Graft-Versus-Leukemia Effects After Allogeneic Bone Marrow Transplantation Are Active Also in the Presence of Clones with Chromosomal Anomalies in Addition to the Ph Chromosome

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ABSTRACT: Two male patients with Philadelphia-chromosome (Ph+) chronic myelogenous leukemia (CML) underwent allogeneic bone marrow transplantation (ABMT) in the first chronic phase after busulfan treatment. In both cases, the donor was a sister, and engrafting was demonstrated by chromosome analyses which showed only donor cells in the BM. Cytogenetic relapse occurred 29 and 30 months after ABMT, respectively, when host cells reappeared: in both cases, the Ph and additional anomalies typical of the blastic phase of CML were evident. We then monitored the chromosome picture for 52 and 39 months, respectively: no striking evolution occurred, and cells with the Ph and additional anomalies persisted together with donor cells, which were a minority in the first patient and a great majority in the second throughout the observation period. A clinical relapse was observed in the first patient, but the disease never progressed to a blastic phase, whereas the second patient has not relapsed 7 years after ABMT. We reviewed data from the literature on cytogenetic relapse after ABMT in CML without clinical relapse, especially the 12 patients in whom cytogenetic relapse included chromosome anomalies in addition to the Ph, as in our patients. We suggest that graft-versus-leukemia (GVL) reactions in such patients are able to arrest progression of the leukemic blastic clone and prevent a possible relapse in blastic phase.

INTRODUCTION

After allogeneic bone marrow transplantation (ABMT) patients with graft-versus-host disease (GVHD) have been repeatedly demonstrated to have a decreased risk of leukemic relapse as compared with patients without GVHD [1, 2]. This is due to an immune-mediated reaction, called graft-versus-leukemia (GVL) effect, whose mechanism is not yet well defined but is mediated by the donor's T-cells. Indeed, when the donor's marrow is T-cell-depleted, the risk of relapse is substantially increased [3].

We report two cases of ABMT in patients with chronic myelogenous leukemia (CML) in whom GVL was demonstrated and monitored by repeated chromosome analyses during a long period of clinical remission, but with a relapse at the cytogenetic level, with reappearance of the Ph together with additional chromosome anomalies.

CASE REPORTS

Case 1
V. S., a man born in 1946, was in good health in March 1980 when a blood examination showed a white blood cell (WBC) count of 21,000/μl. In September 1981, a diagnosis of CML was made on the basis of the following features: enlargement of liver and spleen (both 2 cm from the costal margin); WBC 21,100/μl, with 36% neutrophils, 1% eosinophils, 1% basophils, 49% lymphocytes, 8% metamyelocytes, 4% myelocytes, 1% promyelocytes, and platelet count 700,000/μl; and a leucocyte alkaline phosphatase score of 1 [normal values 20–100]. A bone marrow (BM) trephine biopsy was typical for CML. Since November 1981, he was treated with intermittent courses of busulfan with good results. HLA-typing of family members disclosed an HLA-identical sister, and the patient decided to undergo ABMT, which was performed on February 1, 1983 in the...
Table 1  Patient 1: Results of chromosome analyses

<table>
<thead>
<tr>
<th>Date (days ABMT)</th>
<th>Material</th>
<th>No. of cells</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/16/81 (− 503)</td>
<td>PB</td>
<td>14</td>
<td>46,XY,t(9;22)(q34;q11)</td>
</tr>
<tr>
<td></td>
<td>PB-PHA</td>
<td>5</td>
<td>46,XY,t(9;22)(q34;q11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>46,XY</td>
</tr>
<tr>
<td>2/22/83 (+ 21)</td>
<td>BM</td>
<td>20</td>
<td>46,XX</td>
</tr>
<tr>
<td>2/13/84 (+ 377)</td>
<td>BM</td>
<td>13</td>
<td>46,XX</td>
</tr>
<tr>
<td>7/22/85 (+ 902)</td>
<td>BM</td>
<td>15</td>
<td>46,XX</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>46,XX</td>
</tr>
<tr>
<td></td>
<td></td>
<td>55</td>
<td>46,XY,t(3;9;22)(p21;q34;q11), t(9;12)(q34;p15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>46,XY,t(9;22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>46,XY,t(3;9;22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>46,XY,t(3;9;22),t(9;12)</td>
</tr>
<tr>
<td>4/29/87 (+ 1,548)</td>
<td>PB</td>
<td>6</td>
<td>46,XX</td>
</tr>
<tr>
<td>1/8/88 (+ 1,802)</td>
<td>PB-PHA</td>
<td>1</td>
<td>47,XY,t(9;22), + der(22)t(9;22)</td>
</tr>
<tr>
<td>6/6/89 (+ 2,317)</td>
<td>PB</td>
<td>4</td>
<td>46,XX</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>46,XY,t(9;22)</td>
</tr>
<tr>
<td>11/9/89 (+ 2,473)</td>
<td>PB</td>
<td>26</td>
<td>45,XY,t(3;9;22),t(9;12), − 10, der(10)t(10;17)(q24;q11.2), − 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>47,XY,t(9;22)</td>
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<tr>
<td></td>
<td></td>
<td>2</td>
<td>46,XX</td>
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Abbreviations: ABMT, allogeneic bone marrow transplantation; PB, peripheral blood unstimulated culture; PB-PHA, PB-phytohemagglutinin-stimulated cultures; BM, BM direct preparations and 24-hour cultures.

*Analyses performed at the Fred Hutchinson Cancer Research Center, Seattle, Washington.

Fred Hutchinson Cancer Research Center, Seattle, WA. The conditioning regimen included cyclophosphamide (CY) and total body irradiation (TBI), and he received 250 ml untreated BM. The transplantation was successful; BM aspirates at days +14 and +21 after ABMT showed a good trilineage engraftment, and blood indexes became progressively normal. He received methotrexate as prophylaxis against GVHD. Indeed, although he had complained of pruritis and of a discrete, slightly macular skin rash since day +6, no clear-cut signs of GVHD were present until day +100, in May 1983. At that time, a lip biopsy was positive for GVHD and he was considered affected by chronic GVHD, with two systems (skin and liver) involved. He was placed on an experimental therapy protocol that included prednisone and azathioprine. When evaluated in February 1984 (day +377), the patient was doing well and GVHD appeared to have resolved. Azathioprine was discontinued, but after 2 months was restarted owing to the patient’s deteriorating condition.

In August 1985 the WBC count was 14,700/μl and the platelet count was 880,000/μl, and cytogenetic relapse was observed (Table 1). Busulfan and hydroxyurea were administered, but the platelet number remained high. In October 1986, treatment with recombinant α-interferon (IFN-α) was started, combined with intermittent courses of chemotherapy. Although complete control of thrombocytosis was never achieved, the patient’s condition remained stable, and treatment was continued until the end of 1988.

In January 1989, the patient complained of increasing asthenia: anemia progressed [hemoglobin (Hb) 7.5 g/dl] and transfusions became necessary. Bone pain and fever developed in the following months, and he was treated with hydroxyurea and 6-mercaptopurine. The WBC count remained moderately increased, whereas the platelet count progressively decreased. In November 1989, his blood counts were: Hb level 7.2 g/dl, WBC count 27,800/μl with 22% neutrophils, 22% lymphocytes, 9% monocytes, 22% myelocytes, 25% metamyelocytes; and platelet count 20,000/μl.

The patient’s condition progressively deteriorated, with continuous bone pain and fever, but blood counts remained substantially unchanged. He was admitted to the hospital in March 1990 owing to melena. His Hb level was 7.5 g/dl; RBC count was 2,510,000/μl, and WBC count was 23,310/μl with 50% neutrophils, 12% lymphocytes, 1% monocytes, 12% metamyelocytes, 20% myelocytes, 5% promyelocytes, and platelets 30,000/μl. On March 30, the WBC count was 71,700/μl with 46% neutrophils, 6% lymphocytes, 10% metamyelocytes, 20% myelocytes, 5% promyelocytes, and 13% myeloblasts. He died 2 days later.

Case 2

M. F., a man born in 1942, complained of asthenia in April 1980; a mild splenomegaly was noted (2 cm below costal margin), and blood indexes were: Hb level 13.4 g/dl, RBC count 4,470,000/μl, WBC count 114,600/μl with 60% neutrophils, 1% basophils, 6% lymphocytes, 2% monocytes, 13% myelocytes, 1% metamyelocytes, 2% myeloblasts, and platelet count 85,000/μl. A diagnosis of CML was made, and he was treated with busulfan with a complete remission after 6 months.

HLA-typing of family members disclosed an HLA-identical sister, and ABMT was performed with untreated BM on June 7, 1984 in a Pescara hospital. The conditioning...
regimen included CY and TBI. The engraftment was successful, with normalization of the blood counts.

A grade II GVHD appeared in October 1984 with predominant skin involvement despite CYA given to prevent it. The patient's condition remained satisfactory, until May 1986, when cytogenetic relapse occurred. Blood and BM parameters were normal, but in May 1987 therapy with IFN-α was started and continued until September 1990, although the patient decided to stop IFN-α therapy several times for a few months at a time. He has no major problem and blood counts are normal, except for a slight decrease in platelet number (70,000–100,000/μL).

When last examined in September 1991, the patient was well and blood indexes were Hb 14.8 g/dl, RBC 5,580,000 μL, WBC 5,100/μL with 42% neutrophils, 1% eosinophils, 1% basophils, 52% lymphocytes, 4% monocytes, and a platelet count of 113,000/μL.

Cytogenetic Studies

Chromosome analyses were performed with routine methods on BM direct preparations and 24- and 48-hour cultures and on unstimulated blood cultures (24 and 48 hours). Phytohemagglutinin (PHA)-stimulated cultures were also made in patient 1. QFQ-, CTT-, and CBG-banding techniques were used. The results are shown in Tables 1 and 2.

Patient 1 showed a standard Ph t(9;22) at diagnosis of CML. Two analyses made 21 and 377 days after ABMT at the Fred Hutchinson Cancer Research Center, Seattle, Washington, showed only donor cells in the BM and lymphocytes.

At day +902, we noted only one residual donor cell of 66 analyzed from BM, whereas 65 cells were from the recipient, all with the Ph and additional anomalies. In particular, two distinct clones were present: the first had a duplication of the standard Ph (10 cells), and the second showed a variant three-way Ph rearrangement involving a chromosome 3 besides chromosomes 9 and 22 and an additional translocation between the long arms of the other chromosome 9 at band q34 and the long arms of a #12 (55 cells) (Fig. 1). These findings provided evidence of cytogenetic relapse.

In the following years, the picture remained grossly unchanged, with a few donor cells always present together with a majority of the recipient's cells showing the Ph and the additional anomalies described above. The clone with duplication of the Ph was present in nearly all analyses but never overwhelmed the other clones. Cells with the Ph as sole anomaly were also evident in analyses at days +1,548 and +2,317, whereas the clone with the t(3;9;22) was not apparent in analyses at days +1,602 and +2,317, but appeared at day +2,473 with another aberration superimposed, namely a t(10;17) with loss of the der(17) (Fig. 2).

Patient 2 also had a Ph due to a standard t(9;22) before ABMT. Engraftment was confirmed by analysis 53 days after ABMT at the University of Chieti; all 27 mitoses observed were from the donor. At day +915, the beginning of a cytogenetic relapse was demonstrated, with 20 of 80 BM mitoses belonging to the recipient including a Ph, and with
Figure 1  Q-banded karyotype of one of the clones of patient 1 at cytogenetic relapse (day + 912). Solid arrows indicate t(3;9;22); dotted arrows indicate t(9;12).

Figure 2  Partial karyotype showing the der(10)t(10;17)(q24;q11.2) with loss of the der(17) detected in patient 1 on day +2,473. G-bands (left) and Q-bands (right).
an additional anomaly consisting of the duplication of part of the long arm of #1 (Fig. 3). The cytogenetic picture remained grossly unchanged in this patient during the following years, always with a majority of donor cells and a minority of recipient cells with the Ph and duplication of #1. At day +1,036, we observed further clonal evolution of the Ph+ cell line, which had acquired an apparently balanced translocation between the long arms of the duplicated #1 and a #8, but this rearrangement then disappeared. In analyses at days +1,314 and +2,092, all mitoses were of donor origin.

DISCUSSION

Graft-versus-leukemia reactions cause a decreased risk of relapse after ABMT with unmanipulated BM as compared with T-cell-depleted BM in lymphatic and nonlymphatic leukemias [2]. Recent data indicate that GVL may be at least partially separable from GVHD [4]. With regard to CML, the relative risks of relapse for patients who received T-cell-depleted BM with or without GVHD, as compared with recipients of allografts that are not T-cell-depleted who do not have GVHD are significantly different: 6.91 and 4.45, respectively (p = 0.0001), (data from 2,254 ABMT in leukemia patients included in the International Bone Marrow Transplant Registry) [5]. This indicates that GVL exerts its beneficial effect in CML patients as well. These evaluations are based on a diagnosis of clinical and haematological relapse, however, and do not take into account cytogenetic relapses with reappearance of the recipient’s Ph+ cells during clinical remission. In a group of 67 CML patients who underwent ABMT in the first chronic phase, Thomas et al. [6] reported a cytogenetic relapse rate at 3 years of 0.31 and a clinical relapse rate of 0.22. This difference reflects the fact that in a particular group of patients the reappearance of their own Ph+ cells does not imply clinical relapse and could be related to GVL [7]. The report by Thomas et al. [6] of six patients with only cytogenetic relapse is interesting in this sense because two of them died of infection, whereas four showed the Ph at different periods after ABMT, but subsequently lost the Ph.

The two patients we report had very similar histories, with striking peculiarities at the cytogenetic level. Both were transplanted in the first chronic phase with their sisters’ marrow, and a good engraftment was documented in both (Tables 1 and 2). Cytogenetic relapse occurred at 902 and 915 days after ABMT, respectively, and additional anomalies were detected together with the Ph in both (Ta-
bles 1 and 2). No clinical relapse was evident in either patient at first, but in the following months patient 1 showed slight signs of an accelerated phase, with a persisting thrombocytosis uncontrolled by chemotherapy. The clinical picture, however, remained satisfactory in both patients, and we monitored the cytogenetic relapse until days +2,473 and +2,092, respectively.

Chromosome anomalies in addition to the Ph are considered reliable indicators of blastic or accelerated phase in CML [8], and some of them occur nonrandomly: trisomy 8, duplication of the Ph, isochromosome of the long arm of a #17, and trisomy 19 [9]. The presence of additional anomalies in cytogenetic relapse after ABMT should also be sufficient to categorize the patient as being at least in an accelerated phase. This implies a poor prognosis. Identifying such cases in the literature is difficult, because they are often included in larger series with insufficient information on single cases. We took into account only cases in which available data on the karyotypes were satisfactory to define truly abnormal clones; we were able to find 12 cases in which cytogenetic relapse also included anomalies in addition to the Ph [6, 10–14]. In some of these patients, the clinical course included periods of variable duration in which their BM had cell lines with the Ph and various additional anomalies, whereas the clinical picture was always of remission. We postulate that at least in the patients in whom these periods were quite long, GVL acted to control the leukemic blastic clone. This could be true of the following patients: patients 2 and 4 in the study of Da Silva et al. [10], who showed cytogenetic relapse without signs of clinical relapse for 17 and 13 months, respectively; and patient 15 in the series of Calabrese et al. [11], who in effect is the only patient evaluable in this sense in the sample, and who was monitored for 18 months without clinical relapse; patient B. H. in the series of Lawler et al. [13], who initially had the Ph and an additional anomaly with no sign of relapse and a stable course for about 25 months, at least according to available information, before undergoing a second ABMT.

The possibility that GVL controls a leukemic clone with chromosomal evidence of clonal evolution leading to blastic transformation is better documented by the course of our patients, in whom the GVL appeared to arrest the progression of such a clone and prevent a possible relapse in blastic phase. The additional anomalies evident in these two patients undoubtedly were the signal of a blastic transformation. Among these anomalies, patient 1 showed a duplication of the Ph, one of the most typical changes. This patient, after 1,714 days of cytogenetic relapse with documented clonal evolution (Table 1), died of accelerated disease, but without a true blastic phase; such a development might be conditioned by GVL activity. The proportion of donor cells left in this patient was rather low, at least according to the chromosome data, whereas it was higher in patient 2, whose disease never progressed, although the leukemic clone with the Ph and the additional anomaly never disappeared.

Repeated chromosome analyses in our two patients provided evidence that the donor's engrafted cells in CML may somehow be able to freeze the BM picture for years because of GVL, with the leukemic clone being kept under control even in the presence of chromosome anomalies in addition to the Ph, which usually indicate a beginning or impending blastic transformation.

M. C. is supported by Associazione Studio Malformazioni, Milano. The authors thank Dr. K. Sullivan and Dr. E. Bryant of the Fred Hutchinson Cancer Research Center, Seattle, Washington, and Dr. G. Palko, of Università di Chieti for permission to quote their results of chromosome analyses and Professor M. Fraccaro for help in preparation of this article.

REFERENCES
