Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 464–468, January 1985
Genetics

Isolation and analysis of the 21q+ chromosome in the acute myelogenous leukemia 8;21 translocation: Evidence that c-*mos* is not translocated

[somatic cell hybrids/c-*myc*/gene mapping/superoxide dismutase (soluble)]

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Contributed by Janet D. Rowley, September 4, 1984

ABSTRACT **Acute myelogenous leukemia (AML), subgroup M2, is associated with a nonrandom chromosomal translocation, t(8;21)(q22,q22). The oncogene c-*mos* also has been localized to the q22 band on chromosome 8. There is also evidence that genes on chromosome 21 may be important in the development of leukemia. To determine whether the c-*mos* oncogene has been translocated in AML-M2 with this translocation and to isolate DNA sequences and genes from these two chromosomes that may be important in malignancy, we constructed somatic cell hybrids between a Chinese hamster ovary cell (CHO) mutant defective in glycine metabolism and myeloblasts with an 8;21 translocation from a patient with AML. We isolated the 21q+ chromosome of this translocation in a somatic cell hybrid and showed that the c-*mos* oncogene had not been translocated to chromosome 21, ruling out the possibility that translocation of c-*mos* to chromosome 21 is necessary for development of AML-M2. In addition, there was no detectable rearrangement of the c-*mos* locus within a 12.4-kilobase region surrounding the gene, indicating that rearrangement of the coding region of the gene itself or alteration of proximal 5′ or 3′ flanking sequences is not involved. We used this hybrid to determine whether specific DNA sequences and biochemical markers from chromosomes 8 and 21 had been translocated in this case.**

The discovery of nonrandom chromosomal abnormalities associated with various types of malignant diseases has generated considerable impetus for the isolation and characterization of the abnormal chromosomes and for the determination of the nature of the DNA sequences and genes located at or near the site of chromosomal rearrangement. The specificity of these rearrangements suggests a relationship between the chromosomal abnormalities and the development of specific malignant diseases (1). Recently, this relationship has been extended to the molecular level by a combination of somatic cell genetic and molecular approaches. Thus, the c-*myc* oncogene normally located on chromosome 8 is translocated to chromosome 14 in the region of the immunoglobulin heavy chain locus in Burkitt lymphoma with an 8;14 translocation (2, 3). In some cases, the c-*myc* gene is rearranged, the breakpoint on chromosome 8 being located in the first intron of the gene, while in other cases, the c-*myc* gene is not rearranged, the junction being at least 50 kilobases (kb) from the gene. In relatively rare cases of Burkitt lymphoma involving either a 2;8 or 8;22 translocation, the c-*myc* oncogene remains on chromosome 8, and the immunoglobulin light chain genes are translocated to the 3′ region of c-*myc* (4–7).

Somatic cell genetic approaches have been used also to show that, in Philadelphia (Ph^1)-positive chronic myelogenous leukemia, c-*abl* oncogene sequences are translocated from chromosome 9 to the Ph^1 (22q−) chromosome (8). Analysis of cells from two Ph^1-positive patients has revealed that the breakpoint in chromosome 9 is near c-*abl* (9). Again, the exact breakpoints have proven to be somewhat heterogeneous, although there is good evidence for clustering of the breakpoints on chromosome 22 in various patients.

A nonrandom chromosome translocation, t(8;21)(q22;q22), which results in an 8q− and 21q+ chromosome, is seen almost exclusively in the M2 subtype of acute myeloblastic leukemia (AML with maturation). Eighteen percent of all patients with AML and the M2 morphology have been found to have a 8;21 translocation [t(8;21)] (10). The c-*mos* oncogene, which has been assigned to chromosome 8 by somatic cell genetic techniques (11), has been mapped by using *in situ* hybridization to band 8q22, the band involved in the 8;21 translocation (12). Moreover, band 21q22, the site of the breakpoint on the chromosome 21 involved in this translocation, is a region which, when trisomic, leads to the development of Down syndrome, the most common chromosomal cause of mental retardation in humans and a chromosomal disease with an increased risk of leukemia (13). Thus, there may be genes on chromosome 21 whose abnormal regulation may be significant in leukemia.

To determine what genes and DNA sequences are associated with the 8;21 translocation in AML and, in particular, to determine whether translocation of c-*mos* to chromosome 21 is required for development of AML, we isolated from a patient with this disease somatic cell hybrids that contain the 21q+ portion of this chromosomal rearrangement. Analysis of these hybrids indicates that, at least in this patient, the c-*mos* oncogene is not translocated to the 21q+ chromosome and that no rearrangements can be detected in an area of 12.4 kb around this gene. Thus, neither translocation of c-*mos* to chromosome 21 nor gross rearrangement of the gene is necessary for the occurrence of AML-M2 with the 8;21 translocation.

MATERIALS AND METHODS

Cells and Media. The Chinese hamster ovary (CHO) cell parent, Gly^-B, has a defect in glycine metabolism (14) that is

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Abbreviations: AML, acute myelogenous leukemia; SOD1, superoxide dismutase (soluble); CHO, Chinese hamster ovary; kb, kilobase(s); GSR, glutathione reductase; LDHA, lactose dehydrogenase A; HEXA, hexosaminidase A.

complemented by human chromosome 8 (15). Gly⁻B cells were grown in F12 medium (16) supplemented with 6% (vol/vol) fetal calf serum.

The patient was a 60-year-old man in second relapse, the last chemotherapy having been given 3 months prior as consolidation therapy while still in remission. The complete karyotype of the leukemic cells was 45,X,−Y,t(8;21)(q22;q22), del(9)(q11 or q12q31), which was seen in 97% of the metaphase cells examined. The marrow cells were processed by our usual methods (17).

706B6 clone 17 is a CHO–human hybrid containing chromosome 8 as the only human material (15). 2FUr1 and 72532X-6 are CHO-human hybrids containing the long arm of chromosome 21 and the entire chromosome 21, respectively, as their only cytogenetically detectable human material (18).

Isolation of Somatic Cell Hybrids Between Gly⁻B and AML Cells Containing the 8;21 Translocation. Cell fusions between the Gly⁻B and AML cells were done by using UV-inactivated Sendai virus as described (19). Cells ($\approx 1 \times 10^6$) from each parent were used, and prior to fusions the leukemic cells were separated from mature erythrocytes and granulocytes on a Ficoll/Hypaque gradient. Hybrids were selected by their growth in F12D medium (20) with 6% (vol/vol) dialyzed fetal calf serum that lacks glycine and does not support growth of the Gly⁻B parent.

Cytogenetic Analysis. To identify human chromosomes in CHO–human hybrids, Giemsa/trypsin banding and Giemsa-11 staining were performed as described by Morse *et al.* (21) and Alhadeff *et al.* (22). After Giemsa/trypsin banding, selected metaphase cells were photographed, and the slides were destained with methanol/acetic acid, 3:1 (vol/vol), soaked for 1–2 hr at 60°C, and then restained using the Giemsa-11 technique. With Giemsa-11 staining, human chromosomes appear blue, while the CHO chromosomes appear magenta. The human chromosomes were then identified in the trypsin-banded photographs.

Isozyme Analysis. Isozyme analysis was performed by using Cellogel electrophoresis procedures described by Meera Khan (23) and van Someren *et al.* (24). (The human chromosome assignment of each isozyme marker is indicated in brackets.) The isozymes used were: glutathione reductase (GSR) [8]; lactate dehydrogenase A (LDHA) [11]; hexosaminidase A (HEXA) [15]; and soluble superoxide dismutase (SOD1) [21].

DNA Isolation. High molecular weight DNA was prepared by the method of Gusella *et al.* (25) except that, after the chloroform extractions, T1 RNase (Boehringer Mannheim) was added to a final concentration of 42 units/ml and incubated at 37°C for various periods of time. After this, one phenol and three chloroform extractions were done prior to precipitation with alcohol.

Gel Electrophoresis, Southern Transfer, and Hybridizations. High molecular weight DNA was completely digested with the indicated restriction endonucleases under conditions recommended by the manufacturer. Digested DNA (6–7 μg) was then electrophoresed in a horizontal 0.75% agarose slab gel in a Tris acetate buffer (40 mM Tris-base/1.0 mM EDTA/20 mM sodium acetate, pH 7.6). *Hin*dIII-digested phage λ DNA was used for molecular weight markers. Transfer of DNA to nitrocellulose paper was performed by the method of Southern (26). The nitrocellulose-bound DNA was then hybridized to the indicated ^{32}P-labeled probes, which had been nick-translated to a specific activity of $\approx 10^8$ cpm/μg of DNA in a solution containing 50% (vol/vol) formamide and 5× NaCl/Cit (1× NaCl/Cit is 0.15 M NaCl/0.015 M Na citrate, pH 7.0) at 42°C for 15–18 hr. The filters were washed with 2× NaCl/Cit with 0.1% $NaDodSO_4$ at room temperature, followed by washing in 0.1× NaCl/Cit at 55°C. Filters were air-dried and used to expose Kodak XAR-5 film for various periods.

Molecular Probes. The human c-*myc* cDNA probe, pRyc-7.4 (27) and the immunoglobulin λ heavy chain constant region gene (C_λ) probe (28), which contains the 8-kb *Eco*RI Ke^-Oz^-/Ke^-Oz^+ fragment, were kindly provided by C. Croce. The human *mos* probe used was a 2.7-kb *Eco*RI fragment derived from λHM1 (29) kindly provided by G. Vande Woude. The human chromosome 21-specific probes CP2, CP8, and CP21G1 (18) were kindly provided by J. Davidson.

RESULTS

A Southern blot analysis of DNA from the patient's leukemic bone marrow cells digested with either *Bam*HI or *Bgl*II demonstrated only germ-line size fragments (data not shown). This analysis covers a distance of 12.4 kb around the c-*mos* gene and suggests that the breakpoint must be located outside this region. Thus, alteration in 5′ or 3′ proximal regulatory elements near c-*mos* or alterations in the coding sequence of this gene large enough to be detected by this Southern hybridization analysis are not required for development of AML.

Fusion of CHO Gly⁻B cells with cells from the bone marrow of the patient resulted in 22 separate clones. Because the region of chromosome 8 that complements the Gly⁻B defect is 8q21.1→qter (30), we initially screened 15 clones for the presence of the human GSR gene (*GSR*) which is located at 8p21.1 (31). Four of the 15 clones were negative for human *GSR*, suggesting that they might contain the 8;21 translocation or other fragments of chromosome 8 that allowed growth in deficient media. Two clones contained rearranged human chromosomes and will not be discussed further. An additional clone contained many human chromosomes and was not further analyzed.

Clone 21 was found to contain one recombinant chromosome, namely chromosome 21 with the distal portion of chromosome 8 attached (the 21q+ chromosome), and was further subcloned. Clone 21-4 contained the human chromosomes 11, 15, and 22 in addition to the 21q+ chromosome (Fig. 1). Clone 21-8 was identical except for the spontaneous loss of the human chromosome 22. The human chromosomes 11, 15, and 22 appeared normal by cytogenetic analysis. Table 1 shows the confirmation of the human chromosomes by isozyme analysis. The identity of chromosome 22 was confirmed by probing an *Eco*RI digest with the C_λ probe. The presence of human *SOD1* in clones 21-4 and 21-8 demonstrates that this gene, which has been assigned previously to 21q22.1 (32), must be proximal to the breakpoint on

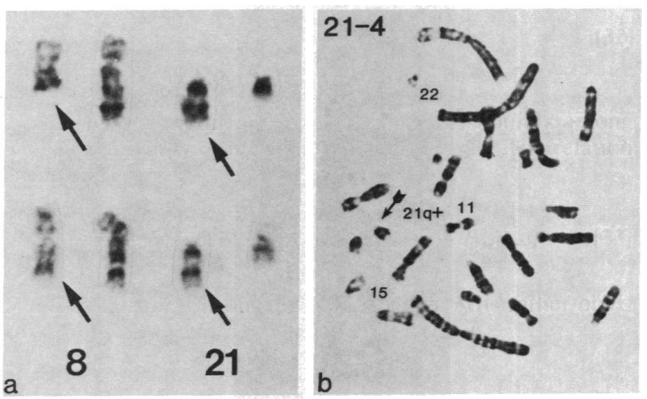

FIG. 1. Giemsa/trypsin/Giemsa-11-banding of metaphase cells. (*a*) Partial karyotypes of chromosome pairs 8 and 21 obtained from two cells from a patient with AML-M2. A reciprocal translocation involving chromosomes 8 and 21 [t(8;21)(q22;q22)] is illustrated. The rearranged chromosomes are identified with arrows and are on the left in each pair. (*b*) CHO–human hybrid 21-4 containing the human chromosomes 11, 15, 21q+, and 22.

Table 1. Isozyme and molecular analysis of hybrids 21-4 and 21-8 and controls

Genes	C21-4	C21-8	Gly⁻B (CHO)	HeLa (human)	AG1522 (human)
LDHA (Chr. 11)	+	+	−	+	ND
HEXA (Chr. 15)	+	+	−	+	ND
SOD1 (Chr. 21)	+	+	−	+	ND
C_λ (Chr. 22)	+	−	−	ND	+

Chr., chromosome; +, human isozyme or DNA sequence present; −, human isozyme or DNA sequence absent; ND, not done.

chromosome 21, an observation consistent with the report by Yunis that the breakpoint is at 21q22.3 (33).

Since the c-*myc* oncogene has been localized to 8q24 (2, 12), which is distal to the breakpoint at 8q22 observed in this disease, human c-*myc* should be present in 21q+ chromosome-containing hybrids. Fig. 2 shows the results of an *Sst* I digest probed with c-*myc* (pRyc-7.4). Clones 21-4 and 21-8, which contain the 21q+ chromosome, show the human c-*myc* bands of 2.8 and 1.5 kb also present in the human fibroblast line AG1522. The CHO parent, Gly⁻B, and two other CHO-human hybrids not containing the 21q+ chromosome do not show the human bands.

To further reduce the number of human chromosomes in clone 21-8 and to verify the identity of the 21q+ chromosome, we performed segregation analysis. We utilized a monoclonal antibody, 4C11, kindly provided by C. Jones that recognizes a human chromosome 11-specific cell surface antigen and, in the presence of antibody and complement, kills cells containing human chromosome 11. Six clones were selected that grew in the presence of antibody with complement. All were found to have lost chromosome 11 by cytogenetic analysis and LDHA activity. All clones also retained the human c-*myc* gene as well as human SOD1 and HEXA activity (data not shown). A bromodeoxyuridine selection procedure (34) was applied to one of these clones to produce a segregant that had lost the 21q+ chromosome. Briefly, exposure of the culture to visible light after growth in deficient medium containing bromodeoxyuridine kills proliferating cells that have incorporated bromodeoxyuridine and allows the growth of these cells that have lost the 21q+ chromosome. Seventeen clones were picked, and 10 of these were analyzed for human SOD1 activity. All ten had lost human *SOD1* activity. Six of these clones were tested for growth in deficient media and all failed to grow, demonstrating that the ability to complement the Gly⁻B defect also has been lost. Southern blot analysis on three of these segregants showed that the human c-*myc* gene also has been lost, and cytogenetic analysis showed that only human chromosome 15 remained. Thus, *SOD1*, c-*myc*, and the ability to correct the Gly⁻B defect all segregate with the chromosome identified as the 21q+ chromosome. Because two of these markers are known to be on chromosome 8 and one is known to be on chromosome 21, this analysis confirms the identification of the 21q+ chromosome.

To determine whether c-*mos* has been translocated to the 21q+ chromosome in the cells from this patient, we probed an *Eco*RI digest of clone 21-8 DNA with c-*mos*. This experiment (Fig. 3) failed to demonstrate human c-*mos*, showing that this oncogene is not translocated to the 21q+ chromosome, strongly suggesting that this gene is retained on chromosome 8q− in this patient. However, c-*mos* was detected in a hybrid, 706B6 clone 17, known to contain an intact human chromosome 8 as its only detectable human chromosomal material. These results demonstrate that translocation of c-*mos* to chromosome 21 is not required for development of AML-M2 with the 8;21 translocation.

To further characterize the translocation chromosome present in hybrid 21-8 and to obtain information on the regional assignment of additional DNA sequences, we analyzed DNA from hybrid 21-8 by Southern blot hybridization, using as probes three cloned DNA sequences isolated from a flow-sorted chromosome 21 library prepared by Krumlauf *et al.* (35) and shown previously to be located on the long arm of chromosome 21 (18). Southern blots of clone 21-8 DNA digested with *Eco*RI and probed with either probe CP2 or CP8 showed that these DNA sequences were present in the hybrid. However, the DNA sequence corresponding to probe CP21G1 was not present (Fig. 4). Therefore, CP21G1 DNA must be located in the region of chromosome 21 that is

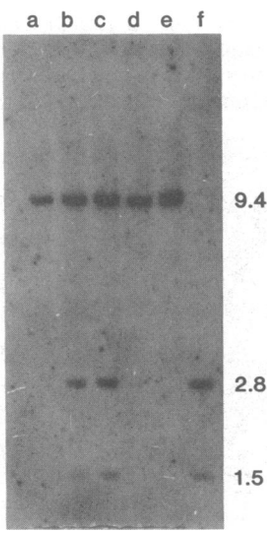

FIG. 2. *Sst* I digests probed with c-*myc* (pRyc-7.4) showing the presence of the human 2.8- and 1.5-kb c-*myc* bands in clones 21-4 and 21-8. The hamster band is 9.4 kb. Lanes: a and e, CHO–human hybrids not containing the 21q+ chromosome; b and c, clones 21-4 and 21-8, respectively, which contain the 21q+ chromosome; d, CHO parent, Gly⁻B; f, human fibroblast cell line AG1522.

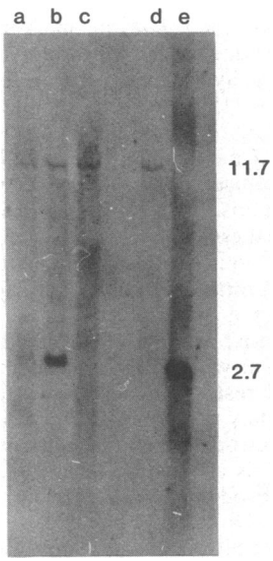

FIG. 3. *Eco*RI digests of DNA probed with c-*mos*, demonstrating that clone 21-8, which contains the 21q+ chromosome, does not contain c-*mos* sequences. The human band is 2.7 kb, and the hamster band is 11.7 kb. Lanes: a, CHO–human hybrid not containing the 21q+ chromosome; b, CHO–human hybrid 706B6 clone 17, which contains chromosome 8 as the only identifiable human material; c, clone 21-8, which contains the 21q+ chromosome; d, CHO–human hybrid 72532X-6, which contains chromosome 21 as the only identifiable human material; e, human fibroblast line AG1522.

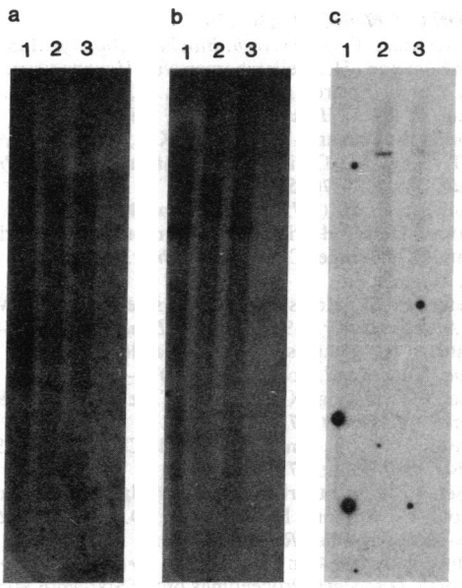

FIG. 4. *Eco*RI digests of DNA used to regionally map the chromosome 21-specific DNA sequences corresponding to probes CP2, CP8, and CP21G1. The sequences of probes CP2 and CP8 are present in the 21q+ chromosome while that of CP21G1 is absent, presumably as a result of the translocation of this region to the 8q− chromosome. (*a*) Probe CP21G1. Lanes: 1, hybrid 21-8 containing the 21q+ chromosome; 2, hybrid 706B6 clone 17 containing human chromosome 8; 3, hybrid 72532X-6 containing human chromosome 21. (*b*) Probe CP2. Lanes: 1, hybrid 21-8; 2, hybrid 706B6 clone 17; 3, hybrid 72532X-6. (*c*) Probe CP8. Lanes: 1, hybrid 706B6 clone 17; 2, hybrid 21-8; 3, hybrid 2FUr1 containing only the long arm of human chromosome 21.

distal to the breakpoint 21q22.3, while the DNA sequences corresponding to probes CP2 and CP8 must lie in the region 21cent→q21q22.3.

DISCUSSION

The localization of the c-*mos* oncogene to 8q22 (12) raises the possibility that c-*mos* may be involved in the pathogenesis of AML-M2. We find that c-*mos* has not been translocated, at least in this instance, and no c-*mos* rearrangement has been detected by Southern blot hybridization analysis. Therefore a c-*mos* must remain on chromosome 8 in this patient, and translocation of c-*mos* to chromosome 21 cannot be responsible for AML in such patients. The lack of a detectable rearrangement within 12.4 kb of the c-*mos* locus suggests that an alteration in 5′ or 3′ proximal regulatory sequences or in the coding region of the locus itself also are not important in this disease. Of course a more detailed analysis of this region of DNA may reveal rearrangements not detected in the present study.

The observed lack of translocation of c-*mos* is consistent with Rowley's postulation that translocation of 21q material to 8q− is the critical event (1) in the pathogenesis of this type of leukemia. If c-*mos* is involved in the pathogenesis of AML, the oncogene may be affected in its function by the transposition to its vicinity of DNA sequences contained in chromosome 21, a situation that would be analogous to the variant translocations in Burkitt lymphoma in which c-*myc* remains on chromosome 8 and the immunoglobulin light chain genes are translocated to the 3′ region of the gene (4–7). Alternatively, c-*mos* function could be affected by the translocation of regulatory elements normally present on chromosome 8 away from c-*mos*.

The only precedent for activation of c-*mos* in a malignancy is the insertion of an intracisternal A particle genome in the coding region of the mouse c-*mos* gene in a mouse plasmacytoma (36). Transcription of c-*mos* in normal cells or in other malignancies has not been reported. It will be of great interest to determine if c-*mos* transcription can be detected in AML cells containing the 8;21 translocation or in hybrids containing the 8q− chromosome, which presumably would contain the c-*mos* gene.

It may be that the location of c-*mos* at 8q22 is fortuitous and that this oncogene is irrelevant to AML. In this case, other genes on chromosomes 8 or 21 would likely be of importance. The importance of genes on chromosome 21 in this regard is suggested by the observation that Down syndrome patients have a significantly increased risk of leukemia. Only band 21q22 need be trisomic to generate the syndrome (37); therefore, these genes would be located in the region of 21 in which the break occurs in the 8;21 translocations. Additionally, newborn infants without Down Syndrome who have a transient leukemia-like disease are also trisomic for chromosome 21 in their "leukemic" cells (38). Therefore, alterations in levels of transcription of a gene or genes on 21q22 might be an important event in leukemia. Translocation of these genes to chromosome 8 in AML-M2 might result in alteration in their regulation or alteration in the regulation of genes on chromosome 8, for example c-*mos*, leading to leukemia. Discovering the identity of chromosome 21-specific genes at or near the 8;21 breakpoint may provide a clue as to the nature of these genes. For this reason, it is important to isolate DNA sequences from chromosome 21 and to regionally assign them.

Therefore, we have used the hybrid described here to assign regionally chromosome 21-specific DNA sequences to the 21q+ chromosome, or, by exclusion, to the 8q− chromosome. We have identified one DNA sequence isolated from the chromosome 21 library of Krumlauf *et al.* (35) that is present in hybrids containing a long arm of chromosome 21 and is not present in clone 21-8. This DNA sequence presumably has been translocated to chromosome 8 and, therefore, is located in the region of 21q22.3→qter. This DNA sequence should prove useful in examining hybrids for the presence of the 8q− chromosome associated with AML-M2. Two other DNA sequences have been assigned to the 21(cen→q22.3) region. This approach should eventually allow isolation and examination of the DNA at the translocation junction in these patients and determination of their importance in leukemogenesis and in normal development.

This work was supported by grants from the National Institute on Aging (AG00029), the Ladies' Auxiliary of the Veterans of Foreign Wars, and National Institute of Child Health and Human Development (HD13432), the Department of Energy (contract DE-AC02-80EV10360), National Cancer Institute (CA 16910 and CA 25568), and the Leukemia Research Foundation. Harry Drabkin is a Fellow of the Damon Runyon–Walter Winchell Cancer Fund (DRG-635) and has received support from the Blood Cancer Research Fund of the University of Colorado Health Sciences Center. M.LeB. is a Fellow of the Leukemia Society of America.

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