism and toxicity in mice that lack CYP2E1 expression. Toxicol Appl Pharmacol 141:205–213 (1996).

55. Snyder R, Hedli CC. An overview of benzene metabolism. Environ Health Perspect 104(Suppl 6):1165–1171 (1996).

56. Smith MT. The mechanism of benzene-induced leukemia: a hypothesis and speculations on the causes of leukemia. Environ Health Perspect 104(Suppl 6):1219–1225 (1996).

57. Wiencke JK, Spitz MR, McMillan A, Kelsey KT. Lung cancer in Mexican-Americans and African-Americans is associated with the wild-type genotype of the NAD(P)H:quinone oxidoreductase polymorphism. Cancer Epidemiol Biomarkers Prev 6:87–92 (1997).

58. Rothman N, Smith MT, Hayes RB, Traver RD, Hoener B-A, Campleman S, Li G-L, Dosemeci M, Linet M, Zhang L, et al. Benzene poisoning, a risk factor for hematologic malignancy, is associated with the NQO1 [609]C→T mutation and rapid fractional excretion of chlorzoxazone. Cancer Res 57:2839–2842 (1997).

59. Rosvold EA, McGlynn KA, Lustbader ED, Buetow KH. Identification of an NAD(P)H:quinone oxidoreductase polymorphism and its association with lung cancer and smoking. Pharmacogenetics 5:199–206 (1995).

60. Ross D, Traver RD, Siegel D, Kuehl BL, Misra V, Rauth AM. A polymorphism in NAD(P)H:quinone oxidoreductase (NQO1): relationship of a homozygous mutation at position 609 of the NQO1 cDNA to NQO1 activity [Letter]. Br J Cancer 74:995–996 (1996).

61. Traver RD, Siegel D, Beall HD, Phillips RM, Gibson NW, Franklin WA, Ross D. Characterization of a polymorphism in NAD(P)H:quinone oxidoreductase (DT-diaphorase). Br J Cancer 75:69–75 (1997).

62. Eastmond DA, Smith MT, Ruzo LO, Ross D. Metabolic activation of phenol by human myeloperoxidase and horseradish peroxidase. Mol Pharmacol 30:674–679 (1986).

63. Smith MT, Yager JW, Steinmetz KL, Eastmond DA. Peroxidase-dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. Environ Health Perspect 82:23–29 (1989).

64. Subrahmanyam VV, Ross D, Eastmond DA, Smith MT. Potential role of free radicals in benzene-induced myelotoxicity and leukemia. Free Radic Biol Med 11:495–515 (1991).

65. Piedrafita FJ, Molander RB, Vansant G, Orlova EA, Pfahl M, Reynolds WF. An *Alu* element in the myeloperoxidase promoter contains a composite SP1-thyroid hormone-retinoic acid response element. J Biol Chem 271:14412–14420 (1996).

66. London SJ, Lehman TA, Taylor JA. Myeloperoxidase genetic polymorphism and lung cancer risk. Cancer Res 57:5001–5003 (1997).

67. Bratton SB, Lau SS, Monks TJ. Evidence for the participation of quinone-thioethers in benzene-mediated hematotoxicity [Abstract]. Toxicologist 36(1):165 (1997).

68. Chen H, Sandler DP, Taylor JA, Shore DL, Liu E, Bloomfield CD, Bell DA. Increased risk for myelodysplastic syndromes in individuals with glutathione transferase theta 1 (*GSTT1*) gene defect. Lancet 347:295–297 (1996).

69. Preudhomme C, Nisse C, Hebbar M, Vanrumbeke M, Brizard A, Lai J, Fenaux P. Glutathione *S*-transferase theta 1 (*GSTT1*) gene defects in myelodysplastic syndromes (MDS) and their correlation with karyotype and exposure to potential carcinogens [Abstract]. Leuk Res 21(Suppl 1):S3 (1997).

70. Compton PJ, Hooper K, Smith MT. Human somatic mutation assays as biomarkers of carcinogenesis. Environ Health Perspect 94:135–141 (1991).

71. Jensen RH, Bigbee WL. Direct immunofluorescence labeling provides an improved method for the glycophorin A somatic cell mutation assay. Cytometry 23:337–343 (1996).

72. Mott MG, Boyse J, Hewitt M, Radford M. Do mutations at the glycophorin A locus in patients treated for childhood Hodgkin's disease predict secondary leukaemia? Lancet 343:828–829 (1994).

73. Rothman N, Haas R, Hayes RB, Li G-L, Wiemels J, Campleman S, Quintana PJE, Xi L-J, Dosimeci M, Titenko-Holland N, et al. Benzene induces gene-duplicating but not gene-inactivating mutations at the glycophorin-A locus. Proc Natl Acad Sci USA 92:4069–4073 (1995).

74. Tough IM, Court Brown WM. Chromosome aberrations and exposure to ambient benzene. Lancet 7387:684–685 (1965).

75. Tough IM, Smith PG, Court Brown WM, Harnden DG. Chromosome studies on workers exposed to atmospheric benzene. The possible influence of age. Eur J Cancer 6:49–55 (1970).

76. Forni A. Chromosome studies in workers exposed to benzene or toluene or both. Arch Environ Health 22:373–378 (1971).

77. Forni AM, Cappellini A, Pacifico E, Vigliani EC. Chromosome changes and their evolution in subjects with past exposure to benzene. Arch Environ Health 23:385–391 (1971).

78. Karacic V, Skender L, Bosner-Cucancic B, Bogadi-Sare A. Possible genotoxicity in low level benzene exposure. Am J Ind Med 27:379–388 (1995).

79. Major J, Jakab M, Kiss G, Tompa A. Chromosome aberration, sister-chromatid exchange, proliferative rate index, and serum thiocyanate concentration in smokers exposed to low-dose benzene. Environ Mol Mutagen 23:137–142 (1994).

80. Tompa A, Major J, Jakab MG. Monitoring of benzene-exposed workers for genotoxic effects of benzene: improved-working-condition-related decrease in the frequencies of chromosomal aberrations in peripheral blood lymphocytes. Mutat Res 304:159–165 (1994).

81. Picciano D. Cytogenetic study of workers exposed to benzene. Environ Res 19:33–38 (1979).

82. Wolman SR. Cytologic and cytogenetic effects of benzene. J Toxicol Environ Health (Suppl)2:63–8 (1977).

83. Yardley-Jones A, Anderson D, Lovell DP, Jenkinson PC. Analysis of chromosomal aberrations in workers exposed to low level benzene. Br J Ind Med 47:48–51 (1990).

84. Sasiadek M. Nonrandom distribution of breakpoints in the karyotypes of workers occupationally exposed to benzene. Environ Health Perspect 97:255–257 (1992).

85. Sasiadek M, Jagielski J, Smolik R. Localization of breakpoints in the karyotype of workers professionally exposed to benzene. Mutat Res 224:235–240 (1989).

86. Ding XJ, Li Y, Ding Y, Yang HZ. Chromosome changes in patients with chronic benzene poisoning. Chin Med J Engl Ed 96:681–685 (1983).

87. Forni A. Chromosome changes and benzene exposure. A review. Rev Environ Health 3:5–17 (1979).

88. Hagmar L, Brogger A, Hansteen IL, Heim S, Hogstedt B, Knudsen L, Lambert B, Linnainmaa K, Mitelman F, Nordenson I, et al. Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. Cancer Res 54:2919–2922 (1994).

89. Bonassi S, Abbondandolo A, Camurri L, Dal Pra L, De Ferrari M, Degrassi F, Forni A, Lamberti L, Lando C, Padovani P, et al. Are chromosome aberrations in circulating lymphocytes predictive of future cancer onset in humans? Preliminary results of an Italian cohort study. Cancer Genet Cytogenet 79:133–135 (1995).

90. Le Beau MM. Chromosomal abnormalities in hematologic malignant diseases. In: Progress in Clinical and Biological Research: Mutation and the Environment. Vol 340 (Mendelsohn ML, Albertini RJ, eds). New York:Wiley-Liss, 1990;325–335.

91. Hagemeijer A, Grosveld G. Molecular cytogenetics of leukemia. In: Leukemia (Henderson E, Lister T, Greaves M, eds). Philadelphia:Saunders, 1996;131–144.

92. Kagan J. Molecular biology of chromosomal aberrations in leukemia/lymphoma. Hematol Pathol 7:159–201 (1993).

93. Look AT. Oncogenic transcription factors in the human acute leukemias. Science 278:1059–1064 (1997).

94. Pedersen-Bjergaard J, Pedersen M, Roulston D, Philip P. Different genetic pathways in leukemogenesis for patients presenting with therapy-related myelodysplasia and therapy-related acute myeloid leukemia. Blood 86:3542–3552 (1995).

95. Smith MA, McCaffrey RP, Karp JE. The secondary leukemias: challenges and research directions. J Natl Cancer Inst 88:407–418 (1996).

96. Mitelman F, Nilsson PG, Brandt L, Alimena G, Gastaldi R, Dallapiccola B. Chromosome pattern, occupation, and clinical features in patients with acute nonlymphocytic leukemia. Cancer Genet Cytogenet 4:197–214 (1981).

97. Crane M, Strom S, Halabi S, Berman E, Fueger J, Spitz M, Keating M. Correlation between selected environmental exposures and karyotype in acute myelocytic leukemia. Cancer Epidemiol Biomarkers Prev 5:639–644 (1996).

98. Forni A, Moreo L. Cytogenetic studies in a case of benzene leukaemia. Eur J Cancer 3:251–255 (1967).

99. Forni A, Moreo L. Chromosome studies in a case of benzene-induced erythroleukaemia. Eur J Cancer 5:459–463 (1969).

100. Sellyei M, Keleman E. Chromosome study in a case of granulocytic leukaemia with 'Pelgerisation' 7 years after benzene pancytopenia. Eur J Cancer 7:83–85 (1971).

101. Erdogan G, Aksoy M. Cytogenetic studies in 20 patients with pancytopenia and leukaemia with long-term exposure to benzene. In: European and American Division International Society of Hematology, 3rd Meeting, London, 1975. [As cited in Aksoy M. Benzene Carcinogenicity. Boca Raton, FL:CRC Press, 1988.]

102. Van den Berghe H, Louwagie A, Broeckaert-Van Orshoven A, David G, Verwilghen R. Chromosome analysis in two unusual malignant blood disorders presumably induced by benzene. Blood 53:558–566 (1979).

103. Xi L, Zhang L, Wang Y, Smith MT. Induction of chromosome-specific aneuploidy and micronuclei in human lymphocytes by metabolites of 1,3-butadiene. Carcinogenesis 18:1687–1693 (1997).

104. Eastmond DA, Pinkel D. Detection of aneuploidy and aneuploidy-inducing agents in human lymphocytes using fluorescence *in situ* hybridization with chromosome-specific DNA probes. Mutat Res 234:303–318 (1990).

105. Cremer T, Lichter P, Borden J, Ward DC, Manuelidis L. Detection of chromosome aberrations in metaphase and interphase tumor cells by *in situ* hybridization using chromosome-specific library probes. Hum Genet 80:235–246 (1988).

106. Gray JW, Pinkel D, Brown JM. Fluorescence *in situ* hybridization in cancer and radiation biology. Radiat Res 137:275–289 (1994).

107. Eastmond DA, Schuler M, Rupa DS. Advantages and limitations of using fluorescence *in situ* hybridization for the detection of aneuploidy in interphase human cells. Mutat Res 348:153–162 (1995).

108. Goldstein BD. Benzene toxicity. Occup Med 3:541–554 (1988).

109. Rothman N, Li G-L, Dosimeci M, Bechtold WE, Marti GE, Wang Y-Z, Linet M, Xi L-Q, Lu W, Smith MT, et al. Hematotoxicity among Chinese workers heavily exposed to benzene. Am J Ind Med 29:236–246 (1996).

110. Erdogan G, Aksoy M. Cytogenetic studies in thirteen patients with pancytopenia and leukaemia associated with long-term exposure to benzene. New Istanbul Contrib Clin Sci 10:230–247 (1973).

111. Zhang L, Venkatesh P, Creek ML, Smith MT. Detection of 1,2,4-benzenetriol induced aneuploidy and microtubule disruption by fluorescence *in situ* hybridization and immunocytochemistry. Mutat Res 320:315–327 (1994).

112. Eastmond DA, Rupa DS, Hasegawa LS. Detection of hyperdiploidy and chromosome breakage in interphase human lymphocytes following exposure to the benzene metabolite hydroquinone using multicolor fluorescence *in situ* hybridization with DNA probes. Mutat Res 322:9–20 (1994).

113. Zhang L, Rothman N, Wang Y, Hayes RB, Bechtold W, Venkatesh P, Yin S, Wang Y, Dosemeci M, Li G, et al. Interphase cytogenetics of workers exposed to benzene. Environ Health Perspect 104(Suppl 6):1325–1329 (1996).

114. Zhang L, Rothman N, Wang Y, Hayes RB, Yin S-N, Li G-L, Smith MT. Aneuploidy of chromosomes 7, 8 and 9 detected by fluorescence *in situ* hybridization in workers exposed to benzene [Abstract]. In: 86th Annual Meeting, American Association for Cancer Research, 18–22 March 1995, Toronto, Ontario, Canada. Proc Am Assoc Cancer Res 36:112 (1995).

115. Smith MT, Zhang L, Wang Y, Hayes RB, Li G, Weimels J, Dosemeci M, Tittenko-Holland N, Xi L, Kolachana P, Yin S, Rothman N. Increased translocations and aneusomy in chromosomes 8 and 21 among workers exposed to benzene. Cancer Res (in press).

116. Zhang L, Rothman N, Wang Y, Hayes RB, Li G-L, Dosemeci M, Yin S-N, Kolachana P, Titenko-Holland N, Smith MT. Benzene exposure is associated with increased aneusomy and long arm deletion of chromosomes 5 and 7 in Chinese workers. In: 88th Annual Meeting: American Association for Cancer Research, 12-16 April 1997, San Diego, California. Proc Am Assoc Cancer Res 38:123 (1997).

117. Downing JR, Head DR, Curcio-Brint AM, Hulshof MG, Motroni TA, Raimondi SC, Carroll AJ, Drabkin HA, Willman C, Theil KS, et al. An AML1/ETO fusion transcript is consistently detected by RNA-based polymerase chain reaction in acute myelogenous leukemia containing the (8;21)(q22;q22) translocation. Blood 81:2860–2865 (1993).

118. Liu Y, Hernandez AM, Shibata D, Cortopassi GA. BCL2 translocation frequency rises with age in humans. Proc Natl Acad Sci USA 91:8910–8914 (1994).

119. van Rhee F, Kasprzyk A, Jamil A, Dickinson H, Lin F, Cross NC, Galvin MC, Goldman JM, Secker-Walker LM. Detection of the *BCR-ABL* gene by reverse transcription/polymerase chain reaction and fluorescence *in situ* hybridization in a patient with

120. Philadelphia chromosome negative acute lymphoblastic leukaemia. Br J Haematol 90:225–228 (1995).

120. Natarajan AT, Boei JJ, Darroudi F, Van Diemen PC, Dulout F, Hande MP, Ramalho AT. Current cytogenetic methods for detecting exposure and effects of mutagens and carcinogens. Environ Health Perspect 104(Suppl 3):445–448 (1996).

121. Biernaux C, Loos M, Sels A, Huez G, Stryckmans P. Detection of major *bcr-abl* gene expression at a very low level in blood cells of some healthy individuals. Blood 86:3118–3122 (1995).

122. National Research Council. Environmental Epidemiology: Public Health and Hazardous Waste. Report. Vol 1. Washington:National Academy Press, 1991.

123. Knox EG. Leukaemia clusters in childhood: geographical analysis in Britain. J Epidemiol Community Health 48:369–376 (1994).

124. Bithell JF, Draper GJ. Apparent association between benzene and childhood leukaemia: methodological doubts concerning a report by Knox. J Epidemiol Community Health 49:437–439 (1995).

125. Knox EG, Gilman EA. Hazard proximities of childhood cancers in Great Britain from 1953-80. J Epidemiol Community Health 51:151–159 (1997).

126. Alexander FE, Leon DA, Cartwright RA. Isolation, car ownership, and small area variation in incidence of acute lymphoblastic leukaemia in children. Paediatr Perinat Epidemiol 10:411–417 (1996).

127. van Steensel-Moll HA, Valkenburg HA, van Zanen GE. Childhood leukemia and parental occupation. A register-based case-control study. Am J Epidemiol 121:216–224 (1985).

128. Shu XO, Gao YT, Brinton LA, Linet MS, Tu JT, Zheng W, Fraumeni JF Jr. A population-based case-control study of childhood leukemia in Shanghai. Cancer 62:635–644 (1988).

129. Buckley JD, Robison LL, Swotinsky R, Garabrant DH, LeBeau M, Manchester P, Nesbit ME, Odom L, Peters JM, Woods WG, et al. Occupational exposures of parents of children with acute nonlymphocytic leukemia: a report from the Childrens Cancer Study Group. Cancer Res 49:4030–4037 (1989).

130. McKinney PA, Alexander FE, Cartwright RA, Parker L. Parental occupations of children with leukaemia in west Cumbria, north Humberside, and Gateshead. Br Med J 302:681–687 (1991).

131. Kishi R, Katakura Y, Yuasa J, Miyake H. Association of parents' occupational exposure to cancer in children: a case-control study of acute lymphoblastic leukemia. Sangyo Igaku 35:515–529 (1993).

132. Ford AM, Ridge SA, Cabrera ME, Mahmoud H, Steel CM, Chan LC, Greaves M. *In utero* rearrangements in the trithorax-related oncogene in infant leukaemias. Nature 363:358–360 (1993).

133. Ford AM, Pombo-de-Oliveira MS, McCarthy KP, MacLean JM, Carrico KC, Vincent RF, Greaves M. Monoclonal origin of concordant T-cell malignancy in identical twins. Blood 89:281–285 (1997).

134. Savitz DA, Whelan EA, Kleckner RC. Effect of parents' occupational exposures on risk of stillbirth, preterm delivery, and small-for-gestational-age infants. Am J Epidemiol 129:1201–1218 (1989).

135. Skalko RG. Reproductive and developmental toxicity of the components of gasoline. Environ Health Perspect 101(Suppl 6):143–149 (1993).

136. Ji BT, Shu XO, Linet MS, Zheng W, Wacholder S, Gao YT, Ying DM, Jin F. Paternal cigarette smoking and the risk of childhood cancer among offspring of nonsmoking mothers. J Natl Cancer Inst 89:238–244 (1997).

137. Kessler II, Lilienfeld AM. Perspectives in the epidemiology of leukemia. Adv Cancer Res 12:225–302 (1969).

138. Gale KB, Ford AM, Repp R, Borkhardt A, Keller C, Eden OB, Greaves MF. Backtracking leukemia to birth: identification of clonotypic gene fusion sequences in neonatal blood spots. Proc Natl Acad Sci USA 94:13950–13954 (1997).