Isochromosome 17q in Ph1-Negative Leukemia: A Clinical, Cytogenetic, and Molecular Study

By Reinhard Becher, Felix Carbonell, and Claus R. Bartram

We report on eight patients who were 35 to 77 years old with an isochromosome 17q as the sole structural chromosomal anomaly. Additional numerical chromosomal changes were a trisomy 8 or 17 in two cases each and a trisomy 19 in one case. Five patients had myelodysplastic syndrome (MDS) diagnosed according to the FAB nomenclature as chronic myelomonocytic leukemia (CMML) in two cases, refractory anemia with excess of blasts in transformation (RAEBt) in two cases, and refractory anemia with excess of blasts (RAEB) in one case. One patient suffered from a myeloproliferative disorder (MPD). All cases progressed to acute nonlymphocytic leukemia (ANLL) type M1, M2, or M4 in a period of 2 to 30 months after initial diagnosis, except one patient with RAEBt who died within 2 months. Two patients presented with ANLL-M2 at time of diagnosis. Treatment during the chronic phase of disease consisted of mild cytoreduction and/or substitution of platelets or red blood cells. One patient with CMML received an allogeneic bone marrow graft and relapsed after 33 months with ANLL-M1. Treatment results for overt leukemia were poor, and survival was short, lasting from 1 to 4 months. Overall survival was 1 to 37 months (median duration, 6.5 months). Molecular studies in two cases revealed neither a BCR rearrangement nor a translocation of the ABL protooncogene, as observed in Ph1-positive chronic myeloid leukemia (CML). Thus, an i(17q) anomaly seems to identify a distinct subgroup of mostly myelodysplastic and, less frequently, myeloproliferative disorders that progress rapidly to ANLL, respond poorly to chemotherapy, and are associated with short survival after transformation.

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NORRANDOM CHROMOSOMAL changes in malignant hematopoietic disorders are increasingly used for the subclassification of these disorders. In acute leukemia they have been shown to play an important role as an independent prognostic parameter. Accordingly, a new nomenclature was recently proposed that uses chromosomal anomalies in combination with immunologic and morphologic features and represents a significant improvement over the standard FAB classification.1,2

The most frequent specific chromosome changes in hematologic disorders are translocations that lead to genetic rearrangements without loss or gain of chromosomal DNA. Other changes include isochromosomes formed by the loss of one arm of a chromosome and duplication of the remainder. The most frequently observed isochromosome in malignant hematologic disorders is isochromosome 17, characterized by loss of the p arm and a subsequent duplication of the q arm at the centromere. This anomaly is a common event, resulting from clonal evolution in Ph1-positive chronic myeloid leukemia (CML), and is closely related to the terminal phase of this disorder.3

In this article we report on hematologic and cytogenetic findings in eight patients and molecular studies in two patients, all with a fatal Ph1-negative hematologic disorder in which an i(17q) remained the only structural chromosomal anomaly throughout the course of disease. This anomaly identifies a poor risk group of patients with acute nonlymphocytic (ANLL) or acute unclassified leukemia (AUL), frequently preceded by a short myelodysplastic or myeloproliferative phase.

PATIENTS AND METHODS

Patients. Cases 1 through 6 were studied in Essen and cases 7 and 8 in Ulm. Hematologic data and information about treatment during the chronic and/or acute phase of disease are summarized in Table 1. The age of patients ranged from 35 to 77 years. Gender type was equally distributed. Six patients presented with a chronic disorder diagnosed as refractory anemia with excess of blasts in transformation (RAEBt) in two cases, chronic myelomonocytic leukemia (CMML) in two cases, and refractory anemia with excess of blasts (RAEB) and a myeloproliferative syndrome (MPS) in one case each. One patient with RAEBt died 2 months after diagnosis; all others progressed to acute nonlymphocytic leukemia (ANLL) type M1 (two cases), type M2 (two cases), and type M4 (one case). Duration of the chronic phase was short, ranging from 2 to 13 months. Patient 2 received allogeneic bone marrow transplantation (BMT) and achieved a remission for 33 months before relapsing with ANLL-M1. All other treatment approaches in the chronic phase were unsuccessful or only temporarily successful and did not prolong survival. Results of intensive chemotherapy in the acute phase were also disappointing, with no case of complete remission. Overall survival seemed improved only in the patient who received BMT (37 months). All other patients survived between 1 and 15 months from time of first diagnosis.

Cytogenetic studies. Heparinized cell material for chromosomal analysis was derived from bone marrow punctures and/or peripheral blood. Cells were cultured either in RPMI 1680 or McCoy's medium containing 20% fetal calf serum and antibiotics and were harvested after 16 to 24 hours. G-banding was performed according to standard procedures after short-term trypsin treatment.

DNA analysis. DNA (10 μg) was digested with Bgl II and HindIII, as well as EcoR I electrophoresed in a 0.7% agarose gel, blotted and hybridized to a 1.2 kb HindIII/Bgl II 3′ bcr and a 2 kb

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0.2 pg/mL in 50% formamide/2x SSC/10% dextran sulphate, added to the chromosomes, and hybridized for 10 hours at 37°C. The probe was denatured for HindIII/EcoRI and SSC, and dehydrated. The probe, a 1:1 mixture of human 3' 1.1 kb BglII/ HindIII probe, as described. Lambda DNAs were included as molecular weight standards (data not shown). After hybridization and subsequent washing, filters were exposed to XAR-2 film (Eastman Kodak) for 2 days at -70°C with Dupont Lightning Plus intensifying screens (Wilmington, DE).

In situ hybridization. Chromosomes obtained from bone marrow were prepared according to standard techniques. After RNase treatment, chromosomes were denatured in 70% formamide/2x SSC at 70°C for 2 minutes, rinsed in 2x SSC, and dehydrated. The probe, as described, was labeled by nick-translation using (32P)dCTP and (32P)dTTP (New England Nuclear, Boston, MA) to a specific activity of 2.4 × 10⁶ cpm/μg. The probe was denatured for 5 minutes at 70°C at a concentration of 0.2 μg/mL in 50% formamide/2x SSC/10% dextran sulphate, added to the chromosomes, and hybridized for 10 hours at 37°C. Slides were rinsed in three changes of 50% formamide/2x SSC at 39°C, followed by a 6-hour washing in 2x SSC at room temperature. Slides were then exposed to Kodak NTB2 emulsion for 18 days at 4°C, developed, and stained with quinacrine mustard, as described.

**RESULTS**

**Cytogenetic findings.** An i(17q) chromosome was detected in seven cases at time of diagnosis. There was no case of an isodicentric i(17q). Additional numerical anomalies were: a trisomy 8 in two cases, an additional chromosome 17 in two cases, and a trisomy 19 in one case. Case 2 presented with a normal karyotype at time of diagnosis and developed the i(17q) anomaly at time of relapse, 33 months after allogeneic BMT. The relapse was considered to be of the recipient type. Residual normal metaphases were detectable only in two patients. Table 2 presents the detailed results of cytogenetic studies, and Fig 1 shows partial karyotypes.

**Molecular findings.** Ph¹-positive CML patients' 3' BCR sequences are translocated to chromosome 9q+, and 5' BCR sequences fuse with ABL sequences on the Ph¹ chromosome. Southern blot analysis of Cases 3 and 4, however, revealed no rearrangement within the BCR gene on chromosome 22. Hybridization of 5' and 3' BCR probes on DNAs detected 5 kb germline bands.

To investigate the possible involvement of abl oncogene sequences in genomic alterations, we performed in situ hybridization studies of ABL sequences to metaphase chromosomes in Case 4. Distribution of silver grains obtained from analysis of 37 well-spread metaphases was uniform and at random on all chromosomes, except for the specific signal on chromosome 9 (P < .01). Of the 182 grains observed on all chromosomes, 16% clustered at band 9q34. Analysis by Poisson distribution with the number of grains per chromosome adjusted for the relative size of the band in a 400-band ideogram revealed a highly significant (P < 10⁻¹⁵) grain accumulation at this band. Thus, in contrast to all Ph¹-positive CML patients, in situ hybridization and Southern analysis of Cases 3 and 4, however, revealed no

| Table 1. Hematologic Findings and Treatment of Cases 1 to 8 During the Chronic and Acute Phase of Disease |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | 1               | 2               | 3               | 4               | 5               | 6               | 7               | 8               |
| Age/sex        | 77/F            | 35/M            | 65/M            | 51/F            | 67/M            | 55/M            | 66/F            | 62/M            |
| Chronic phase  | RAEB            | CMMML           | —               | MPS             | CMMML           | RAEB            | —               | RAEB            |
| Hb (g/dL)      | 4.8             | 7.7             | —               | 12.0            | 5.0             | 8.1             | —               | 9.5             |
| WBC (x 10⁹/L)  | 1.9             | 2.0             | —               | 79.0            | 3.0             | 6.5             | —               | 2.7             |
| % Blasts (blood) | 2               | 1               | 0               | 0               | 5               | 0               | 5               | —               |
| Platelets (x 10⁹/L) | 47              | 20              | —               | 300             | 60              | 84              | —               | 14              |
| % Blasts (marrow) | 25*            | 2*              | —               | 0               | 3*              | 20*             | —               | 10              |
| Treatment      | RC, C           | RC, BMT         | H, RC, BUS       | RC              | NT              | C               | —               | —               |
| Duration (mos) | 2               | 31+330§         | 12              | 6               | 2               | 13              | —               | —               |
| Acute phase (ANLL) | —              | M1              | M2              | M1              | M4              | M2              | M2              | M2              |
| Hb (g/dL)      | —               | 9.7             | 9.3             | 9.4             | 10.5            | 9.6             | 7.7             | 9.5             |
| WBC (x 10⁹/L)  | 249             | 113             | 50.7            | 33              | 6.9             | 1.0             | 2.7             | —               |
| % Blasts (marrow) | —              | ND              | 84*             | 80+             | 80              | 50*             | 90              | 80              |
| Duration (mos) | 1               | 4               | 3               | 2               | 3               | 1               | 2               | —               |
| Survival (mos) | 2               | 37              | 4               | 15              | 8               | 5               | 1               | 13              |

Abbreviations: RC, red cell packs; BUS, busulfan; WBC, white blood cells; Hb, hemoglobin; O, vincristin; P, prednisone; C, cytosine arabinoside; T, thioguanine; D, daunorubicin; M, 6-mercaptopurine; A, adriamycin; Mi, mithramycin; H, hydroxyurea; ND, not determined; NT, not treated.

*Microsomakaryocytes increased.
†Basophilia.
§Complete remission for 33 months after BMT.

**Table 2. Cytogenetic Data**

<table>
<thead>
<tr>
<th>Case (Initials)</th>
<th>Karyotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (St.H.)</td>
<td>47.XX,i(17q)+B: [31]</td>
</tr>
<tr>
<td>2 (Sch.H.)</td>
<td>46.XY,i(17q): [6]</td>
</tr>
<tr>
<td>3 (P.E.)</td>
<td>46.XY,i(17q): [20]</td>
</tr>
<tr>
<td>4 (O.I.)</td>
<td>46.XY,i(17q): [4]</td>
</tr>
<tr>
<td>5 (P.W.)</td>
<td>46.XY,i(17q): [9]</td>
</tr>
<tr>
<td>6 (B.I.)</td>
<td>46.XY,i(17q): +19: [12]</td>
</tr>
<tr>
<td>7 (H.Ch.)</td>
<td>46.XX,i(17q): +17: [40]</td>
</tr>
<tr>
<td>8 (J.W.)</td>
<td>46.XX,i(17q): +17: [17]</td>
</tr>
</tbody>
</table>

Cytogenetic data of patients 1 through 8 at time of first diagnosis, except for Case 2, who developed the i(17q) anomaly at time of transformation to ANLL-M2 and presented with a normal karyotype at time of diagnosis.
ISOCHROME 17q IN Ph'-NEGATIVE LEUKEMIA

1) 2) 3) 4) 5) 6) 7) 8) 17 i(17q) 17 i(17q)

Fig 1. Partial karyotypes of eight cases with an i(17q) as the sole structural chromosomal anomaly.

blot analysis failed to detect a BCR-ABL rearrangement in this patient.

DISCUSSION

In reviewing the literature, we found 30 Ph'-Negative cases with an i(17q) as the only structural anomaly. These cases are summarized in Table 3 with our cases. Numerical anomalies in the published cases were a trisomy 13 (Cases 21, 27, 28), an additional chromosome 21 (Cases 3 and 11), and a monosomy 9 (Case 17). There was no apparent prevalence of gender type, with 18 of 34 female and 16 of 34 male cases. Most patients were 50 or more years old (22 of 30 patients). Only two children showed this anomaly (10 and 12 years old).

Including our cases, 24 patients were diagnosed with a chronic hematologic disease, including various forms of MDS (12 cases), as well as different myeloproliferative disorders (12 cases). The latter were described as Ph'-negative CMLs, myelofibrosis with myeloid metaplasia, essential thrombocytopenia, or polycythemia vera. Accordingly, during the chronic phase, the phenotype of these disorders was heterogeneous, including several cases of MDS that seemed not to fit well into the FAB classification. Only four patients died during the chronic phase of disease, and two cases were in sustained chronic phase at time of the respective report. Sixteen cases presenting with a chronic disorder progressed to ANLL, mostly of type M2.

Fourteen patients already suffered from acute leukemia at time of diagnosis, mostly ANLL except for three cases, which were classified as acute lymphocytic leukemia (ALL; Cases 3, 17, and 23). No increased mutagenic exposure was reported in the literature or observed in our patients.

During the chronic phase of disease, patients received anabolic hormones or low dose cytostatic drugs with little or no substantial benefit. Frequently, treatment was reduced to blood cell substitution only. One of our patients received an allogeneic bone marrow graft but relapsed after almost 3 years. In the terminal phase of disease, treatment consisted of different intensive chemotherapy combinations, such as also given for de novo acute leukemias. The outcome, however, was generally poor. In only one patient in the literature (Table 3, Case 25) was a complete remission reported, which lasted for 13+ months. While the duration of the chronic phase ranged from 2 to 72 months (median, 12 months), survival after onset of the terminal acute phase ranged only from less than 1 to 13+ months (median, 2 months). The biphasic course of disease resembles CML; however, it is more heterogeneous regarding the phenotype of the chronic phase and clearly more aggressive. Frequent clinical features during the chronic course are anemia and splenomegaly, which are relatively unspecific in chronic hematologic disorders.

An i(17q) chromosome is a common finding in CML blast crisis resulting from clonal evolution. It seems to be closely related to a myeloid type of transformation, which is also the case in the large majority of patients with an i(17q) as the sole abnormality.

The numerical changes observed in some of our cases (trisomy 8 and 19) and those reported in the literature (trisomy 13 and 21) have been shown to be associated not only with myeloid CML blast crisis, but also MDS and ANLL. These changes seem to be less specific than the i(17q) and might, therefore, involve the activation of genes that lead to a further growth advantage of leukemic myeloid cells, without a significant impact on the phenotypic expression.

Since most cases with an i(17q) as the only structural anomaly have a biphasic course of disease similar to Ph'-positive CML, it was necessary to study molecular rearrangements of Ph'-positive CML, which have also been described in the absence of structural chromosomal anomalies. Southern blot analyses performed in two of our cases (Cases 3 and 4) excluded genetic rearrangements of the M-BCR region and in situ hybridization a translocation of the ABL proto-oncogene (Case 4). This finding, together with the observation that an i(17q) in this disorder can already be present during the chronic phase of disease, distinguishes it clearly from CML. In rare cases, the i(17q) was not yet detectable during the chronic phase of the i(17q) disorder. Our Case 2, as well as Cases 20 through 22 in the literature (Table 1), revealed the i(17q) only at the time of onset of the terminal phase. This observation might indicate that the i(17q) anomaly is not necessarily the first event in the process of initiation of disease, and that it might be preceded by changes not detectable at the cytogenetic level.
colony-stimulating factor (G-CSF) gene, responsible for the terminal maturation of granulocytes, is located at 17q11-21, and the gene for myeloperoxidase (MPO), which is located at 17q21-q24.

Results of treatment for i(17q) disorders have been poor so far. Red cell packs and/or mild cytoreductive therapy were the only effective modality for temporarily treating the acute phase. In younger patients, the possibility of allogeneic BMT should be further evaluated.

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