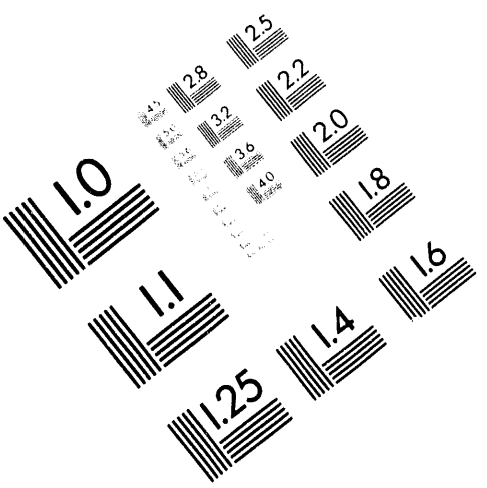
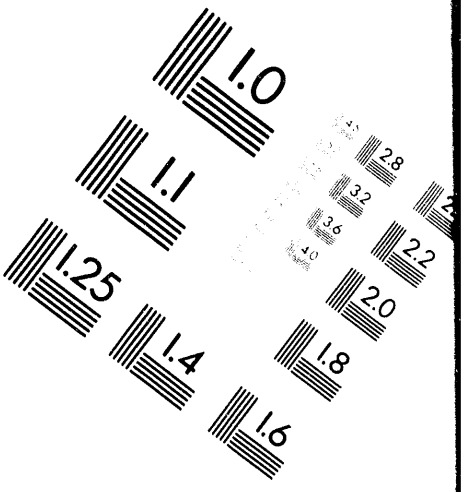
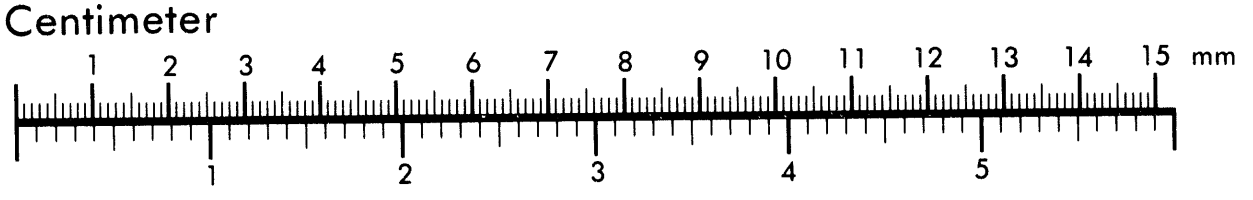


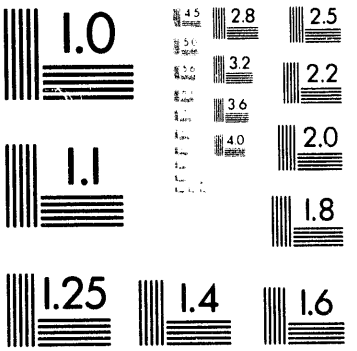
Association for Information and Image Management

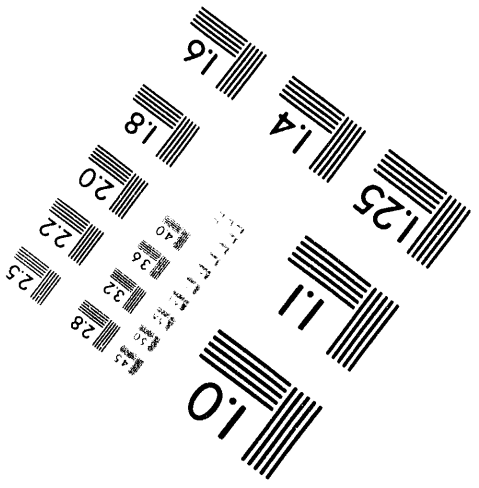
1100 Wayne Avenue, Suite 1100
Silver Spring, Maryland 20910

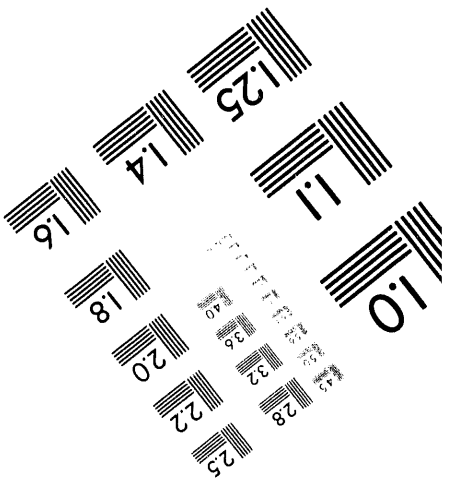
301/587-8202











MANUFACTURED TO AIIM STANDARDS
BY APPLIED IMAGE, INC.

1 of 1

DOE/ER60408-16

Correlation of Chromosome Patterns in Human Leukemic Cells with Exposure to Chemicals and/or Radiation

Comprehensive Progress Report
For the Period July 1991 through June 1994

Janet D. Rowley, M.D.

The University of Chicago
5841 S. Maryland Ave., MC 2115
Chicago, IL 60637-1470

June, 1994

Prepared for:
THE U.S. DEPARTMENT OF ENERGY
UNDER GRANT NO. DE-FG02-86ER60408

DISCLAIMER

TABLE OF CONTENTS

TABLE OF CONTENTS
(cont.)

I. COMPREHENSIVE PROGRESS REPORT: 1991-1994

A. Major Research Accomplishments

1. Introduction

This project began in 1962 when I became a member of the staff of the Argonne Cancer Research Hospital at the University of Chicago, which was fully funded by the Atomic Energy Commission. I was studying chromosome abnormalities with the use of autoradiography. In about 1965, I began to study the chromosome pattern in marrow cells from patients with leukemia and preleukemia. After a sabbatical 1970-71, I used chromosome banding techniques and discovered a number of recurring chromosome translocations in the cells of these patients. I showed that there was a non-random pattern of chromosome gains and losses in leukemia cells and that specific translocations were relatively consistently associated with particular morphologic subtypes of acute myeloid leukemia de novo. Moreover, I showed that these aberrations had some interesting variations in frequency that were related to the age of the patient. Thus recurring translocations tended to be more frequent in children whereas loss of chromosomes 5 and 7 tended to occur in older patients.

In 1977, my colleagues and I reported on the karyotype of leukemia cells in the first large (10 patients) series of Hodgkin's disease patients with a history of prior mutagenic therapy. I noted that 9 of these patients had loss of all or part of the long arm of chromosome 5 and that 5 patients also had loss of chromosome 7. This observation has been confirmed by many other investigators as well as by our continuing analysis of more patients.

I then embarked on an ongoing investigation as to the nature of the chromosome aberrations in t-AML/t-MDS because I felt that a careful analysis of these patients' cells would provide unique insights into the location of genes that were important in leukemogenesis and that this information might help us to determine which patients who appeared to have AML de novo, might, in fact, have mutagen related leukemia. This hope is still just that; however we are getting progressively closer to the gene or genes involved as we use ever more sophisticated molecular and cytogenetic techniques. As is described in this report, we have cloned some of the genes involved in balanced translocations in t-AML and in AML de novo. At least some of the genes involved in deletions in t-AML should be cloned in the next five years.

Since 1974, we have examined the karyotype of over 160 patients with acute myeloid leukemia (AML) who have a history of prior treatment with radiation and chemotherapy usually for a malignant disease. We have sufficient clinical data on 132 patients so that we can correlate the type of prior treatment with the chromosome abnormalities in the patient's leukemic cells. In the past, we have concentrated on the deletions involving chromosomes 5 and/or 7 because these were the most common changes.

In the course of our analysis of these 132 t-AML patients, it became apparent that there was another group of patients who lacked aberrations of chromosomes 5 and/or 7 and whose leukemic cells had certain specific changes that were quite unusual for t-AML. These patients have balanced translocations usually involving

chromosome bands 11q23 or 21q22. Of special interest is the fact that these patients have usually received high doses of topoisomerase II inhibitors, especially the epipodophyllotoxins, etoposide (VP16) or teniposide (VM26). These latter drugs were first used with any frequency in the 1980s. Our group at the University first called attention to this association in 1987, when we reported on the greatly increased risk of t-AML in a series of lung cancer patients treated with very high doses of etoposide.(Ratain, et al., Blood, 70:1412-1417, 1987) It was noteworthy that three patients in this study had balanced translocations involving 11q23. We proposed that this unusual karyotypic pattern was related to the high dose of etoposide received by these patients. This association has now been confirmed by many other laboratory groups. More recently, we and others have found a very close correlation between the presence of balanced translocations involving 11q23 or 21q22 and exposure to topoisomerase II inhibitors.

It has been clear for the last decade that cloning translocation breakpoints in both AML de novo and t-AML would provide the DNA probes required to determine whether the breakpoints in cytogenetically apparently similar translocations were identical at the level of DNA. Therefore we have pursued an analysis of rearrangements in both types of leukemia simultaneously. We have also cloned and sequenced several translocations in acute lymphoblastic leukemia and in chronic lymphatic leukemia. Recently we cloned the breakpoint in a number of translocations involving chromosome bands 11q23 and 21q22. These studies have provided DNA probes that will be very important for diagnosis and monitoring the patient's response to treatment.

2. Major accomplishments with reference to originally stated objectives (January 1, 1992)

a. *Specific objectives of original three year proposal*

1. Defining the chromosome segments associated with radiation and chemically-induced leukemogenesis (treatment-related acute myeloid leukemia, t-AML)

   a. Continued genetic analysis of chromosomes 5 and 7

   b. Correlation of treatment with balanced and unbalanced translocations

2. Cloning the breakpoints in balanced translocations in t-AML

   a. Clone the t(9;11) and t(11;19) breakpoints

   b. Clone the t(3;21)(q26;q22) breakpoint

   c. Determine the relationship of these translocations to prior exposure to topoisomerase II inhibitors

3. Compare the breakpoint junctions in patients who have the same translocations in t-AML and AML de novo

4. Map the scaffold attachment regions in the genes that are involved in balanced translocations in t-AML

b. List of major accomplishments in the last three years

•We cloned the gene on chromosome 11 involved in most of the 11q23 translocations; we called it *MLL* for myeloid-lymphoid leukemia or mixed-lineage leukemia.(Ziemen van der Poel, et al., Proc Natl Acad Sci, 88:10735-10739, 1991; McCabe, et al., Proc Natl Acad Sci, 89:11794-11798, 1992)

•We have cloned the breakpoint in the t(9;11)(p22;q23) and in the t(11;19)(q23;p13.1). The gene on chromosome 9 is *AF-9* (cloned independently by Croce and Canaani).(Nakamura, et al., Proc Natl Acad Sci, 90:4631-4635, 1993) We have cloned the t(9;11) breakpoint from one patient and one cell line. The breakpoint in *MLL* is after exon 6 in one and after exon 8 in the cell line. The breakpoint in *AF-9* is in the same intron as reported by Nakamura, et al. In the t(11;19), the breakpoint on chromosome 19 is more centromeric than the *ENL* gene that was cloned by Cleary's group(Tkachuk, et al., Cell, 71:691-670, 1992) and it shows no sequence homology to *ENL*. A paper reporting this discovery has been submitted.(Thirman, et al.)

•We have cloned the t(3;21) breakpoint and have shown that it is unusual in that it involves *AML1* on chromosome 21 and results in fusion mRNAs from at least two (possibly three) different genes on chromosome 3.(Nucifora, et al., Proc Natl Acad Sci, 91:4004-4008, 1994)

•We have shown that all nine t-AML patients with 11q23 translocations and *MLL* rearrangements on Southern blot analysis had received topoisomerase II inhibitors for treatment of another malignant disease.

•The patients with t-AML and 11q23 translocations have breaks in the same 8.3 kb region of *MLL* as the de novo AML. Our preliminary data suggest that these breaks in the t-AML are relatively evenly distributed in this segment, whereas the breaks in the de novo patients tend to cluster in the 5′ two-thirds of the segment.(Broeker, et al., AACR abstract, 1994)

•We have mapped the scaffold attachment regions in the 8.3 kb breakpoint cluster region of the *MLL* gene. Most of the breakpoints in the de novo AML patients map in a 5 kb DNA loop that is bounded by SARs.

•We have also mapped the location of vertebrate topo II consensus binding sequences in the 8.3 kb genomic fragment. There are eleven of them, eight of which are in the region we have defined as a SAR.

B. Technical Progress of Project during the last three years

1. Defining the chromosome segments associated with radiation and chemically induced leukemogenesis (treatment-related acute myeloid leukemia, t-AML)

a. Continued genetic analysis of chromosomes 5 and 7

Much of the responsibility for this project has been given to Drs. Michelle LeBeau and Carol Westbrook. They have support for this project through a program project grant from NIH. They are presently constructing a YAC contig for the critical region of chromosome 5, namely 5q31. I remain a close collaborator and I am involved in parts of the project as needed.

As I indicated in my earlier annual report, I have access to a patient (patient A) with abnormalities of many of the chromosome regions involved in t-AML. In addition, we also received a sample from a second man (patient B) involved in the same accident. He has many abnormal metaphase cells, but no clonal abnormality in the samples we received. As the appropriate probes become available, I will analyze the DNA from patients A and B for mutations in the appropriate candidate genes (see Proposed Research).

An important related project has been the completion of a major study on the karyotypic pattern seen in patients with a subtype of AML, namely erythroleukemia, AML M6. We have performed a retrospective analysis of the clinical, morphologic, and cytogenetic findings in 26 patients diagnosed between January 1969 and September 1991 with acute erythroblastic leukemia de novo (EL or AML-M6). Clonal chromosomal abnormalities were found in 20 patients (77%). Loss of all or part of the long arm (q) of chromosomes 5 and/or 7 was observed in 17 patients (65%). In addition, the karyotypes were often complex with multiple abnormalities and subclones. Among the remaining 9 patients, 6 had a normal karyotype and one each had trisomy 8, t(3;3), or t(3;5). The overall frequency of abnormalities of chromosomes 5 and/or 7 observed in our M6 patients is similar to that observed in our patients with therapy-related acute myeloid leukemia (t-AML) (99 of 129 patients, 77%) but substantially higher than that noted in our other patients with AML de novo (FAB subtypes M1-M5: 52 of 334 patients, 16%). Our M6 patients with abnormalities of chromosomes 5 and/or 7 were older and had a shorter median survival (16 weeks vs 77 weeks [p = .0047]) than the patients without these abnormalities. We found no correlation between morphologic features and either cytogenetic abnormalities or clinical outcome. Of note was the finding that the percentage of myeloblasts, which may account for only a small fraction of the total marrow elements when the revised FAB criteria are applied, had no bearing on prognosis. We conclude that acute erythroblastic leukemia, when defined by morphologic criteria, consists of two distinctive subgroups: one group tends to be older, has complex cytogenetic abnormalities especially of chromosomes 5 and/or 7, and shares biological and clinical features with t-AML; the other group with simple or no detectable cytogenetic abnormalities has a more favorable prognosis when treated with intensive chemotherapy.(Olopade, et al., Blood, 80:1-9, 1992)

b. Correlation of treatment with balanced and unbalanced translocations

The results of our analysis has been published as a Letter to the Editor of Blood.

We had five patients with balanced translocations of 11q23 and 5 patients with balanced translocations of 21q22. Only one patient in each group did not

receive a topo II inhibitor. This compares with 2 and 8 patients with unbalanced translocations involving these bands; except for one patient with an 11q23 breakpoint, none of these patients had received topo II inhibitors.(See Table 1)

Table 1 RELATIONSHIP BETWEEN PRIOR CHEMOTHERAPY AND CLONAL CHROMOSOMAL TRANSLOCATIONS IN 132 PATIENTS WITH t-MDS/t-AML

	ATTop Only	ATTop + AA	AA Only	Other Treatment	Total
Number Studied	6	40	67	19	132
Balanced translocation band 11q23	2	2	1	0	5
Balanced translocation band 21q22	0	4	1	0	5
Unbalanced translocation band 11q23	0	0	2	0	2
Unbalanced translocation band 21q22	0	1	6	1	8

Abbreviations: ATTop, chemotherapy agents that target DNA topoisomerase II; AA, alkylating agents.

Balanced translocations involving band 11q23 or 21q22 were significantly associated with prior ATTop exposure (p=0.003, Fisher's exact test, two sided).

Larson, R.A., Le Beau, M.M., Ratain, M.J., Rowley, J.D. (Letter to the Editor) Balanced translocations involving chromosome bands 11q23 and 21q22 in therapy-related leukemia. Blood, 79:1892-1893, 1992.

2. Cloning the breakpoints in balanced translocations in t-AML

a. Analysis of 11q23 aberrations

1. Clone the t(9;11) and t(11;19) breakpoints

Our original objective of cloning the t(9;11) and t(11;19) breakpoints in t-AML has been substantially expanded and this is reflected in the altered organization of this section. My colleagues and I have made steady progress in first mapping and now in cloning the breakpoint junctions in the above two translocations, as well as in a related translocation, t(6;11), in patients with de novo acute leukemia. The results of our cloning analysis will be described later in section 2C. We previously showed that the translocations involving 11q23 with chromosomes 4(q21), 6(q27), 9(p22), and 19(p13) both p13.3 (more common) and p13.1 (less common) all had breakpoints within a 330 kb human DNA

insert in a yeast artificial chromosome (YAC) identified with a CD3 gamma probe.(Figure 1A)(Zieman van der Poel, et al., Proc Natl Acad Sci, 88:10735-10739, 1991 We subcloned three fragments from the YAC and showed that one fragment remained on chromosome 11 in all translocations. The other two fragments were translocated to the other chromosome.(4,6,9,or19) All three fragments recognize the same large-sized band (~15 kb) on Northern blot analysis as well as other transcripts of varying size down to 1.5 kb. We subcloned a number of unique sequence probes, especially from the large fragment (clone 14) just telomeric to the breakpoint.(McCabe, et al., Proc Natl Acad Sci, 89:11794-11798, 1992)

We called this gene *MLL* (myeloid-lymphoid, or mixed-lineage leukemia). The gene is estimated to be over 100 kb in size and there are multiple size RNA transcripts. The largest cDNA is 15 kb and it codes for a protein of just over 3,900 amino acids of about 430 kDa. There are a minimum of 21 exons. Exons 8 to 11 code for a series of zinc fingers that show homology to the *Drosophila trithorax* gene. Using subclones of these genomic probes, we have identified a number of cDNA clones of *MLL*, two of which recognize rearrangements on Southern blots of DNA from patients and cell lines with 11q23 translocations. An internal 0.74kb *Bam*H1 subclone of one of the cDNAs, which recognizes an 8.3kb *Bam*H1 genomic fragment, detects rearrangements on Southern blots of *Bam*H1 digested patient and cell line DNA.(Figure 1B,C) This 8.3 kb fragment contains exons 5 through 11.

We analyzed DNA from 61 samples using this 0.74 kilobase *Bam*HI fragment isolated from a cDNA clone of the *MLL* gene. Patient samples were selected on the basis of a karyotype containing an 11q23 abnormality and the availability of cryopreserved bone marrow or peripheral blood. Unique sequences from the 0.74 kb cDNA fragment, corresponding to the centromeric and telomeric ends of the 8.3 kb germline fragment, were amplified by the polymerase chain reaction (PCR) and were used as probes to distinguish the chromosomal origin of rearranged bands on Southern blot analysis. Using the 0.74kb probe, we detected rearrangements in *Bam*HI digested DNA from 61 patients with acute leukemia and 11q23 aberrations, three cell lines derived from such patients, and three patients with non-Hodgkins lymphomas. Nineteen different chromosome breakpoints were associated with the *MLL* gene in these rearrangements, suggesting that *MLL* is juxtaposed to 19 different genes. In 70% of these cases, two rearranged bands, corresponding to the two derivative chromosomes, were detected and in 30%, only one rearranged band was present. In cases with only one rearranged band, it was always detected by only the centromeric probe. Thus, the sequences centromeric to the breakpoint were always preserved, whereas, telomeric sequences were deleted in 30% of cases.(Figure 1D,2)(Thirman, et al., New Engl J Med, 329:909-914, 1993)

Thus we have developed a cDNA probe that detects rearrangements on Southern blot analysis with a single *Bam*HI restriction digest in all patients with the common 11q23 translocations. This cDNA probe will be very useful clinically both in diagnosis of rearrangements of the *MLL* gene especially in infants with leukemia as well as in monitoring patients during the course of their disease. The usefulness of this probe is illustrated by the recent report of Caligiura, et al. (*Cancer Research*, 54:370, 1994) who identified *MLL* rearrangements in patients without 11q23 cytogenetic aberrations using this probe.

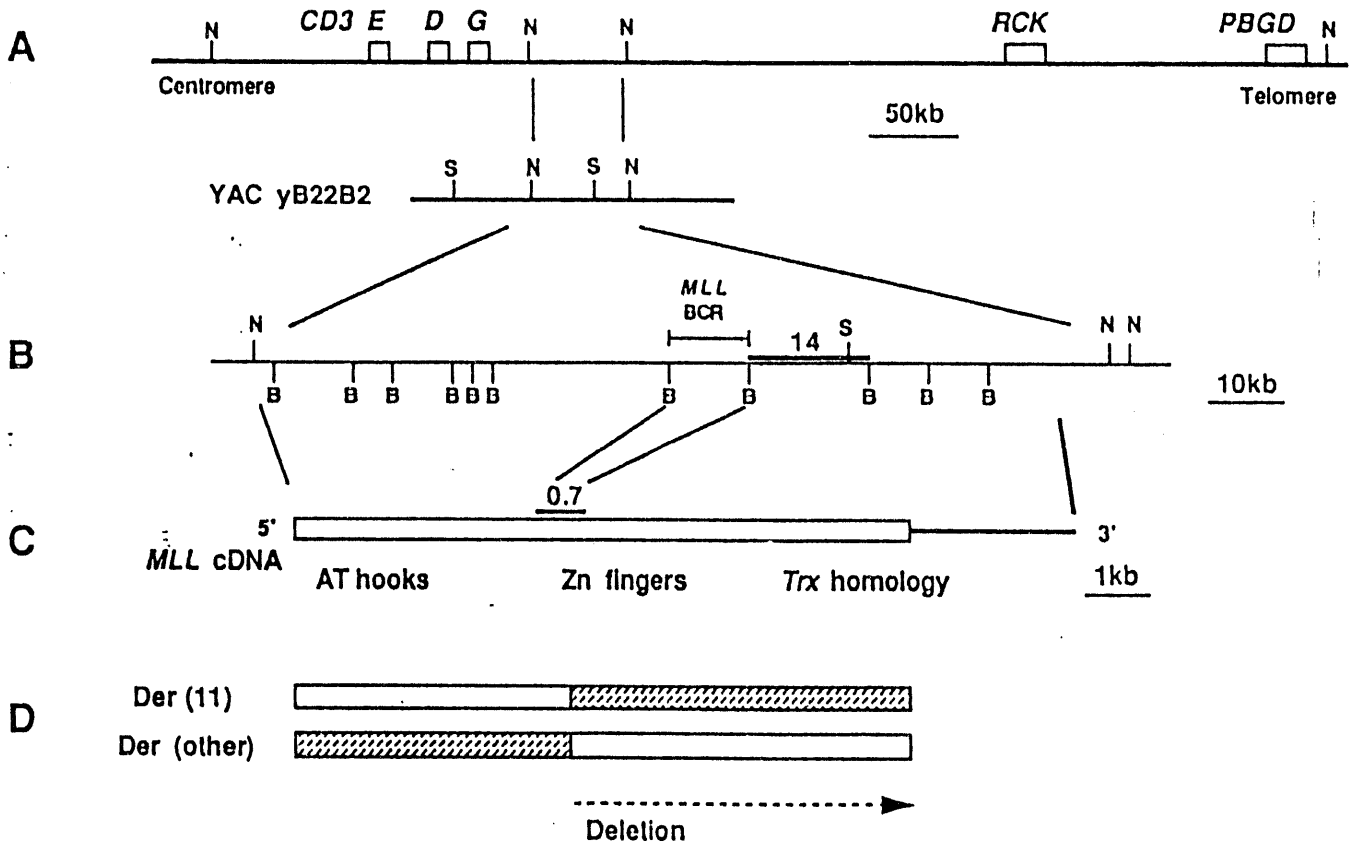


FIGURE 1

A. A partial map of 11q23 showing the location of nearby genes. Below, partial restriction map of YAC showing alignment to larger genomic map above and to the *MLL* gene in B.

B. A partial map of *MLL* showing the location of the 8.3kb breakpoint cluster region (BCR) and the clone 14 probe used in FISH studies.

C. Diagram of the cDNA with the position of the 0.7kb probe and the general location of important motifs.

D. Representation of the two derivative chromosomes formed as a result of the translocation. The zinc fingers are translocated to the der(other) chromosome and part or all of *MLL* telomeric to the breakpoint is deleted in about 25% of de novo acute leukemia patients. N - *Not*I, S - *SFi*I, B - *Bam*HI, Zn fingers - zinc fingers, *trx* - *trithorax*, der(11) - derivative 11 chromosome, der(other) - other derivative chromosome.

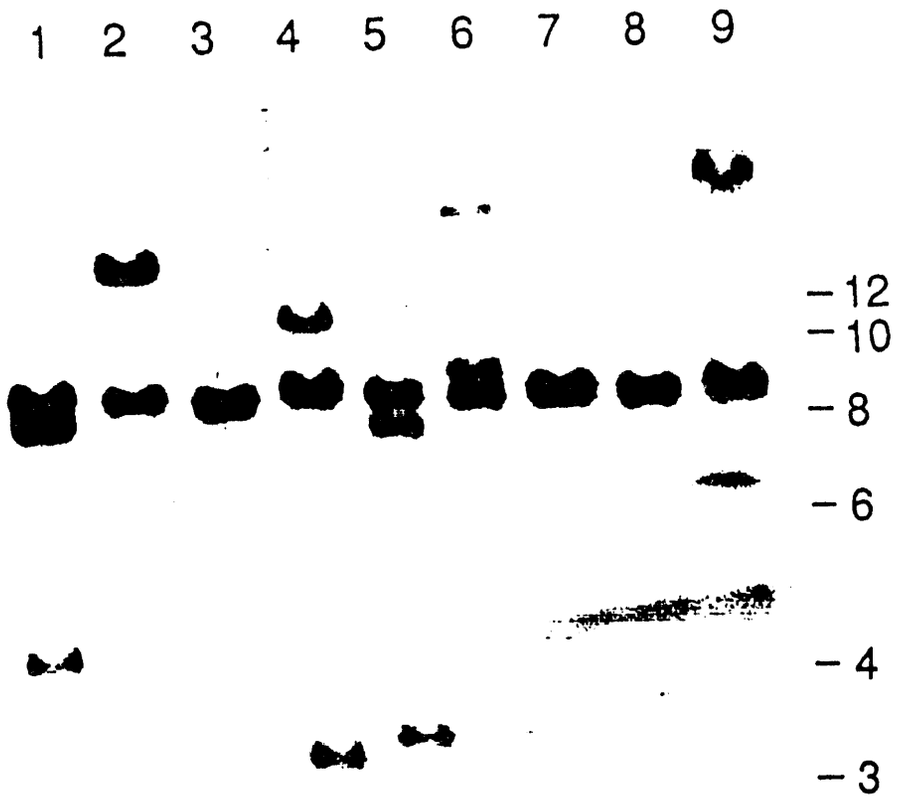


FIGURE 2

*Bam*HI digested DNA from patients with a t(11;19) lanes 1-6, or with a t(6;11) lanes 8-9, probed with the *MLL* 0.7kb *Bam*HI cDNA. Lane 7 is the control. Lanes 1 and 4-6 have two rearranged bands whereas lanes 2, 3, 8 and 9 have a single rearranged band indicating a deletion of *MLL* telomeric to the breakpoint.

This series of translocations involving the *MLL* gene has been a major focus of our group this year. The gene is also called *ALL1* (Croce-Canaani, Gu, et al., Cell, 71:701, 1992), *Htrx* (G. Evans, Diabali, et al., Nature Genetics, 2:113, 1992)), and *HRX* (M. Cleary, Tkachuk, et al., Cell, 71:691, 1992). In fact we have published ten papers, including eight primary research papers in 1993 or 1994. In September, 1993, we published the largest series of patients with 11q23 aberrations.(Thirman, et al., *Op.cit.*)

2. Analysis of 11q23 breakpoints in t-AML patients

We have used the same strategy to examine for *MLL* rearrangements in patients with AML whose cancers were treated with various drugs, especially the epipodophylltoxins. Five to 15% of patients treated with chemotherapy for a primary neoplasm develop therapy-related AML (t-AML) which may show rearrangements, usually translocations involving band 11q23, or less often, 21q22. These leukemias develop after a relatively short latent period and often follow the use of drugs that inhibit the activity of DNA-topoisomerase II (topo II). We have studied 17 patients with t-MDS/t-AML, 12 of whom had cytogenetically detectable 11q23 rearrangements. Ten of the 12 t-AML patients had received topo II inhibitors and nine of these, all with balanced translocations of 11q23, had *MLL* rearrangements on Southern blot analysis. None of the patients who had not received topo II inhibitors showed an *MLL* rearrangement. Of the five patients lacking 11q23 rearrangements, some of whom had monoblastic features, none had an *MLL* rearrangement, although four had received topo II inhibitors. Our study indicates that the *MLL* gene rearrangements are similar in AML which develops *de novo* and in t-AML. The association of exposure to topo- reactive chemotherapy with 11q23 rearrangements involving the *MLL* gene in t-AML suggests that topo II may play a role in the aberrant recombination events that occur in this region in both AML *de novo* and in t-AML.(Super, et al., Blood, 82:3705-3711, 1993) The involvement of *MLL* in t-AML has been reported by others.(Hunger, et al., Blood, 81:3197-3203, 1993; Felix et al., Cancer Res, 53:2954, 1993)

3. Cloning the breakpoints in the t(6;11), t(9;11) and t(11;19)

It is of interest (and a surprise to me) that all of the translocation junctions that have been cloned thus far lead to fusion genes. Using first cytogenetic data(Rowley, Genes Chromosomes and Cancer, 5:264-266, 1992) and more recently the evidence that *MLL* is deleted 3′ of the breakpoint,(Thirman, et al., New Engl J Med, and ASH abstract, 1993) we have shown quite convincingly that the critical genetic event is the juxtaposition of 5′ sequences on *MLL* with 3′ sequences of the various partner genes on the derivative 11 chromosome.(Figure 1,2) These 3′ deletions usually result in the loss of the zinc finger region and the remainder of the *MLL* gene and they can often be as large as several megatoses.

a. Cloning the t(6;11)

With probes that we have used to map other 11q23 translocations with FISH, we showed that the rearrangements in the ML-2 cell line involved both chromosomes 11 including the *MLL* gene. We showed that one chromosome 11 is involved in a heretofore unrecognized translocation with chromosome 6. The break in *MLL* is

just telomeric to exon 6. We cloned the unknown sequences on chromosome 6 using reverse-anchor-PCR.(Corral, et al., Proc Natl Acad Sci, 90:8538-8542, 1993) The breakpoint in the *AF-6* gene is in the same position as reported on by Prasad, et al., Cancer Research, 53:5624, 1993. With our FISH analysis we have shown that there is a deletion of part of *MLL* on the other chromosome 11. Our present mapping data indicate that the break may be 3′ of exon 5 of *MLL*. These data indicate that there is no normal copy of *MLL* in this cell line. As soon as we complete the mapping of the 5′ boundary of the *MLL* deletion we will prepare this paper for publication. ML-2 will be a very useful cell line for our functional studies of the MLL protein. The FISH analysis was done by Dr. Hirofumi Kobayashi and the cloning was done by Dr. Satoru Tanabe, working with Dr. Nancy Zeleznik-Le.

b. Cloning of the t(9;11)

This research is the Ph.D. thesis project of Heidi Gill Super. Using frozen material from one of our patients, Heidi prepared a genomic library which she screened with the *MLL* 0.74 kb cDNA probe. Among the clones she obtained were several that contained material from chromosome 9 because they gave a positive signal on 9 using a filter we had of a somatic cell hybrid panel. With the probe from chromosome 9, she has isolated several cDNA clones that are positive and she has sequenced these. She is using these probes to map the breakpoint on chromosome 9 in a number of patients with t(9;11). It is of interest that this gene, called *AF-9* has been shown to be rearranged in only two of the five patients reported on by Nakamura, et al., Proc Natl Acad Sci, 90:4631-4635, 1993). However, using RT-PCR, Yamamoto, et al. could amplify a fusion message in two cell lines and in four of six patients with a t(9;11).(Blood, 83:2912-2921, 1994)

Because the t(9;11) is common in t-AML, we are especially interested in mapping the genomic breakpoints in these patients. We have the probes available to proceed with this analysis of the breakpoints in both *MLL* and *AF-9*.

c. Cloning the t(11;19)

There are two different breakpoint on chromosome 19 in band 19p13; the more common breakpoint is in 19p13.3 and is seen primarily in children with ALL. The gene at this breakpoint was identified independently by Tkachuk, et al., (Cell, 71:691-700, 1992) who called it *ENL* and by Yamamoto, et al., (Oncogene, 8:479-485, 1993) who called it *LTG19*. We have recently cloned the less common breakpoint at 19p13.1 which is involved in both AML and ALL in children and in adults. We developed a strategy to clone the region from a cDNA library using primer sequences from 5′ *MLL* and a known vector linked to random sequences as the antisense primer. The clones that contained the fusion transcript were isolated and these were used to screen a cDNA library. The largest cDNA was 2.8 kb and it was used for FISH as well as for sequencing. FISH showed that it mapped to 19p13.1 and sequencing revealed that it was a unique previously unreported gene. It has no homology to other partner genes cloned in *MLL* translocation junctions. A paper describing these results is presently being submitted.(Thirman, et al.)

4. Mapping of t(X;11) in the Karpas 45 cell line

Using FISH, we and others showed that the Karpas 45 cell line had a t(X;11)(q13;q23). We also found that *MLL* was rearranged on Southern blot analyses of DNA from Karpas 45. We detected at least two altered transcripts of *MLL* that are derived from the der(11) chromosome in this cell line using cDNA fragments that were centromeric and telomeric to the breakpoint.(McCabe, et al., Genes Chrom Cancer, 9:221-224, 1994)

On Northern analysis, the cDNA fragments detected the normal-sized *MLL* transcripts of 13 and 15 kb in the control cell lines, and to a lesser extent in the Karpas 45 cell line. However, two cDNA fragments, which span or are centromeric to the breakpoint junction, detected two additional altered transcripts (approximately 6 and 8 kb) of the *MLL* gene in the Karpas 45 cell line. As a more telomeric cDNA fragment did not hybridize to these two novel transcripts, we conclude that these transcripts are altered *MLL* transcripts coming from the derivative chromosome 11. Our studies also revealed that the altered transcripts of approximately 6 and 8 kb were very highly expressed relative to the normal transcripts. We are using this cell line for a number of our functional studies. We previously detected one altered *MLL* transcript coming from the derivative chromosome 11 in the RS4;11 cell line that has a t(4;11) (McCabe, et al., Proc Natl Acad Sci USA, 89:11794-11798, 1992), and this was confirmed by others.(Corral, et al., Proc Natl Acad Sci, 90:8538-8542, 1993; Parry, et al., Genes Chromosomes and Cancer, in press)

5. *MLL* rearrangement in twins

Rearrangements of chromosome band 11q23 are common in infant leukemias, comprising more than 70% of the observed chromosome abnormalities in leukemic children less than one year of age. We have detected an identical, clonal, non-constitutional rearrangement of the *MLL* gene in peripheral blood cells from a pair of female infant identical twins with ALL and a t(11;19)(q23;p13.3). It should be noted that the cytogenetic analysis done in another laboratory suggested that the abnormality was a deletion of 11q23. Using FISH and telomeric probes for 11q23, we could show that the correct analysis was t(11;19). The detection of non-identical *IGH* rearrangements suggests that the *MLL* rearrangement took place in a B-cell precursor or hematopoietic stem cell in one twin which was transferred *in utero* to the other fetus resulting in ALL with an identical aneuploid karyotype in both infants. We speculate that the other *MLL*-related infant leukemias may also develop *in utero*, and that the rearrangements may occur consistently in stem cells or early precursor cells, accounting for the mixed-lineage leukemia in infants.(Super, et al., Blood, 83:641-644, 1994)

6. Analysis of various samples with aberrations of 11q with FISH

Complimenting the molecular analyses just described, we have just completed a series of studies that have defined the nature of various structural aberrations involving 11q, most especially those that involve 11q23. These studies were carried out by Dr. Hirofumi Kobayashi, M.D. who returned to Japan in April.

First we mapped and ordered 17 cosmid, phage, and plasmid clones to chromosome 11, bands q22-q24, using FISH. We then analyzed four hematopoietic cell lines with 11q23 rearrangements, Karpas 45, SUP-T13, RC-K8, and Karpas 422, using these probes.(Figure 3) The studies showed that the translocation breakpoints of the Karpas 45 and SUP-T13 cell lines, which were derived from T cell malignancies, were located in the same breakpoint cluster region of the *MLL* gene as the RS4;11 cell line. We also found that the translocation breakpoint of the RC-K8 cell line was located telomeric to the *MLL* gene, and that the derivative 11 chromosome of the Karpas 422 cell line which had been thought to contain a t(4;11)(q21;q23), was in fact formed through a deletion and an inverted tandem repeat of this part 11q. Because these cell lines are used by scientists world wide for a number of molecular and biological studies, information regarding the correct karyotype of these lines is important.(Kobayashi, et al., Blood, 81:3027-3033, 1993)

We then used FISH to analyze cells from four patients who had acute leukemia or MDS who were thought on standard cytogenetic analysis to contain a del(11)(q23q25). Cells from all patients were shown to contain translocations which involved chromosome 6, t(6;11)(q27;q23) in three of them. Our data suggest that a large proportion of presumptive del(11)(q23) or del(11)(q23q25) chromosomes may represent previously unidentified translocations that can now be detected by FISH. Since publication of this paper we have identified six more samples that were erroneously called deletions rather than translocations. This was true for the twins, originally thought to have a del(11)(q23) who were found on FISH to have a t(11;19).(Kobayashi, et al., Genes Chrom Cancer, 7:204-208, 1993)

We studied 24 patients whose cells contained a variety of 11q23 rearrangements, including translocations, insertions, and an inversion using FISH with cosmid, phage, and plasmid probes mapped to 11q22-24. In 17 patients, the breakpoints of the common 11q23 translocations involving chromosomes 4, 6, 9, and 19 as well as some uncommon translocations involving 3q23, 17q25, 10p11, and an insertion 10;11 were all located in the breakpoint cluster region of the *MLL* gene,(Figure 1C) regardless of age, phenotype of disease, or involvement of a third chromosome.(Tables 2-5) The breakpoints in 11q23 in the other seven patients with a t(7;11)(p15q23), inv(11)(p11q23), t(4;11)(q23;q23), der(5)t(5;11)(q13;q23), ins(10;11)(p11;q23q24), t(11;14)(q23;q11), or t(11;18;11)(p15;q21;q23) were located either centromeric to *CD3D* or telomeric to *THY1*.(Tables 3,5) Thus, although most 11q23 rearrangements involve the same breakpoint cluster region of *MLL*, there is heterogeneity in the breakpoint in some of the rare rearrangements. Distinguishing among the translocations that involve *MLL* from those that do not is important. In the future, treatment is likely to be different for rearrangements involving *MLL* from that used for other patients. Based on our total series of patients, it appears that *MLL* is involved in at least 80 to 90% of 11q23 translocations.(Kobayashi, et al., Blood, 82:547-551, 1993)

Deletions of 11q23 are very common in patients with hematopoietic diseases. We were curious whether *MLL* was also the target of these deletions. Cytogenetic analysis of 28 patients and of a cell line showed that all deletions included band 11q23. FISH analysis demonstrated that the proximal part of 11q23, including *NCAM*, was deleted in 14 of 16 patients.(Figure 4) These results

TABLE 2

Clinical, cytogenetic, and FISH data of the 17 patients with translocation breakpoints located between *CD3D* and clone 14.

Patient number	Age,yr/ sex	Hematologic disease	Partial karyotype	Localization of	
				CD3D	clone 14
1#	19/M	ALL	der(4)t(4;11;19)(q21;q23;q13), der(11)t(4;11;19)(q21;q23;q13) del(11)(q1?4q2?3), der(19)t(4;11;19)(q21;q23;q13)	der(11)	deleted
2#	48/M	AML	t(6;11)(q27;q23)	der(11)	deleted
3a#	7/M	AML	t(6;11)(q27;q23)	der(11)	deleted
4a#	14/M	ALL	t(6;11)(q27;q23),i(17)(q10), add(12)(p12)	der(11)	der(6)
5	4/M	AML-M5A	t(9;11)(p22;q23)	der(11)	der(9)
6	29/M	AML-M5	t(9;11)(p22;q23)	der(11)	der(9)
7a#	44/M	AML-M1	t(9;11)(p22;q23)	der(11)	deleted
8#	34/M	AML-M5A	t(9;11)(p22;q23)	der(11)	der(9)
9	31/M	AML-M5A	t(1;9;11;21)(p34;p22;q23;q22.3), del(1)(p34p36)	der(11)	der(21)
10	2/M	AML-M5	t(9;11;13)(p22;q23;q34)	der(11)	der(13)
11#	37/M	AML-M4	t(11;19)(q23;p13.3)	der(11)	der(19)
12	0.3/F	AML-M2	t(11;19)(q23;p13.3)	der(11)	der(19)
13	32/M	AML-M4	t(11;19)(q23;p13.1)	der(11)	der(19)
14	62/F	CLL	t(3;11)(q23;q23),+12	der(11)	der(3)
15	55/F	RAEB	t(11;17)(q23;q25)	der(11)	der(17)
16#	0.4/M	AML-M4	t(10;11)(p11;q23)	der(11)	der(10)
17#	2/M	AML-M5	inv ins(10;11)(p11.2;q23q13), del(1)(p34p36),-9,t(11;15)(q13;q2?3)	der(10)	der(11)

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; RAEB, refractory anemia with excess blasts.

[a] Karyotypes of patients 3, 4 (Kobayashi et al.),[7] and 7 (Rowley et al.)[3] were previously reported.

#*MLL* gene rearranged on Southern blot analysis.

TABLE 3

Clinical and cytogenetic features of patients with uncommon 11q23 translocation breakpoints not involving *MLL*.

Patient number	Age, yr/sex	Hematologic disease	Stage	Karyotype
18	68/F	CML	Blast crisis	46,XX,**t(7;11)(p15;q23)**,t(9;22)(q34;q11) [18]/ non-clonal abnormal cell [1]/ 46,XX[1]
19	30/F	CML	Chronic phase	46,XX,t(9;22)(q34;q11) [15]/ 46,XX,t(9;22)(q34;q11),**inv(11)(p11q23)** [6]/ 46,XX [1]
20	51/F	AML	Diagnosis	43,X,-X,-3,t(3;6)(q23;p25),**t(4;11)(q23;q23)**,der(5)t(X;5)(p11;q15),del(6)(p21p25),der(16)t(?3;16)(p21;p13),der(17)t(?3;17)(q25;q21)dic(17;22)(p13;q13),-22 [1]/ 43,idem,add(21)(q22) [10]/ 45,idem,+2mar [2]/ 45,idem, der(16)ins(16;?)(p13;?),+2mar [4]/ non-clonal abnormal cells [2]/ 46,XX [2]
21	47/M	t-MDS	Diagnosis	46,XY,add(3)(p13),**der(5)t(5;11)(q13;q23)** [19]/ 46,XY [2]
22	4/M	AML-M5	Diagnosis	46,XY,**ins(10;11)(p11;q23q24)** [14]/ 46,XY [1]
23[a]	22/F	ALL	Diagnosis	46,-X,add(X)(q2?8),del(7)(q21q36),t(10;14)(q24;q32), **t(11;14)(q23;q11)** [18]/ 46,XX [11]
24	65/F	NHL	Diagnosis	49,X,t(X;1)(p2?1;p3?1),+5,del(9)(q12q22),**t(11;18;11)(p15;q21;q23)**, del(13)(q12q14),+del(13)(q12q14),add(14)(q32),der(15)t(1;15)(q11;p11), +der(18)t(11;18)(p15;q21) [6]/ 48,X,t(X;1),+11,del(13),add(14), der(15)t(1;15),+mar [4]/ non-clonal abnormal cells [2]/ 46,XX [7]

CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; t-MDS, therapy-related myelodysplastic symdrome; NHL, non-Hodgkin's lymphoma.

[a] Karyotype of patient 23 was described previously.[4,16]

TABLE 4

Mapping of 11q deletions in patients whose cells showed only one rearranged band on Southern blot analysis with the 0.7 kb cDNA probe of *MLL*.

Probe order	Patient number			
	1	2	3	7
cen				
CD3D	der(11)	der(11)	der(11)	der(11)
14	deleted	deleted	deleted	deleted
	→			
9.12	der(19)	deleted	deleted	deleted
		→		
XB1	der(19)	der(6)	deleted	deleted
XH5			deleted	deleted
			→	
9.4			der(6)	deleted
PBGD		der(6)	der(6)	deleted
THY1			der(6)	deleted
				→
SRPR	der(19)		der(6)	der(9)
ETS1		der(6)	der(6)	der(9)
tel				

An arrow shows a telomeric end of each deletion.

Some FISH data from patients 3 (Kobayashi et al.)[7] and 7 (Rowley et al.)[3] were described previously.

TABLE 5 Mapping of the chromosomal rearrangements in patients with uncommon 11q23 rearrangements not involving *MLL*.

Probe order	Patient number						
	18	19	20	21	22	23	24
cen							
3.16	der(11)	inv(11q)	der(11)	(-)[b]	der(11)	der(11)	der(11q)
23.20	der(11)	inv(11q)	der(11)	(-)[b]	der(11)	der(11)	der(11q)
	→	→			→		
NCAM	der(7)	deleted[a]	der(11)	(-)[b]	der(10)	der(11)	der(11q)
		→	→	→			
4.13	der(7)	inv(11p)	der(4)	der(5)	der(10)	der(11)	der(11q)
1.16	der(7)	inv(11p)	der(4)	der(5)	der(10)	der(11)	der(11q)
CD3D	der(7)	inv(11p)	der(4)	der(5)	der(10)	der(11)	der(11q)
14		inv(11p)				der(11)	der(11q)
XB1	der(7)	inv(11p)	der(4)	der(5)	der(10)	der(11)	der(11q)
XH5		inv(11p)	der(4)		der(10)	der(11)	der(11q)
9.4	der(7)	inv(11p)	der(4)	der(5)		der(11)	
PBGD	der(7)	inv(11p)	der(4)	der(5)		der(11)	der(11q)
THY1			der(4)		der(10)	der(11)	der(11q)
L121	der(7)			der(5)	der(10)	der(11)	der(11q)
					→	→	
SRPR	der(7)	inv(11p)	der(4)	der(5)	der(11)	der(14)	der(11q)
ETS1		inv(11p)	der(4)			der(14)	der(11q)
							→
5.8	der(7)	inv(11p)	der(4)	der(5)	der(11)	der(14)	der(11p)
tel							

An arrow shows the breakpoint of each rearrangement.

[a] *NCAM* was deleted from the inv(11) chromosome. [b] 3.16 through *NCAM* were not located on the der(5) chromosome, whereas these probes hybridized to both the normal 11 chromosomes.

The *MLL* gene and the 11q23 breakpoint of the RC-K8 cell line were located between *CD3D* and clone 14 and between clone 14 and XB1, respectively.[6]

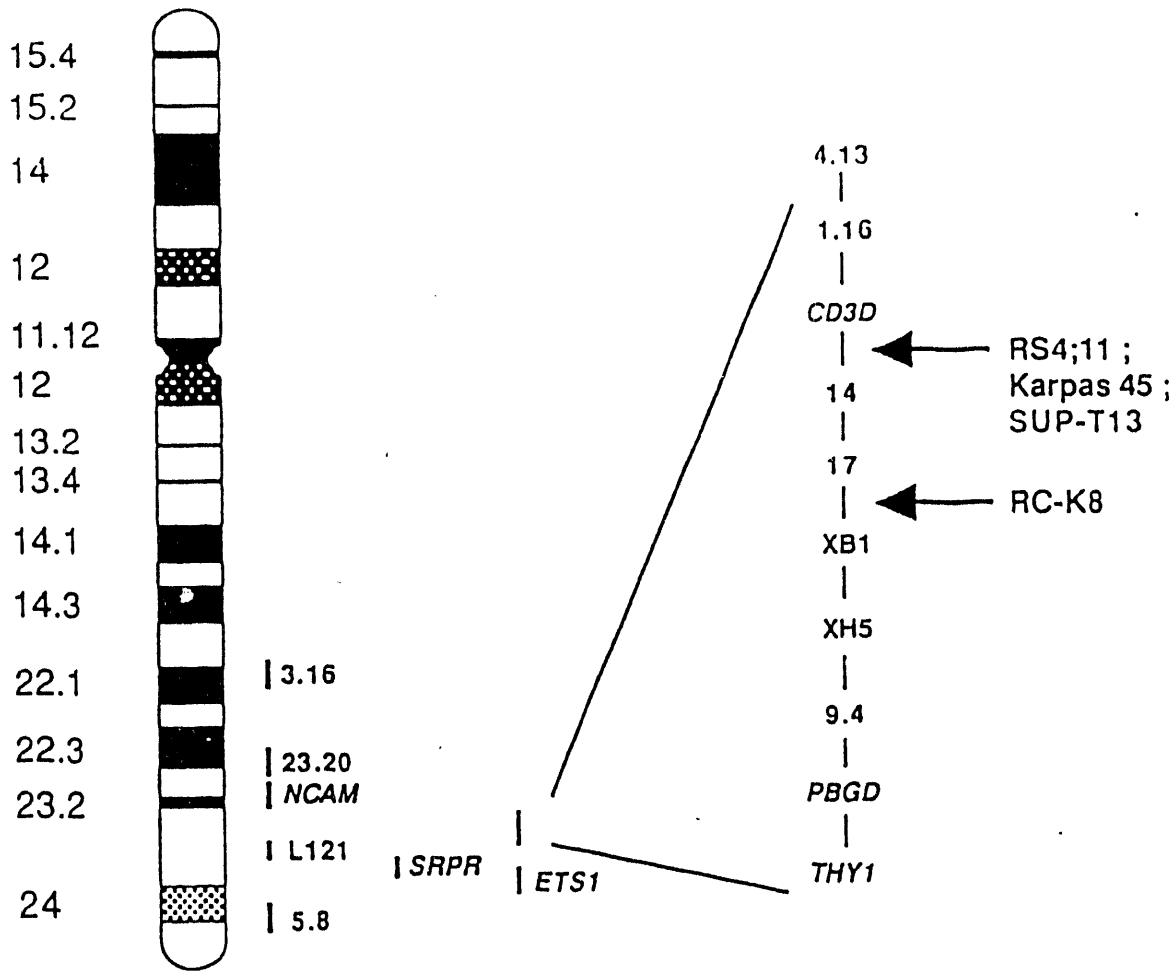


FIGURE 3

A diagrammatic summary of the mapping data for 17 probes used in this study. The vertical lines indicate the approximate location of each probe. The breakpoints in the Karpas 45, SUP-T13, and RS4;11 cell lines and the RC-K8 cell line are identified by horizontal arrows.

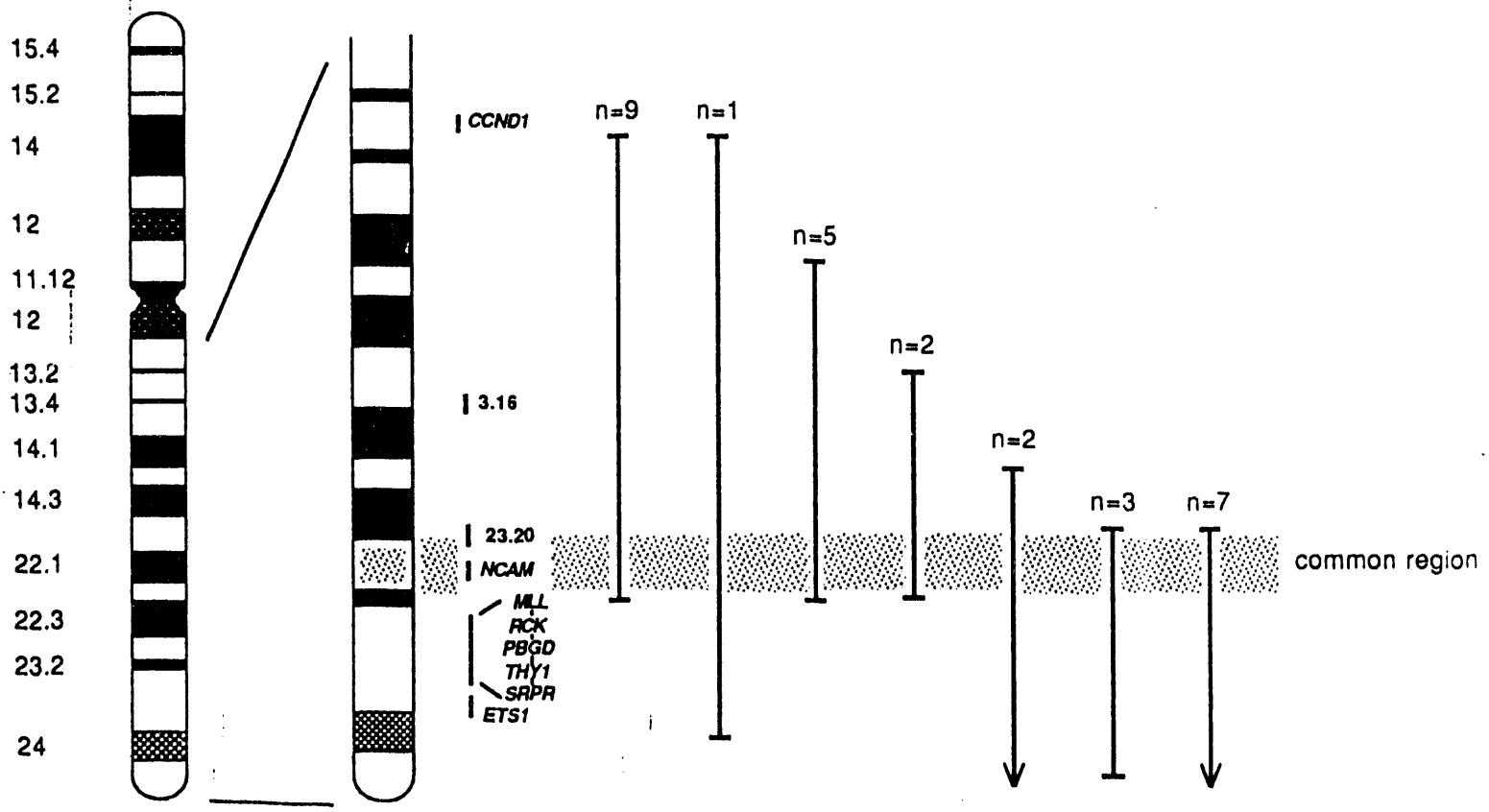


FIGURE 4

Schematic representation of 11q deletions. The approximate location of each probe is indicated by the vertical lines next to the chromosome. The long vertical lines indicate the extent of the various deletions based on cytogenetic analysis; the numbers at the top of the bars indicate the number of patients with each deletion.

suggest that the putative tumor-suppressor gene is proximal to the *MLL* gene which is also located in 11q23.(Kobayashi, et al., Genes Chrom Cancer, 8:246-252, 1993) I have recently written a review of the work published on *MLL* as of June, 1993. A reprint of the article in *Seminars in Cancer Biology*(Rowley JD, 4:377-385, 1994) which tries to put our work in perspective relative to the contributions of other laboratories is included.

b. Clone the t(3;21)(q26;q22) breakpoints

This is a new research project for the laboratory which I think has very exciting implications related to mutagenic agents and leukemogenesis. All of our t-AML patients with 21q balanced translocations have had a 3;21 translocation with breakpoints in 3q26 and 21q22. We have not seen this translocation in any of our patients with AML de novo; a review of the published literature also indicates that this is extremely rare in de novo AML. We have been collaborating with Dr. Harry Drabkin, University of Colorado Medical Center. He isolated probes from a YAC containing DNA from chromosome 21 and we showed that the probes were split in the t(8;21).(Gao, et al., Proc Natl Acad Sci, 88:4882-4886, 1991) Using this information he cloned the breakpoint in the *AML1* gene on chromosome 21 in the 8;21 [t(8;21)(q22;q22)] translocation. The t(8;21) occurs in about 40% of karyotypically abnormal patients with AML-M2 (myeloblastic with maturation). The *AML1* gene is transcribed from telomere to centromere. The translocation results in a fusion on the der(8) chromosome of the 5′ part of *AML1* and the 3′ part of *ETO* on chromosome 8.(Figure 5)(Erickson, et al., Blood, 80:1825-1831, 1992)

AML1 belongs to a new family of heterodimeric transcription regulatory proteins with important roles in processes ranging from segmentation in *Drosophila* to hematopoiesis and leukemia in humans. The three members of this family are AML1, Pebp2αA (mouse) and runt in *Drosophila*. They share a 128 amino acid domain called runt which is a DNA binding protein,(Figure 6) which also has a region for heterodimerization. The AML1 protein is 250 amino acids. Northern blot analysis shows several size transcripts from 8 to 2 kb presumably due to differential splicing. The breakpoint in *AML1* occurs just 3′ of the runt homology domain. It is of interest that the protein that binds to AML1 to form a heterodimer is *CBFB* (core binding factor beta) which is involved in an inversion or translocation affecting chromosome 16 in acute myelomonocytic leukemia with abnormal eosinophils.(Liu, et al., Science 261:1041-1044, 1993; Claxton, et al., Blood 83:1750-1756, 1994)

We used the same DNA probes from chromosome 21 isolated by Dr. Drabkin to analyze the t(3;21). We could show with FISH that these same probes were split by the breakpoint in the t(3;21). We found that *AML1* is also rearranged in the t(3;21).(Figure 7) We have samples of bone marrow from five leukemic patients with CML or t-MDS/t-AML with the t(3;21) which were frozen to maintain cell viability; several hundred million cells are available for each patient. The thawed cells are 90-95% viable, and contain intact DNA and RNA, suitable for construction of genomic DNA libraries, for preparation of RNA for Northern blot analysis, and for RT-PCR of cDNA libraries.

Diagram of AML1/ETO Fusion
in 8;21 Translocation

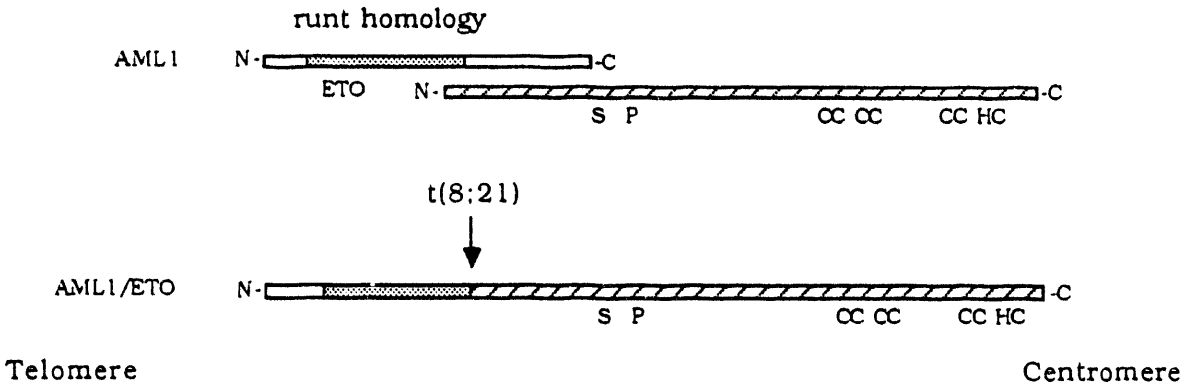


FIGURE 5

Diagram of the fusion gene in the 8;21 translocation. The break in *AML1* is just telomeric to the runt homology region; the break in *ETO* is in the first 27 nucleotides. The fusion gene is in frame.

Relationship of Runt Homology Region in Various Proteins

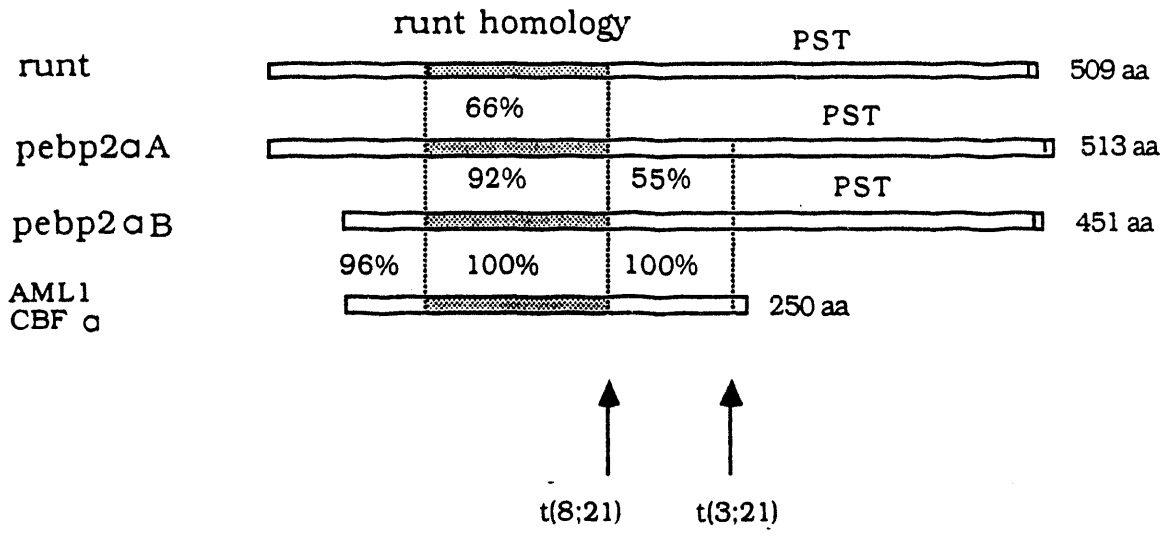


FIGURE 6

Diagram showing the location of the runt homology domain in the various members of the family. The percentages between each gene shows the degree of homology between the different family members. Runt is the gene in *Drosophila*, pebp 2*α*A and *α*B are murine genes and *AML1* (or *CBFA*, its other name) is the human gene.

Location of Translocation Breakpoints in *AML1* Gene

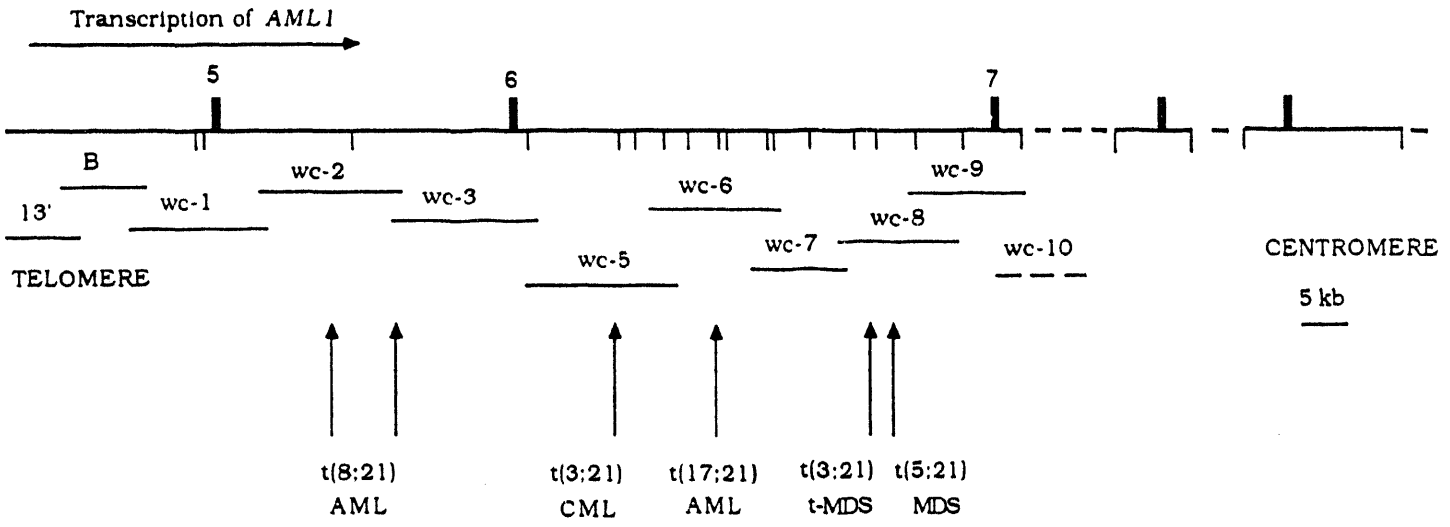


FIGURE 7

*Bam*HI restriction map of chromosome 21 in the region containing the breakpoints of the t(8;21), t(3;21), t(5;21) and t(17;21). The translocation breakpoints are indicated by vertical arrows. The vertical bars below the horizontal line indicate the *Bam*HI sites. The black boxes above the horizontal line indicate numbered *AML1* exons. The direction of transcription of *AML1* is indicated by a horizontal arrow. The recombinant phage clones 13′ through wc-10 are indicated by horizontal lines below the map. Wc-10 and genomic regions indicated by a dashed line have not been mapped.

We screened a cDNA library made from malignant cells of a t-MDS patient with the t(3;21) using an *AML1*-specific probe, and isolated several cDNA clones that hybridized to DNA from a somatic cell hybrid containing chromosome 3 but not chromosome 21. After sequencing, we determined that the clones contained the 5′ region of *AML1* fused to a previously characterized gene *EAP*, which we localized to band 3q26.(Nucifora, et al., Proc Natl Acad Sci USA, 90:7784-7788, 1993) EAP is a highly expressed small protein of 129 residues associated with Epstein-Barr virus (EBV) small RNA and identified as the ribosomal protein L22. As is true for most ribosomal genes, *EAP* belongs to a family of pseudogenes, and Southern blot analysis of germline human DNA showed hybridization of an *EAP*-specific probe to at least 12 *Eco*RI bands varying in size from one to about 20kb. Northern blot analysis showed that *EAP* is expressed in all tissues and cell lines that we tested, and three transcripts of about 1, 1.5 and 2kb were detected. The 3;21 fusion clones contained the region of *AML1* that binds DNA, namely the *runt* homology region, that is fused to *ETO* in the t(8;21) and, in addition, it contained one other *AML1* exon. The 3′ end of *AML1* was replaced by the last 96 codons of *EAP* followed by about 0.9kb of *EAP* 3′ untranslated sequences. We determined that the fusion did not maintain the *EAP* reading frame and that translation of the fusion mRNA was terminated shortly after the *AML1/EAP* junction by an out of frame stop codon introduced by the *EAP* sequence. We suggested that most likely AML1/EAP was devoid of transactivation activity and could act as a constitutive repressor inhibiting the function of the normal protein.(Nucifora, Proc Natl Acad Sci USA, 90:7784-7788, 1993)

After we sequenced the other cDNA clones that hybridized to chromosome 3 DNA, we determined that one of the clones was completely divergent from *EAP* in the region downstream of *AML1*. This second fusion clone contained the same region of *AML1* found in the *AML1/EAP* cDNA fused to new sequences that we localized on chromosome band 3q26 with FISH. We named these sequences *MDS1* (for myelodysplasia syndrome). The region of *MDS1* that we cloned had no significant homology to any sequence deposited in GenBank; it was evolutionarily conserved, and it was represented only once in the genome. The Northern blot analysis of adult human tissues indicated that *MDS1* was expressed only in kidney, lung and pancreas, with two major transcripts of about 5.6 and 6.2kb. We did not detect any transcript in hematopoietic tissues and B, T or myeloid cell lines. These results suggested that *EAP* and *MDS1* were unrelated genes. Whereas *AML1/EAP* produces a truncated form of AML1 consisting mostly of the DNA-binding region of the protein, the fusion of *AML1* with *MDS1* adds 127 residues to the DNA binding runt domain, with a high percentage of proline, serine and acidic residues, suggestive of a transcription regulation domain.(Nucifora, Proc Natl Acad Sci USA, 91:4004-4008, 1994)

To determine whether the production of two chimeric transcripts was an unusual event unique in this patient or a common outcome of the t(3;21), we analyzed peripheral blood samples of four additional t(3;21) patients, one with CML-BC and three with t-AML by RT-PCR; we used two sets of primers: one set specific for the *AML1/EAP* junction, and the second set specific for the *AML1/MDS1* junction. In all cases, we detected a band of the size expected with each set of primers used. By sequencing, we confirmed that the amplified fragments contained the chimeric *AML1/EAP* or *AML1/MDS1* junctions. In addition, small bands were also consistently amplified. These bands represented isoforms of the chimeric junctions involving *AML1* and they lacked exon 6 of *AML1*, as we

determined by sequencing the junctions. These results indicated that the production of two chimeric transcripts was common consequence of the 3;21 translocation and that two different chimeric mRNAs consistently co-existed in these patients. Figure 8 shows a diagram with the position of the primers used for amplification and the various chimeric junctions identified in the patients.

Chromosomal band 3q26 is also the location of *EVI1*, a gene whose transcription can be activated by translocation both upstream or downstream of the gene. The gene encodes a 145kD protein with seven zinc finger motifs at the N-terminal and three zinc finger motifs at the C-terminal. It is oriented on chromosome 3 such that the 5′ end is telomeric and the 3′ end is centromeric. The gene is essential for normal development of kidney, brain and peripheral nervous system. Inappropriate expression of the gene in fibroblasts and macrophages has no obvious effect, but in hematopoietic cells it blocks granulocytic and erythrocytic differentiation. It has been suggested that EVI1 through its zinc fingers at the N-terminal region might activate or repress promoters containing this motif, and thereby interfere with a family of factors which recognize the GATA motif and which have been implicated in the regulation of stem cell differentiation. The transcription factor GATA-1 which has been shown to be essential for erythroid differentiation.

Activation of *EVI1* has been implicated in the leukemogenesis of 7% of AML patients with abnormalities involving band 3q26. Only one of our t-AML patients with a t(3;21) expressed this gene, as determined by use of RT-PCR and *EVI1* primers for detecting the 3′ end of the mRNA. To determine whether *EVI1* was also expressed as a fusion gene with *AML1* in this patient, we designed primers from the published *EVI1* sequence to use with the *AML1* primer in RT-PCR. One of the *EVI1* primers amplified four major bands that were cloned and sequenced. The two smaller fragments contained the junction between *AML1* and either exon 2 or exon 3 of *EVI1*; the two larger fragments represented complex fusion clones, including *AML1* (plus or minus exon 6) fused to *MDS1*, which was fused to exon 2 of *EVI1*.(Figure 8) All of these fusion transcripts maintained the same reading frame, leading to complex fusion peptides.

To localize these involved genes on chromosome 3, we probed filters from pulsed-field gel electrophoresis provided to us by Dr. James Ihle, St. Jude's Children Hospital, Memphis, TN. Previous studies had shown that a CpG island exists 5′ of the *EVI1* gene which contains sites for *BSS*HII cleavage in the 5′ region of the *EVI1* gene. Both the *EAP* and *MDS1* probes were localized on the *BSS*HII fragment that is detected by a 5′ *EVI1* probe.(Figure 9) However, both probes detected an identical but novel *Sfi*I fragment of approximately 300kb. Therefore, both *EAP* and *MDS1* probes are at least 170kb telomeric of *EVI1*. Thus, the order of the probes from the telomere is *EAP-MDS1-EVI1*. We also used fluorescence in situ hybridization to confirm that *EVI1* was centromeric to *MDS1*.

It is generally accepted that, in most translocations associated with leukemia, the genes that are fused across the breakpoint are those directly modified in their expression or function, and thus are critically involved in leukemogenesis. There are only few examples of genes that are affected at a long distance by a translocation, and the effect is limited to altering the expression of the involved gene. These cases of the t(3;21) are unique in containing chimeric transcripts between *AML1* and unrelated genes located over a region of

Diagram of Various Transcripts in t(3;21) Patients

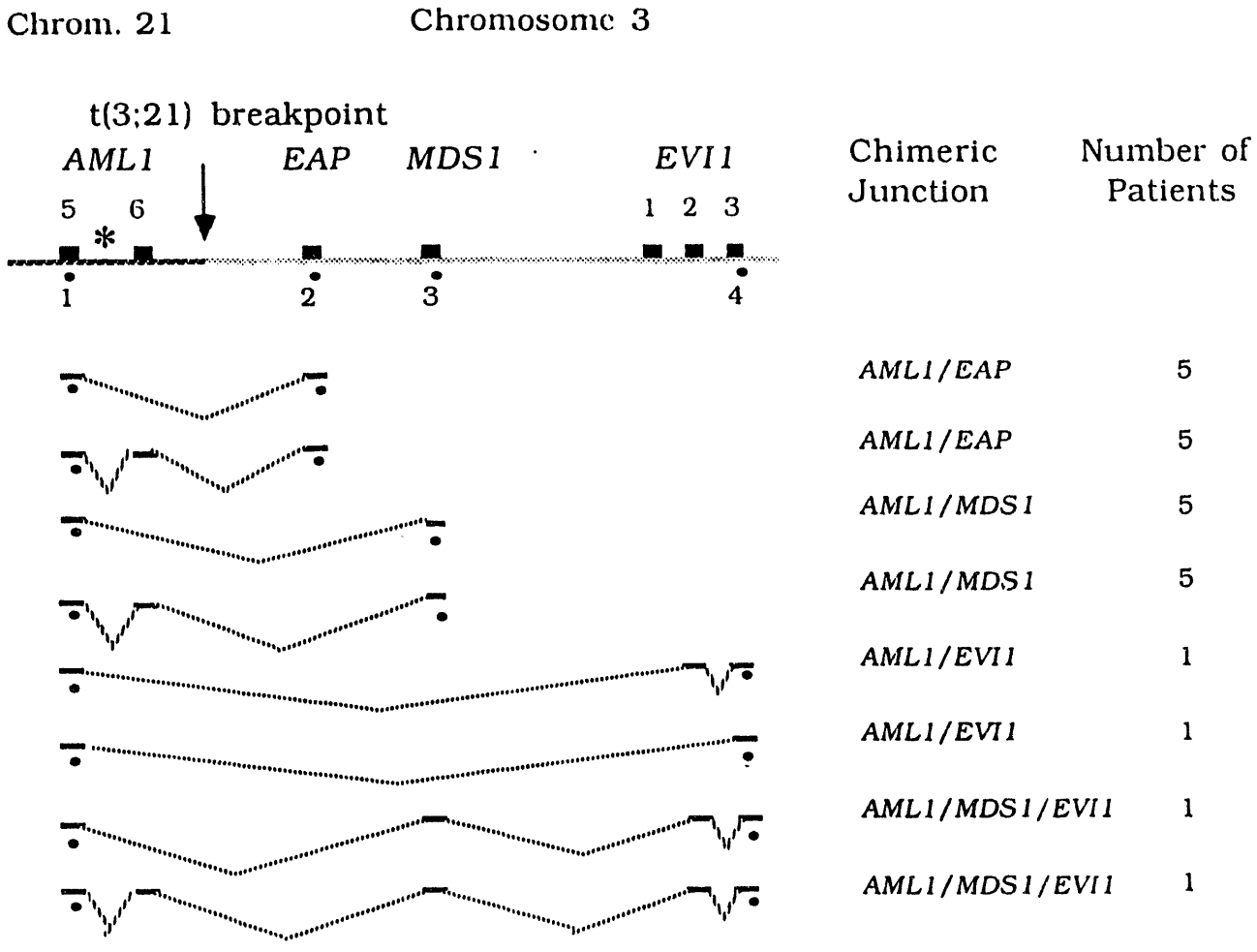


FIGURE 8

Figure 8 diagram illustrating the *AML1/EAP* and *AML1/MDS1* junctions detected by RT-PCR of the five t(3;21) patients, as well as the *AML1/EVI1* and *AML1/MDS1/EVI1* junctions detected in one of these patients. The chimeric junctions were confirmed by nucleotide sequencing. The heavily shaded portion of the horizontal line represents the translocated region of chromosome 21 containing the promoter and the 5′ region of *AML1*. The lightly shaded portion of the horizontal line represents the region of chromosome 3 containing the *EAP*, *MDS1* and *EVI1* probes. The vertical arrow indicates the genomic junction. The black boxes represent the exons involved in the chimeric junctions and identified by nucleotide sequencing; the numbers above the black boxes indicate the published numbering of the *AML1*. The asterisk between exons 5 and 6 of *AML1* indicates the intron where the breakpoints of the t(8;21) have been consistently detected. The black dots below the horizontal line indicate the position of the RT-PCR primers. The short black horizontal segments joined by the V-shaped dotted lines represent the exons amplified by PCR and sequenced. The two columns on the right side indicate the type of junction and the number of patients who had the chimeric junction.

Long Range Map of 3q26.2

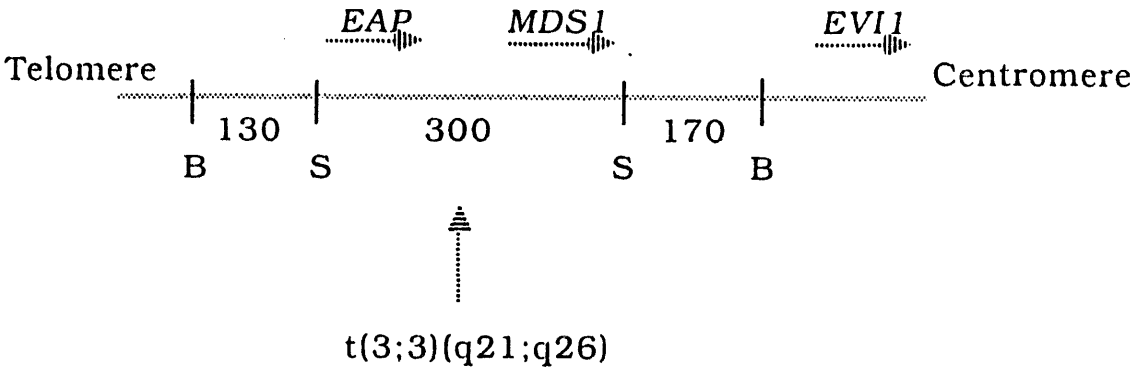


FIGURE 9

Illustration of the PFGE mapping results of the genes on chromosome 3. The horizontal line represents the region on 3q26 where the genes are located. The horizontal arrows indicate the direction of transcription. The short vertical bars indicate the restriction site for *BSS*HII (B) and *Sfi*I (S), and the numbers below the horizontal lines indicate the size of the restriction fragments. The dashed vertical arrow indicates the approximate position of the t(3;3) breakpoint used for mapping of the genes.

at least 400kb at 3q26. Although we have no direct evidence that *AML1* and the partner genes at 3q26 can be transcribed as a single unprocessed nuclear message, the identification of chimeric junctions containing the same exons of *AML1* individually spliced to exons from the other genes would suggest that such nuclear transcripts exist and undergo differential splicing. While 5′ RACE and S1 mapping have shown that in the *EVI1* locus the transcriptional start sites of *EVI1* are within 5kb upstream of exon 1, we cannot rule out that the *MDS1* sequences represent even more 5′ previously unreported exon(s) of *EVI1*.(Nucifora, et al., Proc Natl Acad Sci USA, 91:4004-4008, 1994)

Although the cases which we have examined contain complex fusion transcripts, the roles of the individual transcripts in leukemia are not known. The common occurrence of C-terminal truncations in *AML1* in the t(3;21) and t(8;21) strongly implicates alterations in this gene as an important etiologic component in these cases of AML. It is possible that the translocations also alter the chromosomal structure to allow the inappropriate expression of *MDS1* and *EVI1*. In the case of *MDS1*, we have found that this transcript is not normally expressed in myeloid cells, and therefore it will be important to determine whether *MDS1* is expressed in other cases of AML. In the one case in which we detected *EVI1* transcripts, levels of transcripts detected with internal probes were much higher than those detected with the probes for the fusion transcript. Therefore, in the case of the *EVI1* gene, transcriptional activation may occur at sites other than the *AML1* promoter.

That this complex fusion event is an important component of the leukemogenesis process is supported by the detection of multiple fusions in <u>all</u> of the patients studied. These patients had prior chemotherapy with adriamycin, a topo II inhibitor, either for a previous cancer or for CML in the chronic phase. The t(8;21) has also been observed in AML developing in patients previously treated for cancer. Therefore, *AML1* appears to be susceptible to mutagen-induced translocations.

C. Plans for continuation of present objectives and possible new objectives in consideration of past results

The long term goal of this research project has not changed. We continue to search for the answers to two major questions. (1) Why do patients who are treated with a variety of drugs, often combined with radiation, develop acute myeloid leukemia? (2) Are certain recurring chromosome changes in the leukemic cells of these patients related to the particular actions of these agents in the cells? If so, what is the mechanism relating the target of the drug to the pattern of chromosome change?

In the past, we concentrated on losses of chromosomes 5 and/or 7 because these were the most common recurring changes and they occurred at a high frequency especially in the leukemic cells of patients treated with high doses of alkylating agents, particularly when combined with radiation. As we have had an opportunity to study more patients and as the types of therapeutic agents used by physicians have changed, we are seeing the emergence of a new pattern of karyotypic changes. These aberrations consist of recurring balanced translocations that are usually associated with specific subtypes of acute leukemia de novo and are most common in younger patients. Rather ironically, we

used to comment specifically that these aberrations were very rarely seen in t-AML. The most significant factor contributing to this increased frequency appears to be the introduction of drugs that are topoisomerase II inhibitors, and especially the use of very high doses of these drugs.

Just as we were the first to show that losses of chromosomes 5 and/or 7 were a common feature of leukemia resulting from prior treatment with alkylating agents, we were also the first to suggest that high doses of etoposide (VP16), one of the epipodophyllotoxins, was associated with translocations involving chromosome 11 band q23.(Ratain, et al., Blood, 70:1412-1417, 1987) Not only is there an increased frequency of aberrations of chromosome 11, there is also an increase in 21q22 translocations involving either chromosome 8[t(8;21)(q22;q22)] or chromosome 3 [t(3;21)(q26;q22)]. Finally other recurring translocations, such as the t(15;17)(q21;q11) in acute promyelocytic leukemia (APL) or inv (16)(p13q22) in acute myelomonocytic leukemia with abnormal eosinophils (M4Eo) are also observed in patients previously treated with topoisomerase II inhibitors.(Pedersen-Bjergaard J., and Rowley, J.D, Blood, 83:2780-2786, 1994) This new form of t-AML also differs from the usual form in that it has a much shorter interval between treatment and leukemia, rarely has a preleukemic phase, and appears to have a higher response rate to treatment.

The unfortunate development of the greatly increased frequency of this new type of abnormality in t-AML may provide insights into the mechanism of leukemogenesis. We have concentrated until now on the loss of chromosomal material as a consequence of exposure to alkylating agents, focussing on the loss of all or of the long arm of chromosomes 5 and 7. In collaboration with Drs. Le Beau and Westbrook, we have mapped a number of genes that function as growth factors or their receptors to 5q23 to 5q33. We have worked diligently to develop a YAC contig in the common region of deletion and to identify probes to define translocation involving this region more precisely.

I will continue to collaborate on this project especially in trying to define the translocation breakpoint in chromosome 5 identified in one of three men from El Salvador who received large amounts of radiation from a cobalt^{60} source on February 5, 1989. An 18,000 Curie cobalt^{60} source became stuck in the raised position and one worker went to try to dislodge it. He was unsuccessful and got two other men to move the cobalt^{60} source but without success. It is estimated by Dr. Gayle Littlefield of Oak Ridge Associated Universities, based on her analysis of the chromosome aberrations in mitogen-stimulated peripheral lymphocytes, that the first man (Patient A) received an average dose of 8.2 Gy of whole body radiation, the second man (Patient B) received about 4.4 Gy, and the third man (Patient C) received about 3.2 Gy. (Details are published, Littlefield et al, Radiation Protection Dosimetry, 35:115-123,1991). I received bone marrow and peripheral blood from Patients A and B on July 28, 1989 - (day 175). Patient A, died in El Salvador in August of "viral pneumonia". No autopsy was obtained so whether the patient showed evidence of early leukemia is unknown. We received a second sample of bone marrow and peripheral blood on patients B and C in September 28, 1990, about 19 1/2 months after the accident.

The chromosome changes in the bone marrow cells of patient A are <u>precisely</u> those I would have expected from a mutagen-induced leukemia! He has a

translocation involving chromosome 5 with a break in or near to 5q31. The precise location of the breakpoint will require analysis using fluorescence in situ hybridization (FISH) of DNA probes located in 5q31 to 5q33. He also has an interstitial deletion involving loss of material from the long arm of chromosome 7, del(7)(q22q34). In addition, there is a ring chromosome involving chromosome 17. It is possible that the break in the short arm of chromosome 17 to form the ring chromosome might lead to a deletion of the *TP53* gene. Our tentative karyotype is 46,XY,t(1;3)(p36.1;p25),inv(3)(q12q27),der(5)t(3;5)(q27;q31or q33),del(7)(p13p22),del(7)(q22q34),t(8;14)(p12;q32),t(11;16)(p13;q12),ring(17)(p13q25). The precise localization of the breakpoint in the long arm of chromosome 5 will have a very high priority. My present interpretation of the marker 5 chromosome is as follows: 5pter→5q31::3q27→3q21::3q27→3qter.

These patients, especially Patient A who received greater than 8 Gy, provide a tragic but unique opportunity to study radiation-induced leukemia in man. I assume that similar cytogenetic findings would have been observed in Hiroshima and Nagasaki if cytogenetic analyses had been carried out on affected patients in the late 1940s and 1950s.

However, my major effort will be directed towards exploiting the new opportunities provided by patients who have the recurring abnormalities of 11q23 and 21q22. This is an especially opportune time to begin such a project because, as is described in the Progress Report, we have cloned the gene that is located in 11q23 that is involved in the 6;11, 9;11, and 11;19 translocations that are seen in both AML de novo as well as in t-AML. We already have evidence that the breakpoint in 11q23 in the t(6;11), t(9;11) and t(11;19) is similar in de novo and secondary AML. We have no information about the location of breakpoints in the gene involved on chromosome 9.

In addition, we have cloned the breakpoint in the t(3;21) and we are now in a position to begin studies determining the alterations in functions of the gene involved in the translocation. In fact, the progress we have made in cloning the *MLL* and *AML1* genes involved in the 11q23 and 21q22 translocations, respectively, will provide us with a unique opportunity to investigate the mechanism(s) involved in the genesis of this type of chromosome translocation. Thus *MLL* is involved in translocations that occur in leukemia generally associated with epipodophyllotoxin exposure whereas *AML1* is involved in translocations that occur in leukemias generally associated with prior anthracycline exposure. The questions are clear: What is the mechanism by which exposure to drugs that poison topoisomerase II leads to leukemias in which different topo II inhibitors appear to be associated with translocations involving different genes? How does this relate to the genesis of these translocations in novo leukemia? What is the most creative and effective way to analyze these mechanisms experimentally? Some approaches we are exploring will be described in the Specific Objectives for the Renewal Proposal 1995 to 1997.

D. Graduate Students

Bruce Porterfield; candidate for M.D. and Ph.D. degrees; the latter was received in December, 1993, and the former will be granted in June, 1995.

Sheryl Ziemin van der Poel; received Ph.D. degree in 1992.

Pamela Broeker; received Ph.D. degree in 1993.

Heidi Gill Super; expected to receive Ph.D. degree in 1995.

Susan Perry Gursky; expected to receive Ph.D. degree in 1996.

Because of budgetary constrains, only Bruce Porterfield has been supported with DOE funding.

E. Postdoctoral Fellows

Robert Burnett, Ph.D.; 1989 to 1992

Giuseppina Nucifora, Ph.D.; 1990 to 1993

Michael Thirman, M.D.; 1990 to 1993

Martin Dreyling, M.D.; 1992 to 1994

Jose Martinez-Climent, M.D.; 1992 to 1994

Hirofumi Kobayashi, M.D.; 1992 to 1994

Walter Stadler, M.D.; 1992 to 1994

F. Bibliography

1. Burnett, R.C., David, J.C., Harden, A.M., Le Beau, M.M., Rowley, J.D., Diaz, M.O. The *LCK* gene is involved in the t(1;7)(p34;q34) in the T-cell acute lymphoblastic leukemia derived cell line, HSB-2. Genes Chrom Cancer, 3:461-467, 1991.

2. Park, J.K., Le Beau, M.M., Shows, T.B., Rowley, J.D., Diaz, M.O. A complex genetic rearrangement in a t(10;14)(q24;q11) associated with T-cell acute lymphoblastic leukemia. Genes Chrom Cancer, 4:32-40, 1992.

3. Ziemin-van der Poel, S., McCabe, N.R., Gill, H.J., Espinosa III, R., Patel, Y.D., Harden, A.M., Le Beau, M.M., Smith, S.B., Rowley, J.D., Diaz, M.O. Identification of a gene (*MLL*) which spans the breakpoint in 11q23 translocations associated with human leukemias. Proc Natl Acad Sci USA, 88:10735-10739, 1991.

4. Rubin, C.M., Arthur, D.C., Woods, W.G., Lange, B.J., Nowell, P.C., Vardiman, J.W., Rowley, J.D., Bostrom, B., Nachman, J., Baum, E.S., Suarez, C.R., Shah, N.R., Morgan, E., Maurer, H.S., Larson, R.A., Le Beau, M.M. Therapy-related myelodysplastic syndrome and acute myeloid leukemia in children: Abnormalities of chromosomes 5 and 7 are common. Blood, 78:2982-2988, 1991.

5. Rubin, C.M., Le Beau, M.M., Mick, R., Bitter, M.A., Nachman, J., Rudinsky, R., Appel, H., Morgan, E., Suarez, C.R., Schumacher, H.R., Subramanian, U., Rowley, J.D. Impact of chromosomal translocation on prognosis in childhood acute lymphoblastic leukemia. Jour Clin Oncol, 9:2183-2192, 1991.

6. Neuman, W.L, Rubin, C.M., Rios, R.B., Larson, R.A., Le Beau, M.M., Rowley, J.D., Vardiman, J.W., Schwartz, J.L., Farber, R.A. Chromosomal loss and deletion are the most common mechanisms for loss of heterozygosity from chromosomes 5 and 7 in malignant myeloid disorders. Blood, 79:1501-1510, 1992.

7. Ratain, M.J., Rowley, J.D. Therapy-related acute myeloid leukemia secondary to inhibitors of topoisomerase II: From the bedside to the target genes. Annals Oncol, 3:107-111, 1992.

8. Larson, R.A., Le Beau, M.M., Ratain, M.J., Rowley, J.D. (Letter to the Editor) Balanced translocations involving chromosome bands 11q23 and 21q22 in therapy-related leukemia. Blood, 79:1892-1893, 1992.

9. Erickson, P., Gao, J., Chang, K.S., Look, T., Whisenant, E., Lasher, R., Trujilo, J., Rowley, J.D., Drabkin, H.A. Identification of breakpoints in t(8;21) acute myelogenous leukemia and isolation of a fusion transcript, with similarity to *Drosophila* segmentation gene, *runt*. Blood, 80:1825-1831, 1992.

10. Rowley, J.D. The der(11) chromosome contains the critical breakpoint junction in the 4;11, 9;11, and 11;19 translocations in acute leukemia. Genes Chrom Cancer, 5:264-266, 1992.

11. Bohlander, S.K., Espinosa III, R., Le Beau, M.M., Rowley, J.D., Diaz, M.O. A method for the rapid sequence-independent amplification of microdissected chromosomal material. Genomics, 13:1322-1324, 1992.

12. Olopade, O.I., Thangavelu, M., Larson, R.A., Mick, R., Kowal-Vern, A., Schumacher, H.R., Le Beau, M.M., Vardiman, J.W., Rowley, J.D. Clinical, morphologic, and cytogenetic characteristics of 26 patients with acute erythroblastic leukemia. Blood, 80:1-9, 1992.

13. McCabe, N.R., Burnett, R.C., Gill, H.J., Thirman, M.J., Mbangkollo, D., Kipiniak, M., van Melle, E., Ziemin-van der Poel, S., Rowley, J.D., Diaz, M.O. Cloning of cDNAs of the *MLL* gene that detect DNA rearrangements and altered RNA transcripts in human leukemic cells with 11q23 translocations. Proc Natl Acad Sci USA, 89:11794-11798, 1992.

14. Thirman, M.J., Gill, H.J., Burnett, R.C., Mbangkollo, D., McCabe, N.R., Kobayashi, H., Ziemin-van der Poel, S., Kaneko, Y., Morgan, R., Sandberg, A.A., Chaganti, R.S.K., Larson, R.A., Le Beau, M.M., Diaz, M.O., Rowley, J.D. Rearrangement of the *MLL* gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. New Engl J Med, 329:909-914, 1993.

15. Nucifora, G., Birn, D.J., Erickson, P., Gao, J., Le Beau, M.M., Drabkin, H.A., Rowley, J.D. Detection of DNA rearrangements in the *AML1* and *ETO* loci and of an *AML1/ETO* fusion mRNA in patients with t(8;21) AML. Blood, 81:883-888, 1993.

16. Anastasi, J., Feng, J., Le Beau, M.M., Larson, R.A., Rowley, J.D., Vardiman, J.W. Cytogenetic clonality in myelodysplastic syndromes studied with fluorescence *in situ* hybridization: Lineage, response to growth factor therapy, and clone expansion. Blood, 81:1580-1585, 1993.

17. Burnett, R.C., Espinosa III, R., Shows, T.B., Eddy, R.L., Le Beau, M.M., Rowley, J.D., Diaz, M.O. Molecular analysis of a t(11;14)(q23;q11) from a patient with null-cell acute lymphoblastic leukemia. Genes Chrom Cancer, 7:38-46, 1993.

18. Kobayashi, H., Espinosa III, R., Thirman, M.J., Davis, E.M., Diaz, M.O., Le Beau, M.M., Rowley, J.D. Variability of 11q23 rearrangements in hematopoietic cell lines identified with fluorescence *in situ* hybridization. Blood, 11:3027-3033, 1993.

19. Nucifora, G., Birn, D.J., Espinosa III, R., Erickson, P., Le Beau, M.M., Roulston, D., Drabkin, H.A., Rowley, J.D. Involvement of the *AML1* gene in the t(3;21) in therapy-related leukemia and in chronic myeloid leukemia in blast crisis. Blood, 81:2728-2734, 1993.

20. Kobayashi, H., Espinosa III, R., Thirman, M.J., Fernald, A.A., Shannon, K., Diaz, M.O., Le Beau, M.M., Rowley, J.D. Do terminal deletions of 11q23 exist? Identification of undetected translocations with fluorescence in situ hybridization. Genes Chrom Cancer, 7:204-208, 1993.

21. Kobayashi, H., Espinosa III, R., Thirman, M.J., Gill, H.J., Fernald, A.A., Diaz, M.O., Le Beau, M.M., Rowley, J.D. Heterogeneity of breakpoints of 11q23 rearrangements in hematologic malignancies identified with fluorescence *in situ* hybridization. Blood, 82:547-551, 1993.

22. Kobayashi, H., Espinosa III, R., Fernald, A.A., Begy, C.R., Diaz, M.O., Le Beau, M.M., Rowley, J.D. Analysis of deletions of the long arm of chromosome 11 in hematologic malignancies with fluorescence *in situ* hybridization. Genes Chrom Cancer, 8:246-252, 1993.

23. Nucifora, G., Larson, R.A., Rowley, J.D. Persistence of the 8;21 translocation in patients with AML-M2 in long-term remission. Blood, 82:712-715, 1993.

24. Nucifora, G., Begy, C.R., Erickson, P., Drabkin, H.A., Rowley, J.D. The 3;21 translocation in myelodysplasia results in a fusion transcript between the *AML1* gene and the gene for EAP, a highly conserved protein associated with the Epstein-Barr virus small *RNA EBER1*. Proc Natl Acad Sci USA, 90:7784-7788, 1993.

25. Super, H.G., McCabe, N.R., Thirman, M.J., Larson, R.A., Le Beau, M.M., Pedersen-Bjergaard, J., Preben, P., Diaz, M.O., and Rowley, J.D. Rearrangements of the *MLL* gene in therapy-related acute myeloid leukemia in patients previously treated with agents targeting DNA-topoisomerase II. Blood, 82:3705-3711, 1993.

26. Rowley, J.D. Rearrangements involving chromosome band 11q23 in acute leukemia. In: Seminars in Cancer Biology, 4:377-385, 1994. Editor: Rabbits, T.H., Academic Press, London.

27. Super, H.G., Rothberg, P.G., Kobayashi, H., Freeman, A.I., Diaz, M.O., and Rowley, J.D. Clonal, Non-constitutional Rearrangements of the *MLL* Gene in Infant Twins with ALL: *In utero* Chromosome Rearrangement of 11q23. Blood, 83:641-644, 1994.

28. Rowley, J.D. Cytogenetic and molecular analysis of pediatric neoplasms: Diagnostic and clinical implications. Pediatric Pathology, 14:167-176, 1994.

29. Porterfield, B.W., Pomykala, H., Maltepe, E., Rowley, J.D., and Diaz, M.O. The use of methylthioadenosine phosphorylase activity to select for human chromosome 9 in interspecies and intraspecies hybrid cells. Somatic Cell Molec Genet, 19:469-477, 1993.

30. Bohlander, S.K., Espinosa III, R., Fernald A.A., Rowley, J.D., Le Beau, M.M., and Diaz, M.O. Sequence-independent amplification and labeling of yeast artificial chromosomes for fluorescence in situ hybridization. Cytogenet Cell Genet, 65:108-110, 1994.

31. Claxton, D.F., Liu, P., Hsu, H.B., Marlton, P., Hester, J., Collins, F., Deisseroth A.B., Rowley, J.D., and Siciliano, M.J. Detection of fusion transcripts generated by the inversion 16 chromosome in acute myelogenous leukemia. Blood, 83:1750-1756, 1994.

32. Pedersen-Bjergaard J., and Rowley, J.D. The balanced and the unbalanced chromosome aberrations of acute myeloid leukemia may develop in different ways and may contribute differently to malignant transformation. Blood, 83:2780-2786, 1994.

33. Nucifora, G., Begy, C.R., Kobayashi H., Roulston, D., Claxton, D., Pedersen-Bjergaard, J., Parganas, E., Ihle, J.N., and Rowley, J.D. Consistent intergenic splicing and production of multiple transcripts between *AML1* at 21q22 and unrelated genes at 3q26 in (3;21)(q26;q22) translocations. Proc Natl Acad Sci USA, 91:4004-4008, 1994.

G. Present state of knowledge

The area of chromosome analysis of human malignant diseases and the companion investigations in the molecular analysis of malignant transformation is one of the most rapidly advancing areas of science today. Whereas a few years ago, cytogenetics was viewed as a backwater, its unique contributions to the molecular genetics of cancer are now well recognized. The consistent translocations and

deletions or duplications seen in leukemic cells, for example, pinpoint the location of at least some of the critical genes involved in leukemogenesis of that particular type of leukemia. I use pinpoint loosely, because all we can do is to identify a chromosome band containing the genes, but this is a substantial improvement over trying to search through the entire genome. Many sophisticated molecular laboratories all over the world are now actively cloning chromosome translocation breakpoints, or cloning tumor suppressor genes whose location has been defined by the detection of chromosomal deletions in affected patients or tumors. Graduate students and postdoctoral fellows are now choosing this area of research for their scientific careers. I find this development personally rewarding even though it has resulted in a very competitive environment in which to pursue our own research.

We are, in fact, in an era of scientific discovery that has to be called a Golden Age. We have already seen the impact on cancer biology and genetics of cloning the t(8;14) breakpoint in Burkitt lymphoma reported on in October, 1982, and of the t(9;22) in chronic myeloid leukemia in November, 1983. New translocation breakpoints are continually being defined in the leukemias and lymphomas. Each discovery leads to an enormous amount of research. In the case of the t(14;18) in follicular small cleaved cell lymphoma, the cloning of the translocation led to the identification of a new gene, *BCL2*. This has led to research in many laboratories trying to identify the function of this previously unknown gene in normal cells, and its altered function in malignant cells. The very exciting recent work of Stanley Korsmeyer indicating that *BCL2* may function in programmed cell death of B cells (apoptosis) and that the translocation inactivates this function is an excellent example of the potential impact of discoveries still to be made. These discoveries in turn will lead to imaginative approaches to therapy, targeting cells with the translocation and sparing cells lacking the translocation. Again, the discovery that the retinoic acid receptor alpha (*RARA*) was involved in the 15;17 translocation in acute promyelocytic leukemia, explained why treatment with various retinoic acid derivatives led to remissions in this type of leukemia.

This same process will be repeated many, many times as new translocation breakpoints are cloned and new genes related to cell growth are identified. The cloning of chromosome breakpoints provides a unique approach to the discovery of new growth regulating genes. In fact at least one-half of the genes that have been cloned are transcription factors. The functional categories of the genes that have been cloned at translocation breakpoints are listed in Table 6. Most of the genes in this table were identified only by cloning the translocation breakpoint. Analysis of the chromosome deletions is an equally fruitful area of research; the problem is that it may be somewhat harder to know when the gene you are searching for has been discovered.

This whole area of research will both help and will benefit from the present scientific focus on mapping the genome. New DNA probes will provide immeasurable help for our search for translocation breakpoints and deletions. The use of the YAC to clone the 11q23 breakpoint is an excellent example. At the same time, the probes we generate will benefit those who are mapping the genome by providing DNA probes from defined regions of the human chromosomes as well as DNA sequence data for specific genes that we have cloned.

TABLE 6

FUNCTIONAL CLASSIFICATION OF TRANSFORMING GENES AT TRANSLOCATION JUNCTIONS

	LOCATION	TRANSLOCATION	DISEASE
SRC FAMILY (TYR PROTEIN KINASES)			
ABL	9q34	t(9;22)	CML/ALL
LCK	1p34	t(1;7)	T-ALL
ALK	5q35	t(2;5)	NHL
SERINE PROTEIN KINASE			
BCR	22q11	t(9;22)	CML/ALL
CELL SURFACE RECEPTOR			
TAN1	9q34	t(7;9)	T-ALL
GROWTH FACTOR			
IL2	4q26	t(4;16)	T-NHL
IL3	5q31	t(5;14)	PreB-ALL
MITOCHONDRIAL MEMBRANE PROTEIN			
BCL2	18q21	t(14;18)	NHL
CELL CYCLE REGULATOR			
CCND1 (BCL1-PRAD1)	11q13	t(11;14)	CLL/NHL
MYOSIN FAMILY			
MYH11	16p13	inv(16),t(16;16)	AML-M4Eo
RIBOSOMAL PROTEIN			
EAP (L22)	3q26	t(3;21)	t-AML/CML BC
UNKNOWN			
DEK	6p23	t(6;9)	AML-M2/M4

TABLE 6 (cont.)

STRUCTURAL CLASSIFICATION OF TRANSFORMING GENES AT TRANSLOCATION JUNCTIONS

DNA BINDING FACTORS

	LOCATION	TRANSLOCATION	DISEASE
HOMEOBOX			
PBX	1q23	t(1;19)	PreB-ALL
HOX11	10q24	t(10;14)/t(7;10)	T-ALL
HELIX-LOOP-HELIX			
CAN	9q34	t(6;9)	AML
LYL1	19p13	t(7;19)	T-ALL
MYC[#]	8q24	t(8;14)	B-ALL/T-ALL
TAL1(SCL)	1p32	t(1;14)	T-ALL
TAL2	9p32	t(7;9)	T-ALL
TCF3(E2A)[#]	19p13	t(1;19)	PreB-ALL
ZINC FINGER			
ETO	8q24	t(8;21)	AML-M2
MLL	11q23.3	t(11q23)	ALL/AML
PLZF	11q23.1	t(11;17)	APL
PML	15q22	t(15;17)	APL
RARA	17q12	t(15;17)	APL
EVI1	3q26	inv(3),t(3;3)	AML
BCL6	3q27	t(3;14)	NHL
LIM			
RBTN1(TTG1)	11p15	t(11;14)	T-ALL
RBTN2	11p13	t(11;14)	T-ALL
OTHER			
AML1 (*runt* homology)	21q22	t(8;21),t(3;21)	AML-M2
LYT10 (*rel* homology)	10q24	t(10;14)	B-NHL
UNDEFINED			
MDS1	3q26	t(3;21)	AML

TRANSCRIPTIONAL MODULATORS

BCL3	19q13	t(14;19)	B-CLL
CBFB	16q22	inv(16),t(16;16)	AML-M4Eo

There is no question that this area of research merits significant support in the future. It must, however, be supported with a long-term point of view. This research takes patience and it may be some years before we have successfully cloned a number of translocation breakpoints. In my view, the benefits to be derived from this research, clearly merit this long-term support.

H. Federal Support

I received an Outstanding Investigator Grant from The National Cancer Institute in 1986; it was renewed in 1993. My current year (year 09) budget is $748,612.00. That is my only source of Federal support other than the present grant from the Department of Energy. The grant from NCI is used to support our work on mapping new translocation breakpoints and on the analysis of various malignant lymphoid diseases. At present we are mapping a number of translocation breakpoints on 12p and 1p. We are investigating the possible involvement of the *TEL* gene in our 12p13 translocations. We are also analyzing 12p deletions and are trying to determine whether $p27^{KIP}$ is the target gene of these deletions. We are also mapping the location of a series of 1p36 translocations. All of the freezing of cells and data management and secretarial assistance are supported by the grant from NCI. It also supports our efforts to apply the new DNA probes that we isolate for in situ hybridization with fluorescent labels or with chromogenic detection systems.

DATE
FILMED
7/28/94

END