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

Comprehensive Progress Report
For the Period July, 1992 through August, 1993

Janet D. Rowley, M.D.

The University of Chicago
5801 South Ellis Avenue
Chicago, Illinois

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The specific objectives for the three year proposal January 1992 through December 1995 are listed below.

I. **TECHNICAL PROGRESS OF PROJECT DURING THE PAST YEAR**

A. SPECIFIC OBJECTIVES OF ORIGINAL 3 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) breakpoints.
 - 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. PROGRESS REPORT

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 some support for this research through a program project grant from NIH. I remain a close collaborator and am involved in parts of the project as needed.

An important related project has been a major study on the karyotypic pattern seen in patients with erythroleukemia, AML M6. We have performed a retrospective analysis of the clinical, morphologic, and cytogenetic findings in 26 M6 patients diagnosed between January, 1969 and September, 1991. 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. 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 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 (*Blood*, 80:1-9, 1992)

b. Correlation of treatment with balanced and unbalanced translocations.

The results of our analysis has recently been published as a Letter to the Editor of *Blood* (a copy was enclosed with last year's Progress Report). Virtually all of our patients with balanced translocations involving 11q23 and 21q22 had received prior therapy with topoisomerase II inhibitors.

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

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

As described in my grant proposal, my colleagues and I have been making steady progress in first mapping and now in cloning the breakpoint junctions in the above two translocations, as well as in related translocations t(4;11) and t(6;11). We previously showed that the translocations involving 11q23 with chromosomes 4(q21), 6(q27), 9(p22), and 19(p13) all had breakpoints within a 330 kb human DNA insert in a yeast artificial chromosome (YAC) identified with a CD3 gamma probe. We subcloned genomic fragments from the YAC and have shown that some fragments remain on chromosome 11 in all translocations, whereas the other fragments are translocated to the other chromosome (4,6,9, or 19). 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 have subcloned a number of unique sequence probes, especially from the large fragment (clone 14) just telomeric to the breakpoint.(Figure 1)

We called this gene *MLL* (myeloid/lymphoid, or mixed lineage leukemia). 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 0.7kb BamH1 subclone of one of the cDNAs, which recognizes a 9kb BamH1 genomic fragment, detects rearrangements on Southern blots of BamH1 digested patient and cell line DNA.(Figure 1) To date, using this 0.7kb fragment, all patient material with the 11q23 translocations involving 4, 6, 9, and 19 have shown rearrangements on Southern analysis, indicating that the 11q23 breaks are clustered within 8.5 kb cloning the breakpoint junctions in the t(9;11) and t(11;19) breakpoints continues. We have DNA probes from chromosome 9 which we are in the process of characterizing.

We have used these probes to clarify the nature of rearrangements involving 11q23 in cells from many of our patients using fluorescence in situ hybridization (FISH). Reprints of the three studies reported on to date are enclosed. The first study involved the analysis of cell lines with 11q23 rearrangements. Dr. Kobayashi could show that one cell line, Karpes 45, had an undetected translocation involving *MLL* and an unknown gene on the X chromosome. Many of the patients for whom we did not have DNA for Southern blot analysis were studied with FISH and they were shown to have a break in the *MLL* gene. There was some heterogeneity however and a few translocation breakpoints did not involve *MLL*. An analysis of patients with 11q deletions showed that the consistently deleted region did not involve *MLL* but rather was centered in an area substantially more centromeric than *MLL*.

Relationship of breaks in *MLL* in AML de novo and in t-AML. One of our major interests was to examine patients who had t-MDS or t-AML and 11q23 rearrangements to determine first whether *MLL* was involved and then, second, whether the breaks occurred in the same region of the gene. We have been fortunate to have established a collaboration with Dr. Jens Pedersen-Bjergaard in Copenhagen who has had a long standing interest in hematologic diseases that occur as a consequence of cytotoxic treatment. He sent us DNA from ten patients, six of whom had 11q23 abnormalities and three others had myelomonocytic (M4) leukemia which is closely associated with 11q23 rearrangements.

Chromosome band 11q23 is frequently involved in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) *de novo*, as well as in myelodysplastic syndromes (MDS) and lymphoma. 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 used probes for the *MLL* gene, at 11q23. We have studied 17 patients with t-MDS/t-AML, 12 of whom had cytogenetically detectable 11q23 rearrangements. (Tables 1, 2) 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. (Figure 2) None of the patients who had not received topo II inhibitors showed an *MLL* rearrangement. (Table 3) 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 both AML which develops *de novo* and in t-AML. Most patients who exhibited *MLL* rearrangements presented with overt t-AML following a relatively short latency period. These patients with no *MLL* involvement developed t-MDS or t-AML after a very much longer latent period. (Table 1, Figure 3) The association of exposure to topo II- 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.

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. 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 has become apparent that there is another group of patients who lack aberrations of chromosomes 5 and/or 7 and whose leukemic cells have certain specific changes that are quite unusual for t-AML. These patients have balanced translocations 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. It was noteworthy that 3 patients 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. Dr. Pedersen-Bjergaard observed that balanced translocations involving 11q23 and 21q22 were significantly associated with prior exposure to chemotherapeutic regimens that included topoisomerase II inhibitors. We analyzed our patient data in a similar fashion and confirmed his observation.

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, who has cloned the breakpoint on chromosome 21 in the 8;21 translocation [t(8;21)(q22;q22)]. We used DNA probes from chromosome 21 isolated by Dr. Drabkin to analyze the t(3;21). We showed with fluorescence in situ hybridization (FISH) that these same probes were on either side of the breakpoint in the t(3;21). The *AML1* gene is transcribed from telomere to centromere. In the t(8;21), the 5' part of *AML1* is translocated and fused to the *ETO* gene on chromosome 8 to produce the chimeric *AML1/ETO* on the der(8) chromosome. We found that *AML1* is also rearranged in the t(3;21).

We have frozen samples of bone marrow from six leukemic patients with CML or t-AML with the t(3;21). The thawed cells are 90-95% viable, and contain undegraded DNA and RNA, suitable for construction of genomic DNA libraries, and for preparation of RNA for Northern blot analysis and construction of cDNA libraries. The clinical and cytogenetic information are listed in Table 4.

One probe, wc-8, detected a rearrangement in the DNA from one patient (Figure 4). The results indicate that the t(3;21) breakpoint is located in the segment between probe 3 and wc-9. The genomic mapping of the region between the t(8;21) and t(3;21) breakpoints indicates that the two breakpoints appear to be about 60kb apart. The translocation in the t(8;21) results in a fusion gene on the der(8) chromosome and a fusion mRNA. There is at least one exon between the

two clusters of t(8;21) and t(3;21) breakpoints, as determined by using an *AML1* cDNA as a probe in Southern blot analysis.(Figure 5) We subsequently showed that a chimeric gene is formed in the 3;21 translocation and that it contains a larger portion of *AML1* than the t(8;21). The breakpoints on chromosome 21 in the t(3;21) patients appear to be relatively variable. In two patients the breakpoints were telomeric to the t(8;21) breakpoint and to all FISH probes used in the analysis, whereas in three patients they were centromeric to the t(8;21) breakpoints.(Table 5)

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

This was a long-range objective which cannot even be approached until we have cloned and sequenced the breakpoints in the 11q23 and 21q22 translocations. When we have completed the sequencing, we will be able to analyze the sequence for topo II consensus sequences and for scaffold attachment sites, as well as for other canonical motifs that would provide us with some insights as to the potential function of these regions and their susceptibility to breakage when exposed to topo II inhibitors.

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

We have analyzed the samples from our 11q23 translocation patients together regardless of whether they are treatment related or de novo AML. Of the samples, we have studied to date. We have found no difference in the breakpoint in of patients with AML de novo or treatment-related.. We have established a collaboration with Dr. Pedersen-Bjergaard in Denmark who has been extremely active in studying t-AML. He recently brought us DNA from 13 patients with either 11q23 or 21q22 breakpoints. DNA from these samples has been analyzed and there is basically no difference in the breakpoint within the 8.5kb DNA fragment.

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

Again, this is a very long-range project. We have begun to explore this problem with the genomic and cDNA probes that we currently have for the 11q23 breakpoint region. However, we must develop a more detailed restriction map of this region, and obtain a series of small, unique sequence probes before we can map the scaffold attachment sites in the region of the breakpoint with any precision.

II. SECTION ON CHANGES IN SPECIFIC AIMS

Microdissection, amplification and cloning of unknown DNA from defined chromosome bands or structures.

This aim was not included in the renewal proposal in 1991 because it was the subject of a separate grant request to the DOE Genome Project. It did not receive funding and thus some of the goals of this research are being continued under this grant.

We have developed a simple, efficient method by which microdissected material can be amplified directly in the collection container in a few hours. The procedure involves two initial rounds of DNA synthesis with T7 DNA polymerase, using a primer that contains a random pentanucleotide sequence as the primer. The resulting products can be biotinylated and used for fluorescence in situ hybridization (FISH) to confirm their chromosomal location. As few as 17 dissected chromosomal regions provide sufficient material or a specific FISH signal on the appropriate band of metaphase chromosomes. We have obtained a chromosome 6q25-qter-specific painting probe in this way as well as phage probes for the short arm of chromosome 9.

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TABLE 1. Clinical patient information

A. PATIENTS WITH *MLL* REARRANGEMENT

<u>Patient No.</u>	<u>Sex/ Age</u>	<u>Primary Tumor</u>	<u>Treatment for Primary Tumor</u>	<u>Mths to Secondary Disease</u>	<u>Diagnosis of Secondary Disease</u>	<u>Survival (Months)</u>
1 (10665)	M 64	Large cell lymphoma	CHOP	9 (9)	t-AML	1
2 (12215)	F 51	Breast	Dox/Ctx/ Tam;RT	18 (18)	t-MDS	10
3 (8222)	M 29	Germ cell	Cispl/Bleo/ Vbl;Cispl/ Etop/Bleo	31 (25)	t-AML (M4)	12
4 (6944)	F 48	Ovarian	Ctx/Dox 5FU;Cispl/ Hex	24 (24)	t-AML (M4)	6
5 (10849)	F 69	Lung	Etop/Cispl	35 (35)	t-AML	1
6 (3820)	F 33	Breast	RT; 4-epidox/ Cispl	18 (17)	t-AML (M5a)	45+
7 (169)	F 52	Lung	Etop/Ctx/ CCNU/ VCR;RT	15 (15)	t-AML (M4)	1
8 (13753)	F 25	Rhabdomy- osarcoma	ActD/Vcr/ Ctx;RT; Ifos/Etop	31 (17)	t-AML	0
9 (467)	M 25	Non- Hodgkin's lymphoma	CHOP;RT	24 (24)	t-AML (M5b)	5

B. PATIENTS WITH NO *MLL* REARRANGEMENT

10 (15195)	F 48	Polycyth- emia vera	³² P;HU	unknown	t-AML	1
11 (421)	M 48	Hodgkin's lymphoma	RT;MOPP	53(none)	t-MDS	98
12 (11287)	M 43	Hodgkin's lymphoma	ABVD/ MOPP	40 (40)	t-MDS	9
13 (13085)	F 26	Hodgkin's lymphoma	MOPP; RT	17(none)	t-MDS	16
14 (13504)	M 53	Non- Hodgkin's lymphoma	Ctx/Vcr/ Pred; CHOP/ Bleo/CCNU; RT; BCNU/Vbl	72 (60)	t-MDS	7
15 (5329)	F 53	Breast	Ctx/Mtx/ 5FU/Tam; 4-epidox/ Cispl	56 (33)	t-AML (M4)	0
16 (496)	F 39	Germ cell	Etop/Cispl/ Bleo;RT	68 (68)	t-MDS	30+
17 (6006)	M 78	Rectal	BCNU/Vcr/ DTIC/5FU	104(none)	t-AML (M4)	1
18 (823)	F 52	Non- Hodgkin's lymphoma	CHOP	52 (52)	t-AML (M4)	9

Table 2. Cytogenetic and molecular analysis of patient samples

A. PATIENTS WITH *MLL* REARRANGEMENT

Patient number	Karyotypic abnormality	<i>MLL</i> 7B hybridizing fragments
1	t(9;11)(p22;q23)	G / R1/ R2
2	t(9;11)(p22;q23)	G / R1/ R2
3	t(9;11)(p22;q23)	G / R1
4	t(9;11)(p22;q23)	G / R1/ R2
5	t(9;11;18)(p22;q23;q12)	G / R1/ R2
6	t(6;11)(q27;q23)	G / R1/ R2
7	t(11;19)(q23;p13)	G / R1 (mono) G / R1 (gran)
8	t(11;19)(q23;p13)	G / R1/ R2
9	t(11;19)(q23;p13)	G / R1 (mono) G / R1 (gran)

B. PATIENTS WITH NO *MLL* REARRANGEMENT

Patient number	Karyotypic abnormality	<i>MLL</i> 7B hybridizing fragments
10	del (11)(q13q23)	G
11	del (11)(q14q23)	G (mono) G (gran)
12	der(5) t(5;11)(q13;q23)	G
13	t(11;17;12)(q23;p13;p13)	G
14	complex--del(5)(q13q31) del(7)(q11q34)	G
15	-7	G
16	der(1)t(1;7)(p11;p11),-7	G
17	normal	G
18	normal	G

TABLE 1--Clinical patient information

^aTopoisomerase II inhibitors in bold characters.

^bTotal months from initial treatment; () months from first exposure to Topo-II inhibitors.

Abbreviations:

CHOP, cyclophosphamide (Ctx), doxorubicin (Dox), vincristine (Vcr), prednisone; Tam, tamoxifen; RT, radiotherapy; Cispl, cisplatin; Bleo, bleomycin; Vbl, vinblastine; Etop, etoposide; 5FU, 5-fluoro-uracil; Hex, hexamethylmelamine; 4-epidox, 4-epidoxorubicin; CCNU lomustine; ActD, actinomycin D; Ifos, ifosamide; HU, hydroxyurea; MOPP, nitrogen mustard, vincristine, procarbazine, prednisone; ABVD, Dox, Bleo, Vbl, dacarbazine; BCNU, carmustine; Mtx, methotrexate; DTIC, dacarbazine

TABLE 2. --Cytogenetic molecular analysis of patient samples-

Abbreviations:

mono, mononuclear cells; gran, granulocytes

G--germline fragment

R--rearranged fragment(s)

TABLE 3 Association of *MLL* rearrangements and prior therapy with topoisomerase II reactive drugs.

		Prior treatment with topo II inhibitor	
		+	-
<i>MLL</i> rearrangement	+	9*	0
	-	1#	2†

The numbers of patients in each category is listed. All patients in Table 3 had cytogenetically recognized 11q23 rearrangements. The association of *MLL* rearrangements with prior topo II exposure was statistically significant ($p=0.01$, Fischer's exact test).

* - All patients had balanced translocations

- Patient had an unbalanced translocation

† - Both patients had del(11)(q23)

TABLE 4

CYTOGENETIC ANALYSIS				DNA ANALYSIS		
Pt	Age	Diagnosis ^a	No. (%) of normal cells	Abnormal Karyotype	Southern Blot	FISH
1	48	t-MDS	7(23)	46,XY,t(3;21)(q26;q22),add(7)(q21)[24]	N.A.	breakpoint is within wc-8
2	32	t-MDS	9(31)	46,XY,t(3;21)(q26;q22),del(5)(q13q34)[2]/ 46,XY,t(3;21),-5,+mar[18]	rearranged#	breakpoint is within wc-8
3	63	t-AML	0	46,XX,+der(1)t(1;21)(p11;q21),t(3;21) (q26;q22),-6,der(12)t(6;12)(q22;q21), -21,+mar[19]	polymorphism with <i>EcoRI</i>	breakpoint distal to telomeric cosmid contig
4	69	CML-BC	0	48,Y,t(X;9;22)(p22.1;q34;q11),t(3;21) (q26;q22),+8,i(8q),+12[21]/ 49,idem,+der(22)t(X;9;22)[1]	N.A.	breakpoint is within wc-5
5	44	CML-ACC	0	46,XY,t(9;22)(q34;q11)[2]/ 47,idem,+8[3]/ 46,idem,t(3;21)(q26;q22)[16]	polymorphism with <i>EcoRI</i>	breakpoint distal to telomeric cosmid contig
6	41	CML-BC	0	46,XY,t(9;22)(q34;q11)[6]/46,idem, t(3;21)(q26;q22)[7]	germline*	no cells available
7	48	CML-BC	2(20)	46,XY,t(9;22)(q34;q11)[4]/46, idem,t(3;21)(q26;q22)[4]	germline*	no cells available

^at-MDS - Therapy-related myelodysplastic syndrome; t-AML - Therapy-related acute myeloid leukemia

CML-BC - Chronic myelogenous leukemia in blast crisis; CML-ACC - Chronic myelogenous leukemia in accelerated phase

idem - Same as the first clone.

[] - Number of cells in each clone.

[#]Rearrangement using the 4.5 kb *HindIII* fragment as a probe.

*No rearrangement observed in the *EcoRI*, *HindIII* and *BamHI* digested DNA. See text for probes used.

N.A. - No DNA available for Southern blot analysis

TABLE 5. Chromosomal localization of probes.

Probe	Patient				
	1	2	3	4	5
centromeric contig		der(21)		der(21)	der(21)
wc-10	der(21)	der(21)			
wc-9		der(21)		der(21)	der(21)
wc-8	der(21),der(3)*	der(21)&der(3)*	der(21)		
wc-7	der(3)	der(3)		der(21)	der(21)
wc-6	der(3)	der(3)		der(21)	der(21)
wc-5	der(3)	der(3)		der(21)&der(3)*	
wc-3	der(3)			der(3)	
wc-2	der(3)	der(3)			der(21)
wc-1		der(3)			
probe B	der(3)			der(3)	
13'	der(3)				der(21)
telomeric contig	der(3)	der(3)	der(21)	der(3)	der(21)

* Wc-8 alone in patient No. 2 and wc-5 alone in patient No. 4 hybridized weakly to both derivative chromosomes. For patient No. 1, wc-8 was used in combination with wc-10 and signal was detected on the der(21). In combination with wc-7, signal was detected on the der(3). Wc-8 alone produced marginal signal on the der(21) only.

---> General location of breakpoint in the t(8;21).

Fig. 1 Map of breakpoint region of *MLL* gene.

Top, YAC containing CD3D/G genes. Middle, genomic region of breakpoint showing *BamHI* fragments which flank the breakpoint region. Bottom, partial DNA of the *MLL* gene, including the 0.7kb *BamHI* fragment MLL0.7B and its relationship to the genomic sequences. B, *BamHI*; E, *EcoRI*; P, *PstI*; H, *HindIII*.

Fig. 2 Southern blot analysis of t-AML and t-MDS patient DNA.

Samples were digested with *Bam*HI and Southern blot was hybridized to the MLL0.7B probe. Lanes are marked above with patient numbers from Table 1. Patients 3, 7, and 9 show one rearranged band in addition to the germline 8.3kb *Bam*HI fragment. Patients 4 and 6 show two rearranged bands. None of the other samples exhibited rearrangements. (Data for other patients in study not shown.) C, control DNA; mo, mononuclear cells; gr, granulocytes.

Fig. 3 Interval from primary chemotherapy to t-AML and duration of survival.

Thick horizontal lines indicate the total length of time between first treatment for primary neoplasm and the diagnosis of t-AML. Thin vertical line indicates the time at which the patient received topo II inhibitor. Short, thick vertical lines mark diagnosis of t-AML. Dotted lines indicated duration of survival after diagnosis of t-AML. Patients with *MLL* rearrangement are Nos. 1-9 in order from top to bottom. Patients with no *MLL* rearrangement are Nos. 11-17 in order from top to bottom. (Patient No. 10 is not represented in the figure because of incomplete medical records.) *Patients who received no topo II inhibitors.

Fig. 4

*Bam*HI restriction map of chromosome 21 in the region containing the breakpoints of the t(8;21) (AML) and t(3;21) (t-MDS/t-AML). The breakpoints are indicated by vertical arrows. Upper line pF4-7b is a normal *AML1* cDNA. The vertical bars indicated the *Bam*HI sites. The direction of transcription of *AML1* is indicated by a horizontal arrow. The recombinant phage clones 13' through wc-10 and the 4.5kb *Hind*III probe are indicated by horizontal lines below the map. Wc-10 was not precisely mapped so is indicated by a dashed line. The 4.5kb *Hind*III fragment was used as a probe in the Southern blot analysis shown in Fig. 4. The genomic *Bam*HI bands hybridizing to selected PCR-amplified sections of the *AML1* cDNA clone pF4-7b are indicated by the shaded areas. Genomic regions indicated by a dashed line have not been mapped.

Fig. 5

Detection of the t(3;21) breakpoint by Southern blot analysis of DNA from a t-MDS patient (lanes 2, 4 and 6, patient No. 2, Table 1). Human placental DNA (lanes 1, 3 and 5) was used as control. The DNAs of the patient and of the control were digested with *Hind*III (lanes 1 and 2), *Eco*RI (lanes 3 and 4) and *Bam*HI (lanes 5 and 6), and probed with the 4.5kb *Hind*III probe. DNA size markers are indicated at the left. The sizes of the germline fragments that are rearranged are: *Hind*III, 4.5kb; *Eco*RI, 2.9kb; *Bam*HI, 4.9kb. The sizes of the rearranged fragments are: *Hind*III, 5.2 and 7.3kb; *Eco*RI, 5.9 and 6.4kb; *Bam*HI, 15kb.

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