The Translocation (6;9) (p23;q34) Shows Consistent Rearrangement of Two Genes and Defines a Myeloproliferative Disorder With Specific Clinical Features


Translocation (6;9)(p23;q34) is a cytogenetic aberration that can be found in specific subtypes of both acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). This translocation is associated with an unfavorable prognosis. Recently, the genes involved in the t(6;9) were isolated and characterized. Breakpoints in both the dek gene on chromosome 6 and the can gene on chromosome 9 appear to occur in defined regions, which allows us to diagnose this type of leukemia at the molecular level. Moreover, because of the translocation a chimeric dek-can mRNA is formed which, as we show here, is an additional target for diagnosis via cDNA-preparation and the polymerase chain reaction (PCR). We studied 17 patients whose blood cells and/or bone marrow cells showed a t(6;9) with karyotypic analysis. Fourteen patients suffered from AML, one patient had a refractory anemia with excess of blasts in transformation (RAEBt), one patient had an acute myelofibrosis (AMF), and one patient a chronic myeloid leukemia (CML). In nine cases studies at the DNA and RNA levels were possible while in seven cases only the DNA could be analyzed. In one case only RNA was available. Conventional Southern blot analysis showed the presence of rearrangements of both the dek gene and the can gene. In both genes, breakpoints cluster in one intron in the patients investigated. The presence of a consistent chimeric dek-can product after cDNA preparation followed by the PCR was demonstrated. We conclude from our data that the t(6;9) is found in myeloproliferative disorders with typical clinical characteristics. This translocation results in highly consistent abnormalities at the molecular level.

Since the discovery in 1960 of the Philadelphia chromosome in cases of chronic myeloid leukemia (CML) by Nowell and Hungerford, a large number of leukemias has been associated with specific chromosomal translocations.\(^1,2\) The development of new techniques enabled molecular biologists to isolate and characterize a number of genes involved in reciprocal chromosome translocations. Well-known examples are the t(8;14) in Burkitt's lymphoma (BL) in which the myc gene on chromosome 8 is linked to the IgH-chain locus on chromosome 14, and t(9;22) in CML in which breakpoints occur in the abl gene on chromosome 9 and the bcr gene on chromosome 22.\(^3,5\)

In a specific subgroup of acute myeloid leukemia (AML) a t(6;9)(p23;q34) can be found.\(^6,10\) Patients with this type of leukemia are usually quite young and their prognosis is poor. Blast cells are mostly classified as French-American-British (FAB)-M2 or M4 (90%) and in a minority as M1 (10%). At the time of diagnosis the t(6;9) is usually the sole cytogenetic aberration. Additional karyotypic abnormalities are rare but may occur during progression of the disease.\(^9,18\) Recently, the genes located at the chromosomal breakpoints of this translocation were isolated and characterized.\(^10,20\) The gene on chromosome 6 that participates in the reciprocal exchange is called dek and encompasses 40 kb. Southern blot analysis of four patients with t(6;9) indicated that breakpoints are located in one intron of 9 kb, which is called ‘intron containing breakpoints on chromosome 6’ or icb-6. The can gene on chromosome 9 is more than 130 kb in length. Here, breakpoints occur in one intron of 7.5 kb (icb-9) that is located in the middle of the gene. The can gene is transcribed into a 6.6-kb mRNA. Because of the translocation the 3' part of the can gene is fused to the 5' part of dek, resulting in a chimeric dek-can gene on the 6p− derivative.\(^20\) This chimeric gene is transcribed into an aberrant 5.5 kb-mRNA. The functions of the normal dek and can gene products are as yet unknown and it is equally unclear in which way the hybrid product may be involved in leukemogenesis.

In this study we analyzed 14 patients with AML, 1 patient with refractory anemia with excess of blasts in transformation (RAEBt), 1 patient with acute myelofibrosis (AMF), and 1 patient with CML whose blood or bone marrow cells carried a t(6;9). Investigation of the leukemic cells at the DNA and RNA level confirms the highly consistent involvement of both the dek and can genes in this translocation. The myeloproliferative disorder marked by a t(6;9) appears as a distinct clinical entity which, as we show here, can now be diagnosed and monitored at the molecular level.

MATERIALS AND METHODS

Patients. Clinical and hematologic data of the patients are given in Table 1. In the cases of patients 1 through 5, various data were published previously.\(^13,19,21,22\) Patients 6 through 17 were newly admitted cases and fresh or frozen samples were sent to us for molecular investigations by the following centers: Regional Cancer Center Marseille, France (patients 6 and 17); University Hospital Groningen, The Netherlands (patient 8); Centre Regional de Transfusion Sanguine et de Génétique Humaine Bois-Guillaume, France (patient 9); Free University Hospital Amsterdam, The Netherlands and Stichting Nederlandse Werkgroep Leukemie bij Kinderen The Hague, The Netherlands (patient 10); Medical Center of the University of Amsterdam, The Netherlands (patients 11 and 15); Imperial Cancer Research Fund, Saint Bartholomew's Hospital, London, United Kingdom (patients 12

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Blood, Vol 79, No 11 (June 1), 1992: pp 2990-2997
MOLECULAR DETECTION OF TRANSLOCATION (6;9) IN AMLL 2991

EcoRI fragment) are used for the detection of breakpoints in

stored in liquid nitrogen until used.

or a Ficoll-Hypaque gradient, cells were frozen and

collected in heparinized tubes. After isolation of the white fraction

onto nylon filters (Zeta-probe; Biorad Lab, Richmond, CA)

and MF2BH (an 800-bp BglII-HindIII fragment). Probes AL1F6EP

short, rearrangement of the dek gene is detectable using

Southern blot analysis shows rearrangement of the

dek-can gene. DNA n.a., DNA was not available.

can gene. DNA n.a., DNA was not available.

no amplification by PCR.  

and 13); Children’s Cancer Research Institute Vienna, Austria  

(14). Samples. Bone marrow aspirates and blood samples were

collected in heparinized tubes. After isolation of the white fraction

dextran or a Ficoll-Hypaque gradient, cells were frozen and

and stored in liquid nitrogen until used.

hybridization and washing conditions were described previously.19

According to the manufacturers instructions. Probes used for

radio-labeled probes MF1E5 (a 500-bp EcoRI-EcoRI fragment) and

M28H (an 800-bp BglII-HindIII fragment). Probes AL1F6EP

(a 200-bp EcoRI-FstI fragment) and AL1F6E.3 (a 300-bp EcoRI-

EcoRI fragment) are used for the detection of breakpoints in the

can gene. In Fig 1A and B, a schematic representation is given of a

simplified restriction map of both ibc-6 and ibc-9 and the localization

of the probes used is indicated.

Polymerase chain reaction (PCR). RNA isolation, cDNA prepa-

ration, and PCR conditions were described previously.25 As a

control for the cDNA synthesis and PCR reaction, not only the

chimeric dek-can cDNA was amplified from the patient RNA but

also the normal can cDNA. One unit of Taq polymerase was added

for the cDNA synthesis and PCR amplification consisted of either of the two

following sets of sequences: (1) Primer-set I: 3′ primer in can

5′ GGCCAGTGCTAACT-  

5′ 5′ CCTACAGAT-  

3′ = 3′ AAGAGAC-  

3′ = 3′ CTGAAAA-

The PCR was performed using 24 cycles of denaturation (1 minute,  

Cutters Corp, Emeryville, CA or BRL, GIBCO Lab,

Life Techno. Inc, Grand Island, NY). Primers used for cDNA

synthesis and PCR amplification consisted of either of the two

following sets of sequences: (1) Primer-set I: 3′ primer in can = 5′

ACCAGGTTGATTCGCCCT 3′; 5′ primer in can = 5′ CTGAAAA-

CACTTATTCTGG 3′; 5′ primer in dek = 5′ CCTACAGAT-

GAAGAGTTAA 3′; or (2) Primer-set II: 3′ primer in can = 5′

GTGTCCTCGACTGTTG 3′; 5′ primer in can = 5′ AAGACAGAC-

CAGAGTTAA 3′; 5′ primer in dek = 5′ GGCCAGTGCTAAC-

TG 3′.

The anneal temperature for these primers was chosen at 45°C.

‘ †Diagn., at diagnosis; after ther., after therapy: patient 8, chemotherapy: patient 17, IL-2 therapy.

†+ Southern blot analysis shows rearrangement of the dek and the can gene. DNA n.a., DNA was not available.

<table>
<thead>
<tr>
<th>Patient Age/Sex*</th>
<th>FAB</th>
<th>Clinical Phase†</th>
<th>Karyotypic Abnormality</th>
<th>% Abnormal Metaphase</th>
<th>Basophilia‡</th>
<th>WBC 10^9/L</th>
<th>BM</th>
<th>PB</th>
<th>Blot§</th>
<th>Southern</th>
<th>PCR</th>
<th>Response to Therapy</th>
<th>Survival After Diagnosis</th>
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<tbody>
<tr>
<td>15</td>
<td>13/F</td>
<td>RAEB</td>
<td>Diagn.</td>
<td>t(6;9)</td>
<td>100</td>
<td>No</td>
<td>1,66</td>
<td></td>
<td></td>
<td>RNA n.a.</td>
<td></td>
<td>CR</td>
<td>18 mo</td>
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<tr>
<td>16</td>
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<td>M4</td>
<td>Relapse</td>
<td>t(6;9)</td>
<td>87</td>
<td>47</td>
<td>30</td>
<td>+</td>
<td></td>
<td>No ampl.</td>
<td></td>
<td>CR</td>
<td>29 mo</td>
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<tr>
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<td>63/F</td>
<td>M4</td>
<td>Diagn.</td>
<td>t(6;9)</td>
<td>97</td>
<td>No</td>
<td>21,2</td>
<td>59</td>
<td>18</td>
<td>No therapy</td>
<td></td>
<td>CR</td>
<td>3/4 mo</td>
</tr>
<tr>
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<td>38/M</td>
<td>AMF</td>
<td>Diagn.</td>
<td>NM</td>
<td>No</td>
<td>5,2</td>
<td>9</td>
<td>5</td>
<td></td>
<td>No ampl.</td>
<td></td>
<td>CR</td>
<td>30 mo</td>
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<tr>
<td>31</td>
<td>17/F</td>
<td>M4</td>
<td>Diagn.</td>
<td>t(6;9)</td>
<td>87</td>
<td>No</td>
<td>29,4</td>
<td>73</td>
<td>58</td>
<td>DNA n.a.</td>
<td></td>
<td>CR</td>
<td>30 mo</td>
</tr>
<tr>
<td>41</td>
<td>13/F</td>
<td>M4</td>
<td>Diagn.</td>
<td>t(6;9)</td>
<td>80</td>
<td>Yes</td>
<td>12,6</td>
<td>58</td>
<td>35</td>
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<td></td>
<td>CR</td>
<td>18 mo</td>
</tr>
<tr>
<td>6</td>
<td>35/M</td>
<td>M2</td>
<td>Diagn.</td>
<td>t(6;9)</td>
<td>80</td>
<td>Yes</td>
<td>12,6</td>
<td>58</td>
<td>35</td>
<td>RNA n.a.</td>
<td></td>
<td>CR</td>
<td>18 mo</td>
</tr>
<tr>
<td>7</td>
<td>28/F</td>
<td>M4</td>
<td>Relapse</td>
<td>t(6;9)</td>
<td>100</td>
<td>92</td>
<td>95</td>
<td>+</td>
<td></td>
<td>No ampl.</td>
<td></td>
<td>CR</td>
<td>17 mo</td>
</tr>
<tr>
<td>8</td>
<td>18/M</td>
<td>M2</td>
<td>Diagn. after ther.</td>
<td>t(6;9), inv.1</td>
<td>35</td>
<td>No</td>
<td>10</td>
<td>25</td>
<td>&lt;1</td>
<td>RNA n.a.</td>
<td></td>
<td>CR</td>
<td>&gt;24 mo</td>
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<tr>
<td>9</td>
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<td>Relapse</td>
<td>t(6;9)</td>
<td>100</td>
<td>Yes</td>
<td>100</td>
<td>87</td>
<td>94</td>
<td>+</td>
<td>CR</td>
<td>14 mo</td>
<td></td>
</tr>
<tr>
<td>10</td>
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<td>M4</td>
<td>Diagn.</td>
<td>t(6;9)</td>
<td>100</td>
<td>No</td>
<td>262</td>
<td>80</td>
<td>45</td>
<td>+</td>
<td>+</td>
<td>CR</td>
<td>&gt;36 mo</td>
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<tr>
<td>11</td>
<td>6/F</td>
<td>M4</td>
<td>Diagn.</td>
<td>t(6;9)</td>
<td>100</td>
<td>65,9</td>
<td>79</td>
<td>+</td>
<td></td>
<td>No ampl.</td>
<td></td>
<td>CR</td>
<td>2 mo</td>
</tr>
<tr>
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<td>28/M</td>
<td>M1</td>
<td>Diagn.</td>
<td>t(6;9)</td>
<td>100</td>
<td>65,9</td>
<td>79</td>
<td>+</td>
<td></td>
<td>No ampl.</td>
<td></td>
<td>CR</td>
<td>2 mo</td>
</tr>
<tr>
<td>13</td>
<td>24/F</td>
<td>M1</td>
<td>Diagn.</td>
<td>t(6;9), del.7q</td>
<td>40</td>
<td>54,8</td>
<td>95</td>
<td>+</td>
<td>RNA n.a.</td>
<td>+</td>
<td></td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>10/M</td>
<td>M2</td>
<td>Diagn.</td>
<td>t(6;9)</td>
<td>100</td>
<td>No</td>
<td>47,5</td>
<td>52</td>
<td>37</td>
<td>+</td>
<td>No ampl.</td>
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<tr>
<td>15</td>
<td>53/M</td>
<td>M4</td>
<td>Relapse</td>
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<td>Yes</td>
<td>100</td>
<td>41</td>
<td>31</td>
<td>+</td>
<td>+</td>
<td>PR</td>
<td>&gt;4 mo</td>
</tr>
<tr>
<td>16</td>
<td>54/F</td>
<td>CML</td>
<td>Diagn.</td>
<td>t(6;9)</td>
<td>100</td>
<td>No</td>
<td>92</td>
<td>9</td>
<td></td>
<td>+</td>
<td>RNA n.a.</td>
<td>11 mo</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>53/F</td>
<td>RAEB</td>
<td>Diagn. after ther.</td>
<td>t(6;9)</td>
<td>100</td>
<td>No</td>
<td>22</td>
<td></td>
<td></td>
<td>No chemoth.</td>
<td></td>
<td>ND</td>
<td>15 mo</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete remission; PR, partial remission; NR, no remission; NM, no mitosis; ND, not done; WBC, white blood cell count; BM, bone marrow; PB, peripheral blood.

*Age in years at diagnosis. Sex: M, male; F, female.

†Diagn., at diagnosis; after ther., after therapy: patient 8, chemotherapy: patient 17, IL-2 therapy.

‡+ 25% basophils in the bone marrow.

§+, Southern blot analysis shows rearrangement of the dek and the can gene. DNA n.a., DNA was not available.

∥+, PCR analysis shows amplification of a chimeric dek-can fragment. –, no amplification of dek-can while can is amplified. RNA n.a., RNA was not available; no ampl., despite intact RNA no amplification by PCR.

#Data from Von Lindern et al.19

Data from Adriaansen et al.22

and 13); Children’s Cancer Research Institute Vienna, Austria

(patient 14).

Conventional Southern blot analysis. DNA was isolated from

cells according to standard procedures23 or high molecular weight DNA was prepared in agarose plugs as described previously.24 The following restriction enzymes were used for digestion of DNA: EcoRV, BamHI, HindIII and/or BglII. DNA fragments were separated on a 0.7% agarose gel and blotted onto nylon filters (Zeta-probe; Biorad Lab, Richmond, CA) according to the manufacturers instructions. Probes used for hybridization and washing conditions were described previously.19

In short, rearrangement of the dek gene is detectable using radio-labeled probes MF1E5 (a 500-bp EcoRI-EcoRI fragment) and MF28H (an 800-bp BglII-HindIII fragment). Probes AL1F6EP (a 200-bp EcoRI-FstI fragment) and AL1F6E.3 (a 300-bp EcoRI-EcoRI fragment) are used for the detection of breakpoints in the

conclusion, the detection of dek can be achieved using Southern blot analysis.
Localization of the breakpoint in the can gene of the patients that are numbered according to Table 1. The breakpoint-containing fragments of patients 1 and 4 were cloned and the precise breakpoints were localized. The breakpoints of patients 9 and 11 fall into probe MFlE.5. RV, HindIII; B, BamHI.

Although we do not know yet the exact copy number of the can and dek-can mRNA per leukemic cell, we assume that both show comparable levels of expression based on data obtained by Northern blot analysis (twofold to threefold more expression than can mRNA). Amplification by PCR of the can- and dek-can fragment occurs with the same efficiency. We compared PCR results for can with dek-can. If 100% of cells with can mRNA also express dek-can mRNA all dilution samples will be positive for both can and dek-can. If 10% of cells carry a t(6;9) no dek-can signal is present in the highest dilution while this still contains can signal. For the sequence-specific detection of amplified fragments two oligomers were designed: (1) detection of normal can-cDNA: 5'- GTTATCTGCATITTGCT 3'; (2) detection of dek-can fusion-cDNA: 5'- GCAAAAAAGGAAAATTGC 3'.

Both 32P end-labeled probes were hybridized at 39°C for 3 hours and filters were washed in 1X SSPE for 1 hour at the same temperature. A schematic representation of the PCR procedure is given in Fig 2.

RESULTS

Karyotyping of blood or bone marrow cells of patients was performed in the centers that sent samples for molecular analysis. In metaphases of all patients a t(6;9) (p23;q34) had been found. Patients 1 through 4 were analyzed previously with Southern blot (Table 1, ref 19). Additionally, we obtained DNA from 12 newly admitted cases (patients 6 through 17, Table 1). DNA of each patient was digested using a minimum of three different restriction enzymes. In Figs 3 and 4, some results of conventional Southern blot analysis in various patients are shown. A combination of three different restriction enzymes and four probes enabled us to localize breakpoints in the dek and the can gene in all 16 patients. Moreover, digestion with an additional restriction enzyme (BglII or EcoRI) (or results of PCR experiments, see below) narrowed down the breakpoint localization in most patients to the icb-6 and icb-9. An example was patient 10: exact localization of the breakpoint in the can gene was not possible using Southern blot alone because only the EcoRV digest generated an aberrant fragment with probes AL1F4EP and AL1F6E.3 (Fig 4), while the HindIII and BamHI digests showed germline bands (data not shown). However, a PCR experiment generated a chimeric dek-can fragment (see below), indicating that in-frame joining of dek and can had occurred. In the EcoRV digest of this patient, 3' dek probe MF2BH detects an aberrant band of 7.5 kb (Fig 3). However, a much larger fragment should be expected because the first EcoRV site 5' of icb-9 in can is located 16 kb upstream of this intron.

5.5 kb dek-can mRNA

6.6 kb can mRNA

Fig 2. Schematic representation of the reversed PCR of dek-can and can mRNA. dek is represented as a stippled bar while can is shown as an open bar. A 3' primer in can (1) is used to make cDNA while the PCR is performed with this primer in combination with a 5' primer either in dek (2) or in can (3). This results in amplification of a dek-can or can fragment that can be detected by the specific oligo-probes 4 and 5. The size of the fragments generated by the various primer-sets are indicated beneath each drawing.
MOLECULAR DETECTION OF TRANSLOCATION (6;9) IN AML

Fig 3. Detection of breakpoints in the dek gene using EcoRV (patients 6, 9, 11, 12, 13, 14, 10, 15) or HindIII (patient 8) as restriction enzymes in combination with probes MF1E.5 and MF2BH. The patients are numbered according to Table 1. The size of the germ line bands is indicated in kilobases (kb). Aberrant fragments have the following sizes: patient 6, 4.4 kb; patient 9, 7.5 kb and >23 kb; patient 11, 8 kb and >23 kb; patient 12, 16 kb; patient 13, >23 kb; patient 14, 16 kb; patient 10, 7.5 kb; patient 15, >23 kb; patient 8, 9.5 kb. 8h, DNA of peripheral blood cells from patient 8; 8b, DNA of bone marrow cells from patient 8; N, DNA of thymus or white blood cells from a nonleukemic individual.

(Fig 1B). Therefore, we assume that patient 10 has a large deletion of the 5' can gene which has not been mapped more precisely. The most accurate prediction for the position of the icb-9 breakpoint in this patient is between the 5' end of icb-9 and probe AL1F4EP (results not shown).

Patient 8 was diagnosed in a preleukemic phase preceding overt AML. His bone marrow contained 25% blasts, while in the peripheral blood only 1% blasts were observed (Table 1). He suffered from paraneoplastic neutrophilic dermatosis, also called variant Sweet's syndrome. Blood differential counts indicated that more than 95% of his leukocytes were granulocytes. The marrow also contained variably high numbers of granulocytes that diluted the blasts; therefore, it was difficult to diagnose AML on purely morphologic grounds. We determined whether the peripheral blood granulocytes carried the t(6:9). Indeed, Southern blot analysis of both blood and bone marrow generated germ line and aberrant fragments of equal intensity, indicating the presence of the t(6;9) in the vast majority of the marrow and blood cells and implying that the granulocytes carried the t(6;9) as well (Fig 3).

In 14 cases (patients 2 through 12, 14, 15, 17, Table 1) sufficient material was available for RNA isolation and subsequent PCR amplification of chimeric dek-can cDNA. As shown in Fig 2, primer-set I generates a fragment of 290 bp when a chimeric dek-can mRNA is present and a 335-bp fragment from the can mRNA template. However, these primers were not optimal and gave rise to aspecific priming on the can mRNA (see Fig 6). Therefore, primer set II was made. The dek-can product of primer set II is 222 bp while the can fragment is 305 bp (Fig 2). Results of the analysis of four patients using primer-set II are shown in Fig 5A. Via PCR a hybrid dek-can mRNA was detected in 10 patients with a t(6;9), while RNA from leukemia patients without a t(6;9) or healthy controls yielded only the fragment derived from the can mRNA. No conclusions can be drawn for

Fig 4. Detection of breakpoints in the can gene of eight patients using restriction enzymes BamHI, HindIII, or EcoRV in combination with probes AL1F4EP and AL1F6E.3. The patients are numbered according to Table 1. The size of the germ line fragments is indicated in kilobases (kb). Aberrant fragments have the following sizes: patient 12, >23 kb; patient 6, 9.5 kb; patient 13, 10 kb; patient 15, 2.4 kb; patient 14, 5 kb; patient 9, 6.5 kb; patient 11, 8 kb; patient 10, 11 kb. N, DNA of thymus or white blood cells from a nonleukemic individual.
patients 2, 3, 6, and 14. Although intact RNA of these patients was available, neither a dek-can nor a can fragment was detected after cDNA preparation followed by PCR.

Because PCR is a sensitive diagnostic method we used this technique for analysis of material containing a minority of cells carrying the t(6;9). Comparison of amplification of can with amplification of dek-can in a dilution series gives an estimation of the fraction of cells in a sample from a patient with t(6;9). Both blood and bone marrow cells from patient 8 were investigated after chemotherapy. Hematomorphologic studies indicated that still 10% blasts were present in the bone marrow (Table 1). With PCR the presence of residual disease in bone marrow cells was seen in dilutions of 10^{-1} and 10^{0}. The signal of dek-can in the 10^{0} dilution is comparable with the can signal in the 10^{-2} dilution, indicating that roughly 1/100 cells carry the t(6;9) (see Materials and Methods). No t(6;9) carrying cells could be detected in the blood (Fig 6).

Patient 17 had been diagnosed as an RAEBt and in diagnostic phase all metaphases showed a t(6;9). Blood cells were analyzed by us from a period after 4 days of interleukin-2 (IL-2) therapy, which was given for activation of the T cells. The karyotype of this sample was unknown, but 3% blasts had been observed in the peripheral blood. Because this low amount of blasts may be difficult to detect by Southern blotting we decided to use PCR. Clearly, a chimeric dek-can fragment was generated, indicating persistence of the translocation (Fig 5B). Despite the low number of blast cells, Southern blot analysis confirmed this result (data not shown).

![Blood and Bone Marrow](image)
DISCUSSION

The entity of AML consists of a heterogeneous group of diseases. Subclassification according to the FAB facilitates diagnosis and enables physicians from different hematologic centers to exchange and compare data. Moreover, the prognosis for a patient depends on the FAB subtype which is found. Additional independent prognostic factors are the various chromosomal abnormalities that are linked to specific FAB subtypes. A t(6;9)(p23;q34) can be found in 0.5% to 4% of patients with AML. To date, 34 patients have been reported in the literature whose karyotype showed a t(6;9). When the 17 patients who were analyzed by us at the molecular level, some general features emerge for this group of 51 patients. Diagnosis of this disease is usually made in the second or third decade of life in contrast to AML as a whole group in which the median age is above 60 years. No striking male or female preponderance is found. FAB classification of the AML was frequently reported as M2 or M4 with a minority of M1. However, Cuneo et al pointed out that a number of patients with t(6;9) were reported who were diagnosed as RAEB, which is a subtype of myelodysplastic syndrome (MDS). Close scrutiny of the literature confirmed this observation, and we estimated RAEB with t(6;9) to be third in rank after, AML-M2 and M4 with this translocation. Patient 3 presented with AMF, which eventually evolved into AML-M4. One of our 17 patients was diagnosed as a Ph- BCR- CML. CML with t(6;9) was reported once before by Fleischman et al. But the role of this feature involves careful examination of marrow morphology.

To date, the immunophenotype of blast cells has only been studied in two patients and this showed HLA-DR/ TdT/CD13-positivity in both cases. It would be interesting to investigate a larger group of patients with t(6;9) for TdT-positivity because its presence in leukemias of myeloid origin indicates an unfavorable prognosis. One of our patients (patient 8) presented with a variant of Sweet’s syndrome, a paraneoplastic syndrome characterized by high fever, cutaneous lesions, and granulocytosis. At diagnosis, his blood contained mainly mature granulocytes. Southern blot analysis of the blood and bone marrow cells of this patient showed germ line and aberrant fragments of similar size. This is a cytogenetic indication that the 6p- derivative that carries the dek-can fusion gene is important in this type of leukemia. Additional aberrations are rare but may occur, especially during progression of the leukemia. The most frequently observed extra cytogenetic abnormalities are trisomy 8 and trisomy 13. Trisomy 8 is often seen during progression of myeloid leukemias and is found in many cases of AML and CML in accelerated or blast phase. Trisomy 13 is a rare event in leukemias but has been reported to occur as a sole aberration in AUL, AML, and RAEB. The finding of a trisomy 8 or a trisomy 13 in any leukemia is an ominous sign and heralds an unfavorable outcome of the disease. One of the patients studied here (patient 13) showed deletion of the long arm of chromosome 7 in addition to a t(6;9). This abnormality is associated with the so-called secondary AML or MDS and also predicts an unfavorable prognosis. To what extent all these secondary cytogenetic changes contribute to the overall prognosis of patients with a t(6;9) is unknown at present.

The discovery of a translocation (6;9) in metaphases of a patient with leukemia is alarming because such patients respond poorly to therapy: in our group of patients only half of the recorded cases achieved a complete remission after therapy, which is in concordance with the data in the literature. This forms a sharp contrast to a comparable age group of patients with AML in whom a 77% complete remission rate can be achieved with chemotherapy. Mostly, survival does not exceed 3 years and in the group we analyzed only one case, patient 10, survived 3 years after a bone marrow transplantation and is still in remission. Correct diagnosis of t(6;9) is of utmost importance in trying to improve the prognosis for these patients in future. Because the reciprocal translocation involves small chromosomal fragments of similar morphology, cytogenetic diagnosis of this disease can be difficult. Initially, von Lindern et al showed that four of four t(6;9) patients contained breakpoints in both icb-6 and icb-9. Because icb-6 and icb-9 represent introns in the dek and can genes, respectively, the translocation apparently fuses the same dek “donor” exon to the same can “acceptor” exon, resulting in the formation of uniform dek-can fusion genes on the 6p- chromosome of these patients. The data presented in this report corroborate and further extend the initial observation: the translocation is amazingly precise and highly consistent in 17 t(6;9) patients analyzed at the molecular level. Since the total target size for the translocation per haploid genome amounts to less than 20 kb of DNA (icb-6 and icb-9), this may well explain the low incidence of the translocation in AML (0.5% to 4%). Standardized Southern blot analysis, using restriction enzymes EcoRV, HindIII, and BamHI in combination with the limited number of four probes, is a reliable
method for diagnosis of t(6;9) and is therefore clinically applicable.

Because of the limited amount of material, intact RNA could only be analyzed in 14 cases (Table 1). In 10 of 14 cases the PCR generated a dek-can chimeric fragment using one primer set and confirmed the results obtained by Southern blot analysis. Intact RNA of four patients yielded neither can- nor dek-can fragments after amplification. This is probably due to technical insufficiency, because inhibition of the Taq polymerase by substances in blood has been described. We infer from our results that the chimeric gene is expressed in the leukemic cells of all of the t(6;9) patients that could be analyzed. Moreover, the chimeric fragment is detected in all of these patients using the same dek-can oligomer, indicating that it originates from the same exon fusion product in these 10 cases.

The uniform findings at the DNA and RNA level in 17 patients with t(6;9) indicate that this translocation is highly suitable for molecular detection by Southern blotting and reversed PCR. The latter technique allows sensitive detection of residual leukemic cells after chemotherapy and after bone marrow transplantation through monitoring the presence of the dek-can mRNA. The consistent finding of the chimeric product in patients with a t(6;9) also strongly argues for a distinct causative role of the dek-can fusion gene in this myeloproliferative disorder. Occurrence of this translocation in various subtypes of AML (M1, 2, 4, AMF) and the observations in patient 8 indicate that the dek-can gene product does not cause maturation block. It remains to be established whether the translocation invariably leads to leukemia.

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