

Cytogenetic Analysis of Chimerism and Leukemia Relapse in Chronic Myelogenous Leukemia Patients After T Cell-Depleted Bone Marrow Transplantation

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Serial cytogenetic studies were performed on 64 patients with chronic myelogenous leukemia (CML) after T cell-depleted allogeneic bone marrow transplantation (BMT). Forty patients with CML in chronic phase (CP) received cytoreduction followed by BMT with HLA-matched T cell-depleted allogeneic marrow. The remaining 24 patients were transplanted in second chronic, accelerated, or blastic phase, or received T cell-depleted grafts with a dose of T cells added back. The Y chromosome and autosomal heteromorphisms were used to distinguish between donor and host cells. Mixed hematopoietic chimerism (presence of donor and host cells) was identified in 90% of patients in first CP. The Philadelphia (Ph) chromosome reappeared in 16 of the 40 first CP CML patients. As expected, patients who had detectable Ph chromosome positive cells at any time during the posttransplant period had a high likelihood

of subsequent clinical relapse. Transient disappearance of the Ph positive clone was rarely observed, and was followed by reappearance of the Ph chromosome or clinical relapse. A subset of engrafted patients with greater than 25% host cells within 3 months post-BMT had a significantly shorter survival time free of cytogenetic or clinical relapse compared with other patients. In patients who had received donor T cells added to the T cell-depleted graft, there was a higher proportion of complete chimerism. Clonal progression of Ph positive as well as negative cells was observed and may be the result of radiation induced breakage. Serial cytogenetic studies of patients post-BMT can provide useful information regarding the biologic and clinical behavior of CML.

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BONE MARROW transplant (BMT) as a treatment of chronic myelogenous leukemia (CML) in chronic phase (CP) has resulted in 45% to 70% long-term disease free survival with relapse of disease observed in 7% to 24% of patients.¹⁻⁴ A higher incidence of relapse and a lower incidence of graft-versus-host disease (GVHD) has been reported for T cell-depleted grafts as compared with conventional BMT.¹⁻⁴ Bone marrow metaphase karyotypes studied serially after successful transplantation at various centers have shown several cytogenetic patterns: complete eradication of the Philadelphia (Ph) positive clone, recurrence of Ph positive cells (cytogenetic relapse) in the presence or absence of clinical relapse,¹ transient reappearance of Ph positive cells in the absence of clinical relapse,³ and late (greater than 1 year) disappearance of Ph positive cells in the absence of further therapy.⁴ Studies of genetic chimerism of hematopoietic cells have confirmed the observation of complex cytogenetic patterns posttransplantation. These studies,⁵⁻¹³ using a variety of cytogenetic, molecular genetic, and immunologic techniques, have reported an incidence of mixed hematopoi-

etic chimerism of up to 80% after T cell-depleted BMT for CML in CP.¹³ A negative prognostic significance has been attached to the late emergence of mixed chimerism post-BMT.¹¹ We report here the patterns of chimerism, Ph chromosome status, karyotypic abnormality, and clinical behavior in 64 CML patients and the prognostic significance of cytogenetic findings in 40 patients with CML in first CP treated with T cell-depleted allogeneic BMT.

MATERIALS AND METHODS

Patient population, cytoreduction, and BMT. From January 1984 until December 1987 HLA-matched allogeneic BMTs were given to 67 patients with CML. Post-BMT cytogenetic studies were not performed in three cases, and they are excluded from this analysis. Of the 64 evaluable patients on whom cytogenetic analysis was performed before and after BMT, all received pretransplant cytoreduction with hyperfractionated total body irradiation (TBI; 1,375 to 1,500 cGy) and cyclophosphamide (60 mg/kg twice) as previously described.¹⁴ Fifty-seven patients received HLA-matched marrow depleted of T cells by a modification of the lectin agglutination and E rosette (SBA-E⁻) technique of Reisner et al.¹⁵ The other seven patients received either SBA-E⁻ grafts to which had been added a dose of peripheral T cells calculated to provide 0.4×10^5 cells/kg (five patients), or SBA⁻ marrow (two patients). Of the evaluable group, 40 were transplanted in first CP of CML, 7 in accelerated phase, 5 in blastic phase, and 5 in second CP. The median age of the 64 patients was 32.5 years. There were 54 adults (ages 20 to 48) and 10 children (ages 1 to 19), of whom 31 were male and 33 were female.

Cytogenetic analysis. Chromosomal analysis of bone marrow (BM) samples was performed following previously described methods.¹⁶ Cytogenetic analysis was performed before BMT and at 1/2, 1, 2 to 3, 6, 12, and 24 months post-BMT. In all, 6,053 cells from 278 samples (mean, 22 ± 8.9 metaphases per sample) were evaluated during this study. Q banding was routinely used to characterize chromosomal complements and to determine host and donor heteromorphisms.¹⁷ At some time point before BMT, a Ph chromosome derived from the standard translocation t(9;22)(q34;q21) or a variant or complex translocation was identified in all but three patients; one of these patients has subsequently demonstrated the standard Ph chromosome at relapse. The standard Ph translocation was identified pre-BMT in 57 patients; one patient each had

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t(12;22)(q13;q11) and t(4;9;22)(q21;q34;q11), while two patients had t(9;10;22)(q34;q22;q11).

Karyotypes of clonally abnormal host cells, if any, and the quinacrine-fluorescent heteromorphisms at which the host and donor normal karyotypes differed were determined posttransplant in each case. The origin of each presumptive donor cell was thus established by using the following cytogenetic markers: autosomal heteromorphisms and sex chromosome difference, if any. Using these methods, it was possible to clearly distinguish between host and donor cells in each of the transplant combinations studied. Clonality of abnormal karyotypes was established by identifying at least two metaphases with identical structural changes or three metaphases with identical gains or losses of chromosomes.

Patients with hematologic evidence of recurrent disease were classified as clinical relapses. Evidence of the Ph chromosome in the absence of hematologic relapse was classified as cytogenetic relapse. More specifically, the presence of a Ph positive cell at any time post-BMT was considered a cytogenetic relapse. Chimerism was classified as complete if donor cells only were observed during the entire course from time of BMT to time of clinical relapse (if any) or last follow-up. If all of the non-Ph cells were of host origin, the sample was classified as "all host." Cases that presented post-BMT or eventually developed mixtures of normal donor and non-Ph-bearing host cells were classified as mixed chimeras. In retrospect, many cases of mixed chimeras developed Ph positive clones (cytogenetic relapse), de novo clonal abnormalities in host cells, or nonclonal abnormalities. Patients developing persistent cytopenias in the absence of disease post-BMT, regardless of chimerism status, were referred to as clinical graft failures.

Statistical analysis. Overall and disease free survival from BMT were analyzed for subsets of patients with selected cytogenetic abnormalities using the life table method of Kaplan and Meier,¹⁸ and the log rank test for significance was applied in making statistical comparisons. Cytogenetic subgroups were analyzed for survival with or without the presence of disease (overall survival), for duration of complete remission terminating in hematologic relapse of disease or death (disease free survival), and for survival without presence of cytogenetic or clinical relapse (remission duration). For patients who received more than one transplant, chimerism status was judged from the time of the first transplant, relapse status was judged at first occurrence of relapse, and survival was judged from last follow-up. Subsets of patients with various cytogenetic abnormalities were compared using the method of inference from proportions.¹⁹

RESULTS

Chimerism, relapse, and graft failure in CML first CP. The results of the serial cytogenetic analysis of the 40 chronic phase patients are shown Fig 1. Of the 40 patients, 30 are alive, 19 are free of clinical disease, and 16 are free of clinical or cytogenetic relapse at a median follow-up for survivors of 34 months. Overall actuarial survival for this group of patients was 73.2% (95% confidence interval [CI] \pm 14.6%) at 2 years posttransplantation. Actuarial survival free of clinical relapse was 40.4% (95% CI \pm 17.2%) at 2 years. Duration of disease greater or less than 16 months from time of diagnosis to time of transplantation was not associated with a difference in overall or disease free survival ($P = .2$). While survival rates were not different (83.5% versus 63.2%, $P = .17$), there was a significantly increased disease free survival for the 20 patients younger than the median age of 32.6 years compared with the 20 patients older than 32.6 ($P = .008$). Other patient characteristics and the clinical results of the transplants have been presented previously.²⁰

Of the 40 patients in first CP transplanted with SBA⁺ E⁻ T cell-depleted grafts, 20 had donors of the opposite sex. In the other 20 cases, autosomal heteromorphisms that were most frequently informative in distinguishing between donor and host were, in decreasing order, 22p13, 3q11, 13p13, 21p13, 13p11, 22p11, 9q12, and 15p13.

Of the 40 patients, 36 became mixed chimeras, and 4 became complete chimeras. One patient, a complete chimera, developed grade 2 acute GVHD, the only such occurrence in this group. Twenty-two mixed chimeras started out as complete chimeras and went on to exhibit host cells, while the remaining 14 patients presented initially as mixed chimeras post-BMT.

There was no difference in disease free survival or survival free of hematologic or cytogenetic relapse between the mixed chimeras and the smaller cohort of patients demonstrating complete chimerism ($P > .3$ in both cases). Among the mixed chimeras, the median disease free survival for Ph negative patients has not been reached. For mixed chimeras demonstrating a Ph clone at any time posttransplant, disease free survival was 18 months ($P = .05$; Fig 2). There was no difference in disease free survival or overall survival between patients demonstrating early (less than 1 month) versus late (greater than 1 month) appearance of mixed chimerism, or mixed chimeras presenting initially as complete chimeras and those presenting as mixed chimeras ($P > .2$ in all cases). In 18 of the 36 mixed chimeras, host heteromorphisms were seen in more than 25% of normal marrow cells at some time post-BMT; only 1 of the 18 patients is alive free of cytogenetic or clinical relapse. In contrast, of the 18 patients with less than 25% host cells and the 4 complete chimeras, 15 are alive free of cytogenetic or clinical relapse ($P < .001$; Fig 3). With respect to remission duration, there was a significantly increased median survival free from cytogenetic or clinical relapse for the group with less than 25% host cells, compared with the group with greater than 25% host cells ($P < .001$). There was no significant difference in the probability of durable engraftment post-BMT between these groups ($P = .16$). Despite a trend in favor of the cohort demonstrating less than 25% host cells at all times posttransplant, the overall survival of these two groups was not significantly different ($P = .07$; Fig 4).

We wished to determine whether the chimeric state detected early posttransplant was predictive of subsequent clinical outcome. Nine patients showed greater than 25% host cells within the first 3 months posttransplant, and before evidence of either graft failure or cytogenetic relapse. Of these, three subsequently showed graft failure, four relapsed, and two died of interstitial pneumonia. Of the 31 patients exhibiting less than 25% host cells within this time period and before either graft failure or clinical relapse, 6 subsequently suffered graft failure, and 8 had relapse of disease. Actuarial survival free of cytogenetic or clinical relapse for the latter group was 48.1%, a significantly different outcome than that of the group with greater than 25% host cells ($P = .002$; Fig 5). This difference was reflected by a decreased median survival free of clinical relapse for the nine patients with greater than 25% host cells within 3 months posttransplant ($P = .01$), but only a trend for decreased survival free of

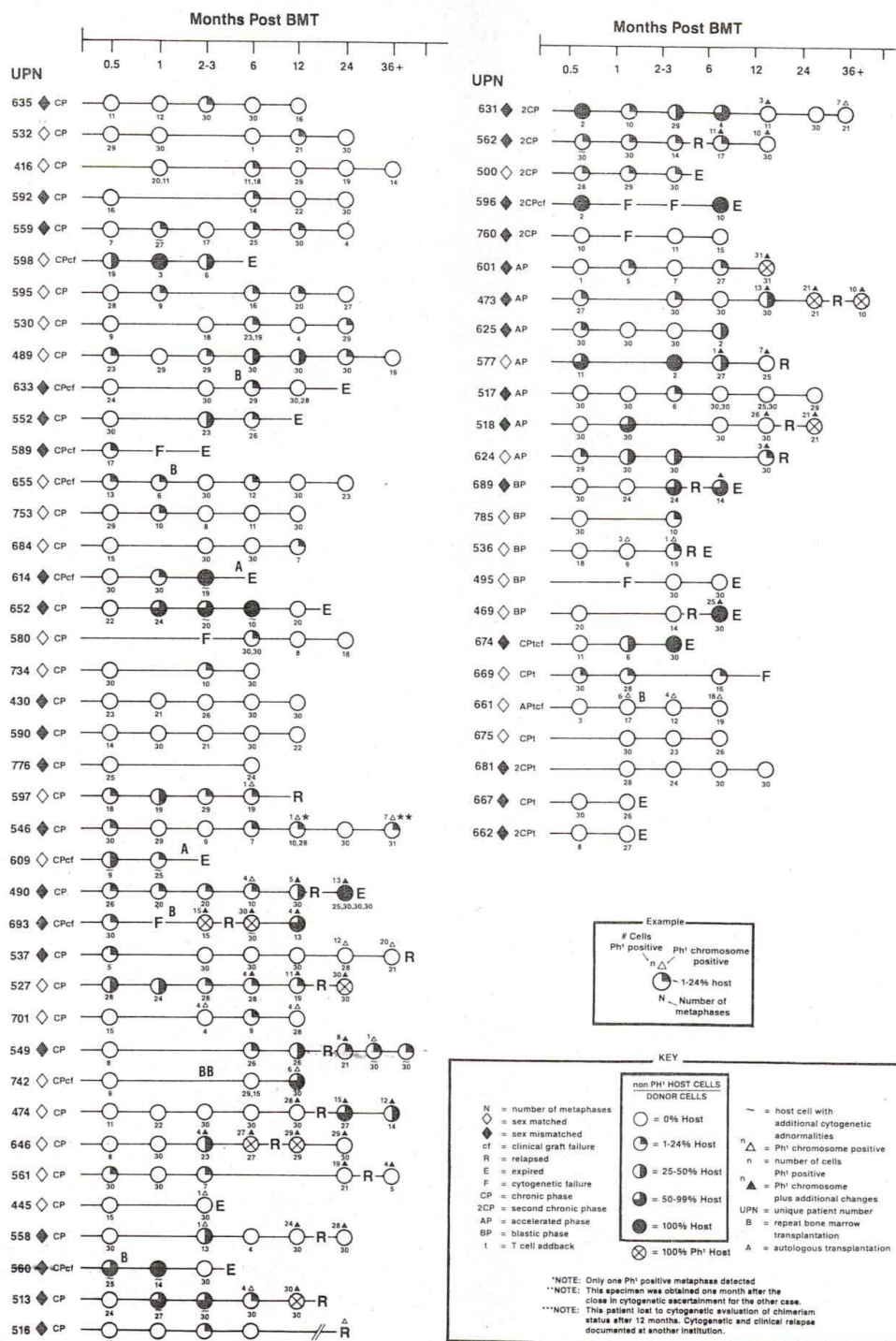


Fig 1. Results of serial cytogenetic studies on 64 patients with CML after T cell-depleted BMT.

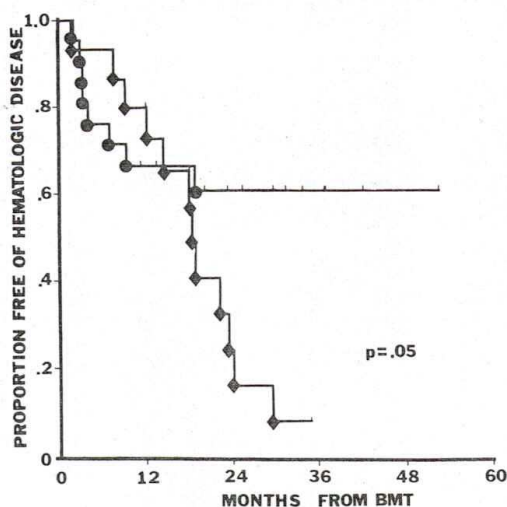


Fig 2. Survival free of hematologic evidence of disease for 15 patients demonstrating evidence of Ph positive cells (♦) in addition to mixtures of non-Ph-bearing host and donor cells posttransplant (mixed hematopoietic chimerism) compared with 21 patients demonstrating mixed chimerism in the absence of Ph-bearing cells (●). All patients received HLA-matched allogeneic BMT for the first CP of CML.

cytogenetic relapse ($P = .1$) and no difference in probability of graft failure for this subgroup ($P = .37$).

In view of the correlation between older age and decreased probability of disease free survival of recipients of SBA⁻E⁻T cell-depleted grafts in this series, we examined whether the age of the recipient was correlated with the level of chimerism observed. The median age of patients with greater than 25% host cells was 36.3 years, compared with 30.7 years for those with less than 25% host cells at all times posttransplant ($P = .04$). Conversely, of the 20 patients below the median age of 32.6 years, 14 showed less than 25% host cells at all times posttransplant, compared with 8 of 20 patients older than 32.6 ($P < .05$).

The dose of T cells given to patients receiving SBA⁻E⁻ marrow grafts was relatively uniform with a median dose of 5.1×10^4 clonable T cells/kg, as measured by limiting dilution assay.²¹ In this group of patients, there was no relationship between T cell dose and chimerism status or T cell dose and subsequent recurrence of Ph host cells ($P = .7$, $P = .8$, respectively). The Ph chromosome was seen post-BMT in 16 cases at 1 to 24 months posttransplant. In five cases, it appeared earlier than 6 months and, in 11 cases, 6 months or later post-BMT. The Ph chromosome was never observed in the first sample immediately post-BMT. In four patients, sequential analysis identified a transient appearance of the Ph chromosome that could not be detected on a subsequent analysis. All four of these patients ultimately experienced reappearance of the Ph positive clone or clinical relapse.

Of the 16 patients with cytogenetic relapse, 12 also relapsed clinically. In this group, the median interval be-

tween cytogenetic and clinical relapse was 6 months (range, 0 to 12 months). Three patients with cytogenetic relapse remain free of clinical relapse 6, 12, and 22 months from time of appearance of the Ph positive clone. The other patient in cytogenetic relapse died of other causes while still in clinical remission.

No patient transplanted for CML in first CP demonstrated exclusively host cells during the full post BMT course. However, five patients progressed from a mixed chimeric state to all host metaphases in the marrow. In two of these cases, the patients exhibited persistent full recovery of marrow cellularity and hematologic function throughout the time period in which reversion to host karyotype occurred. Of these, one also demonstrated cytogenetic relapse at the time of the appearance of all host heteromorphisms in non-Ph-bearing hematopoietic cells. The other patient subsequently reverted spontaneously to complete donor heteromorphisms but died of transplant-related complications. The remaining three patients who reverted to all host karyotypes did so at the time of clinical graft failure. These three patients were among nine patients who experienced clinical graft failures; the other six patients had persistence of some marrow elements of donor origin detected at the onset of pancytopenia. Thus, reversion to exclusively host karyotype in the marrow occurred in only a minority of the patients with this transplant complication.

Chimerism, relapse, and graft failure in CML patients in second CP. Five patients in second CP received T cell-depleted HLA-matched grafts. One patient became a complete chimera, three became mixed chimeras. One patient who had only host cells detected posttransplant experienced graft failure. One patient remains alive free of clinical or

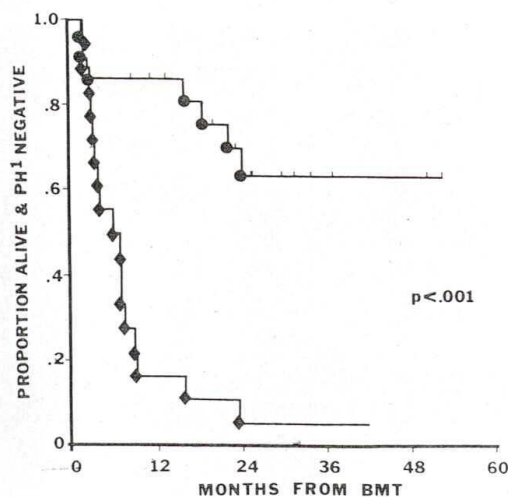


Fig 3. Survival free of hematologic or cytogenetic evidence of disease for 18 mixed chimeras with a fraction of non-Ph chromosome bearing host cells $\geq 25\%$ of non-Ph chromosome bearing metaphase cells (♦) versus 22 patients (18 mixed chimeras and 4 complete chimeras) with $< 25\%$ non-Ph chromosome bearing host cells (●) post-T cell-depleted BMT for first CP of CML.

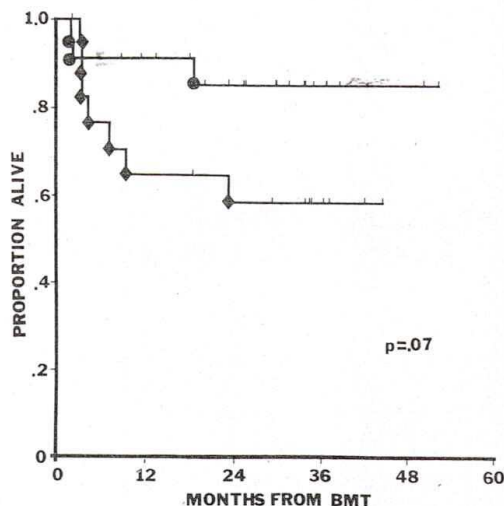


Fig 4. Overall survival for the two subgroups defined in Fig 3. ♦, BM \geq 25% host post-BMT; ●, BM < 25% host post-BMT.

cytogenetic evidence of disease 4 months post-BMT. One patient transplanted in second CP developed a Ph positive clone 12 months after BMT. This clone was not detected 2 years posttransplant but reappeared on subsequent analysis; this patient remains free of hematologic evidence of disease. The proportion of mixed chimeras in this subgroup was not significantly different from the first CP patients.

Chimerism, relapse, and graft failure in CML patients in accelerated and blastic phase. Seven patients in accelerated and five in blastic phase received HLA-matched T cell-depleted grafts. One of these patients became a complete chimera, the other 11 demonstrated non-Ph-bearing host cells at some time posttransplant. None of these patients developed graft failure; however, the proportion of patients with greater than 25% host cells did not differ from that of patients transplanted in a CP of their disease. Three patients are alive without clinical or cytogenetic evidence of disease at 3, 22, and 34 months post-BMT; one patient died with no evidence of disease.

Chimerism, relapse, and graft failure in patients receiving grafts partially depleted of T cells. Seven patients received grafts partially depleted of T cells as described previously. The dose of T cells provided by these transplants was higher than that of SBA⁻E⁻ marrow grafts, with a median of 11.7×10^4 cells/kg. Four of these patients were in first CP, two in second CP, and one in accelerated phase. Five of the seven became complete chimeras, and two became mixed chimeras post-BMT. The proportion of patients becoming complete chimeras (71%) was significantly higher in this group compared with the 11% (6/53) of patients receiving grafts fully depleted of T cells ($P < .05$). However, of the seven patients receiving partially depleted grafts, four developed grade 2 or greater acute GVHD, including three of the five patients who were complete chimeras. Of the seven

patients, four survive and three are disease-free 6 to 12 months posttransplant.

Cytogenetics of leukemia relapse. Of the total of 64 cases of post-BMT CML on whom cytogenetic studies were performed, 27 experienced cytogenetic relapses, and 18 of these exhibited clonal evolution with newly acquired chromosomal changes in addition to the Ph chromosome (Table 1). These were translocations and marker chromosomes with nonrandom involvement of certain chromosomal regions, especially 1p22-p36, 1q21-q25, 9q22, 10q22, and 11q13 (Fig 6). Although 55 breaks were recorded at 35 different chromosomal bands, 20 of them were clustered on chromosome 1 (Fig 6). The majority of sites of breaks corresponded with either nonrandom sites of breaks associated with chromosome aberrations in cancer or chromosomal fragile sites.²² Only chromosome 7 exhibited nonrandom loss, presenting as monosomy in three cases.

Of the 18 patients relapsing with cytogenetic abnormalities in addition to the Ph, four have died (two in documented blastic phase), 13 are in stable chronic phase, and two are without clinical evidence of disease. Of the nine patients with the Ph chromosome and no other abnormalities, three have died (one in documented blast phase), three are alive with clinical evidence of disease, and three are alive with no hematologic evidence of disease (one patient successfully retransplanted). There was no difference in median age or median survival from BMT between those with additional cytogenetic abnormalities and those relapsing with only the Ph chromosome, at a median follow-up of 27 months.

De novo clonal chromosome abnormalities in host cells. Ten patients exhibited de novo clonal chromosome abnormalities in host cells that were Ph negative (Table 2). These abnormalities also exhibited considerable clonal evolution; in addition, the pattern of breaks associated with these abnor-

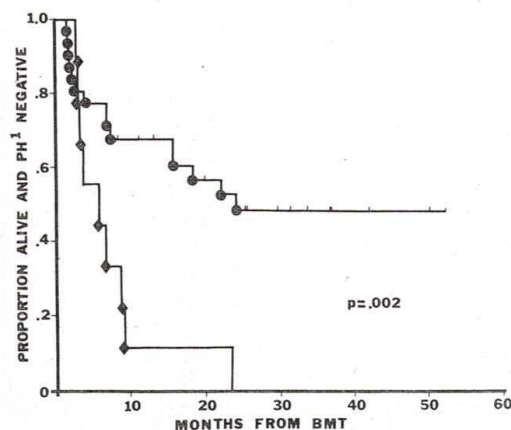


Fig 5. Survival free of hematologic or cytogenetic evidence of disease for nine patients with a fraction of non-Ph chromosome bearing host cells \geq 25% of non-Ph chromosome bearing metaphase cells (♦) detected within 3 months posttransplant and before clinical relapse or graft failure compared with 31 patients with < 25% host cells (●) within 3 months posttransplant. Patients received T cell-depleted transplants for first CP of CML.

Table 1. Karyotypic Evolution Associated With Cytogenetic Release in Ph Positive Patients

UPN	Dx	Pretransplant Karyotype	Posttransplant Karyotype
577	AP	46,XY,t(9;10;22)(q34;q22;q11)	46,XY,t(9;10;22)(q34;q22;q11),t(8;15)(q22;q22)
601	AP	46,XY,t(9;22)(q34;q11)	46,XY,der(Y)t(Y;7)(q11;7),t(9;22)(q34;q11)
624	AP	46,XY,t(9;22)(q34;q11)t(6;8)(p12;q24)	46,XY,t(9;22)(q34;q11),t(6;8)(p12;q24),t(9;19)(q22;q13)
631	2CP	46,XY,t(9;22)(q34;q11)	46,XY,t(9;22)(q34;q11),t(9;11)(q34;q13)
549	1CP	46,XY,t(4;9;22)(q21;q34;q11)	46,XY,t(4;9;22)(q21;q34;q11), + random changes
561	1CP	46,XY,t(9;10;22)(q34;q22;q11)	46,XY,-1,t(9;10;22)(q34;q22;q11),t(1;9)(p31;q34),del(2)(p21→pter),der(16)t(16;7)(q24;7), + mar1
646	1CP	NA	46,XY,t(9;22)(q34;q11),t(1;2)(p13;q21)
693	1CP	46,XX,t(9;22)(q34;q11)	(1) 46,XX,t(9;22)(q34;q11),del(1)(q31→qter) (2) 46,XX,t(9;22)(q34;q11),del(2)(p21→pter)
474	1CP	46,XX,t(12;22)(q13;q11)	(1) 46,XX, (12;22)(q13;q11),t(1;14)(q13;q32) (2) 46,XX,-4,t(12;22)(q13;q11), + mar1 (3) 46,XX,t(12;22)(q13;q11),del(6)(q23→qter) (4) 44-45,XX,-4,t(12;22)(q13;q11),der(9)t(9;7)(p21;7), + mar1 (5) 46,XX,t(12;22)(q13;q11),t(1;14)(q23;p13)
473	AP	47,XY,+8,t(9;22)(q34;q11)	(1) 46,XY,-2,-13,t(9;22)(q34;q11)t(1;16)(p13;q22),der(1)t(1;7)(q32;7) + mar1, + mar2 mar3 (2) 46,XY,t(9;22)(q34;q11),t(1;1)(q21;q32),random markers
513	1CP	46,XY,t(9;22)(q34;q11)	46,XY,t(9;22)(q34;q11),del(1)(q32→qter),del(3)(q13→qter)
527	1CP	46,XX,t(9;22)(q34;q11)	(1) 46,XX,-7,t(9;22)(q34;q11),t(1;12)(q25;q24),der(5)t(5;7)(p11;q11) (2) 46,XX,t(9;22)(q34;q11),der(4)t(4;7)(q35;7)
518	AP	47,XY,+8,t(9;22)(q34;q11),(qter→cen→qter)	47,XY,t(9;22)(q34;q11),del(3)(p21→pter),i(17)(qter→cen→qter), + mar1
490	1CP	46,XY,t(9;22)(q34;q11)	(1) 46,XY,-9,del(22)(q11→qter), + der(9)t(9;7)(q22;7), + mar1, + mar2, + random changes (2) 46,XY,-5,-15,-18,t(9;22)(q34;q11),der(1)(pter→cen::?),inv(1)(p22q21),del(1)(p22→pter), + mar1, + mar2, + mar3 (3) 42-46,XY,t(9;22)(q34;q11),t(1;1)(p22;p32) (4) 43-46,XY,t(9;22)(q34;q11),t(1;16)(q21;p13) (5) 46,XY,-4,t(9;22)(q34;q11),t(1;16)(p21;p13),t(7;10)(p15;p15)
562	2CP	46,XY,t(9;22)(q34;q11)	46,XY,t(9;22)(q34;q11),t(1;14)(q25;q21),t(10;18)(q22;q12)
689	BP	46,XX,t(9;22)(q34;q11)/47,XX,t(9;22)(q34;q11), + 11	46,XX,t(9;22)(q34;q11),t(1;1)(p32;q21),del(7)(q22→qter)
469	BP	46,XX,t(9;22)(q34;q11)	46,XX,-7,t(9;22)(q34;q11),del(3)(p25→pter), + random loss
558	1CP	NA	46,XY,t(9;22)(q34;q11),t(4;12)(q33;q15)

Abbreviations: Dx, diagnosis at BMT; 1CP, first chronic phase; 2CP, second chronic phase; AP, accelerated phase; BP, blastic phase; UPN, unique patient number; NA, not available.

malities closely paralleled that seen in the Ph positive clones. Thus, breaks again were predominantly clustered in the regions 1p13-p36, 1q21-q25, 9q22, 10q22, and 11q13.

DISCUSSION

Recently several groups, using a variety of cytogenetic, molecular genetic, and immunologic techniques, have described the occurrence of mixed chimerism in patients after allogeneic marrow transplants for chronic phase CML.^{5-13,23,24} The incidence of mixed chimerism has ranged from 18% to 80% after T cell-depleted transplants^{5,12,13}

compared with 0 to 15% after conventional transplants for CML.^{5,6,12,13,23,24} This difference potentially reflects the ability of T cells in a conventional marrow graft to eradicate host progenitor cells surviving the myeloablative doses of TBI and chemotherapy used for cytoreduction before transplant.

In the present series, the incidence of persistent mixed chimerism (90%) is particularly high. It is possible that this high incidence merely reflects differences in the techniques used for establishing mixed chimeric states, the proportion of donor-recipient pairs (both sex-matched and sex-mismatched) analyzed, or the frequency of cytogenetic analyses

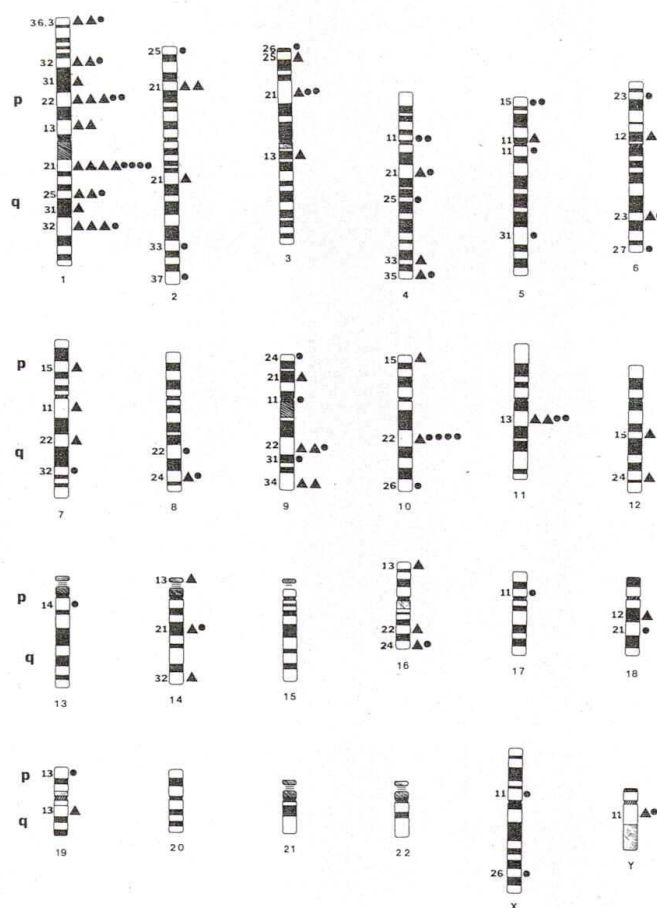


Fig 6. Ideogram of human chromosomal complement showing the banding pattern³² and the positions of individual breaks (excluding those associated with the generation of the Ph chromosome) in the Ph positive and Ph negative host clonal chromosome abnormalities. Each triangle represents one break in a Ph positive clone and each dot represents one break in a Ph negative clone. The ISCN band numbers are shown on the left of each chromosome.

performed in the posttransplant period. Alternatively, it might reflect differences in the efficiency of the T cell-depletion technique or the myeloablative potential of the cytoreductive regimen used. For example, SBA agglutination and E rosette depletion regularly result in a 2.5 to 3.0 \log_{10} depletion of clonable T cells, compared with the 1.5 to 2.5 \log_{10} reduction produced with antibody based techniques.²⁵ While such a level of T cell-depletion may be advantageous for preventing GVHD (only one patient developed a grade 2 acute GVHD, and no patient developed chronic GVHD in this series), it may reduce the potential of the graft to eradicate surviving host hematopoietic progenitors. In this regard, it is of interest that in this series, five of seven patients who received either SBA⁻ grafts or SBA⁻E⁻ grafts with added T cells achieved full donor chimerism. The difference in the dose of T cells given by this approach, quantitated by limiting dilution analyses of the final marrow inocula, was approximately 5×10^4 T cells per kilogram body weight.

Previous reports differ in their interpretations of the significance of mixed chimerism after BMT for leukemia.

One report documented stable mixed chimerism lasting several years post-BMT without recurrence⁶ while another report ascribed an unfavorable prognostic significance both to late emergence of mixed chimerism and any increments in the number of host cells detected post-BMT for leukemia.¹¹ Contrary to this report,¹¹ the data from our study do not indicate poor prognosis for patients developing mixed chimerism late in the post-BMT period.¹¹ Rather, our results are consistent with other reports of prolonged relapse-free survival for mixed chimeras after T cell-depleted marrow transplants.²⁶ While mixed chimerism per se did not predict imminent graft failure or cytogenetic or clinical relapse, mixed chimeras with greater than 25% normal host cells in the marrow at any time posttransplant had a significantly lower probability of disease free survival and a higher probability of clinical relapse. The strikingly poor clinical outcome of the nine patients with greater than 25% host cells in the first 3 months post-BMT suggests the importance of establishing the dominance of donor hematopoietic cells early posttransplant. If further studies confirm this observa-

Table 2. De Novo Clonal Chromosome Abnormalities in Ph Negative Host Cells

UPN	Dx	Karyotype
559	1CP	46,XY,t(4;10)(q21;q22)
549*	1CP	43-45,XY,t(9;11)(p24;q13),del(1)(q21→qter), + random loss
693*	1CP	46,XX,del(2)(q33→qter),der(4)t(4;7)(q35;7),der(8)t(8;7)(q24;7)
513*	1CP	(1) 46,X,-Y,der(4)t(4;Y)(p11;q11), + random changes (2) 46,XY,-5,der(X)t(13;X)(q14;p11),der(2)t(2;7)(q37;7), + mar 1, + random changes (3) 45,XY,t(3;X)(p21;q26),der(3)t(3;7)(p22;7),del(4)(q25→qter), + random loss
609*	1CP	46,XY,t(1;9)(p32;q22),t(11;19)(q13;p13),del(5)(q31→qter), der(10)t(10;7)(q26;7)
562*	2CP	46,XY,-21,der(5)t(5;7)(p15;7), + mar 1
564	1CP	(1) 38-46,XY,t(1;17)(q21;p11), + mar 1, + random loss (2) 45-46,XY,-19, + mar 2 (3) 43-46,XY,-9, + mar 3
552	1CP	(1) 43-46,XX,t(7;10)(q32;q22), + random loss (2) 43-45,XX,del(1)(q21→q25),del(6)(q23→qter)
652	1CP	(1) 46,XX,-2,t(1;3)(p36;p21),der(13) (pter→cen→q14::?:q14→qter) (2) 46,XX,del(1)(q21→qter),del(5)(q11→qter),der(6)t(6;7)(q27;7), + der(1)t(1;7)(p22;7), + mar 1, + mar 2, + random loss
614	1CP	(1) 46,XX,t(1;14)(p22;q21),t(10;18)(q22;q21) (2) 46,XX,del(1)(p22;q21),t(10;18)(q22;q21), + random markers
560	1CP	(1) 46,XX,del(1)(q32→qter) (2) 46,XX,t(9;16)(q11;q24),del(1)(q32→qter), + mar 1, + random changes
569	1CP	(1) 46,XY,-7,t(6;10)(p23;q22),del(4)(p11→pter),del(8)(q22→qter), + mar 1 (2) 46,XY,t(5;9)(p15;q31),der(2)t(2;7)(p25;7)

Abbreviations as in Table 1.

*Co-existing Ph positive clone(s), see Fig 1.

tion, cytogenetic monitoring during the period immediately post-BMT could prove useful in identifying patients appropriate for posttransplant interventions to insure the dominance of donor cells.

The incidence of cytogenetic relapse in this series of first CP patients is somewhat lower than that reported by other groups for T cell-depleted transplants but higher than that reported for conventional marrow grafts.^{1-6,12,13,25,26} In a large series of cytogenetically evaluated recipients of Campath monoclonal antibody-treated grafts,⁵ the Ph positive clone did not clear after conditioning in eight patients. In contrast, persistence of Ph cells through the preparative cytoreduction regimen and into the early posttransplant period was not seen in our series. Possibly, the lower incidence of relapse reported in this series reflects differences in patient populations, preparative cytoreduction regimens used, or the absence of posttransplant GVHD prophylaxis.

In the current series, leukemia relapse was more frequent in older patients. After receiving conventional marrow grafts, such patients have been noted to be more likely to succumb to GVHD-associated complications in the early posttransplant period,^{2,4} and may thereby be excluded from the population at risk for relapse. However, in other series in which marrow grafts have been depleted of T cells with monoclonal antibodies, this age-associated risk of relapse has not been observed,²⁷ suggesting an alternate basis for the increased incidence of relapse detected. Hale et al²⁸ observed that CML patients transplanted with T cell-depleted (Campath-

1-treated) marrow grafts who exhibited delayed or incomplete recovery of post-BMT hematopoietic function were at high risk for relapse. They suggested that the T cells in a conventional graft ensure the early and full establishment of donor hematopoiesis and thereby enhance the graft's capacity to compete with residual host leukemic cells for space and nutrients. Our data would support this hypothesis, since patients with a higher proportion of residual host hematopoietic cells posttransplant had a higher risk of clinical relapse. The quality of engraftment achieved might also explain the high incidence of relapse in older patients; in our series, patients older than the median age were more likely to have a high proportion of host hematopoietic cells in the posttransplant period. As previously reported by our group, older patients are also at greater risk for graft failure and/or rejection.²⁰

Recent reports of the appearance and subsequent spontaneous disappearance of the Ph chromosome post-BMT^{1,3,4,23,24} have cast doubt on the assumption of imminent clinical relapse after cytogenetic relapse. In our series, the cytoreductive regimen used to prepare patients for transplantation at least transiently cleared detectable Ph positive clones from the marrow in each case. In 16 patients in whom Ph positive cells reappeared posttransplant, 12 went on to clinical relapse, usually within 6 months of cytogenetic relapse. Each of the five patients (four in first CP, one in second CP) who appeared to clear Ph positive cells posttransplant ultimately developed clinical or persistent cytogenetic relapse. Thus, our

results appear to differ from those recently reported by the European Cooperative Study Group²³ and Zaccaria et al.²⁴ In those series, 15 of 76 (20%) of patients transplanted for CML in CP who had received unfractionated marrow grafts exhibited Ph positive cells in the marrow posttransplant but did not go on to clinical relapse. Seven of these patients either died after initial detection of the Ph positive clone or have had no cytogenetic follow-up. However, in eight patients the translocation was not detected in samples subsequent to those in which the Ph positive cells were initially observed. Similarly, in the series reported by Thomas et al.,⁴ 6 of 67 patients developed cytogenetic but not clinical evidence of relapse. In 3 of these cases the Ph positive cells disappeared for periods of 1 to 5 years without further therapeutic intervention. In both of these series, however, almost all patients studied showed evidence of normal donor cells in the marrow before or after the relapse of Ph chromosome-positive host cells. In such patients, dominance of the donor hematopoietic graft either by direct competition for space and nutrients,²⁸ or by other mechanisms, including cellular interactions contributing to GVHD,²⁴ may permit elimination of residual Ph positive cells.

Graft failure, defined clinically by the persistence of marrow aplasia after transplantation or its recurrence after initial hematopoietic recovery, was observed in nine patients in this series. At the time of graft failure, marrow cells were of host origin exclusively in three patients; in the other six patients, both donor and host cells were detected in the marrow before or at the time of graft failure. Thus, cytogenetic analyses of the marrow at onset of cytopenias may not provide information that would permit discrimination between drug or infection-induced cytopenias or graft failure. However, as reported by Bordignon et al.²⁹ in our series, T cells detected in the circulation at the time of true graft rejection are of host type exclusively, and exhibit donor-specific myelosuppressive activity.

Of the total number of patients manifesting cytogenetic relapse in this series, over half also exhibited further clonal evolution represented by multiple related clones and highly nonrandom distribution of chromosome breaks. Such clonal evolution with nonrandom cytogenetic abnormalities has been noted during relapse after chemotherapy for acute nonlymphocytic leukemia (ANLL)³⁰ and has recently been documented after BMT for CML.^{23,24} It is interesting to note that in our study the distribution of chromosome breaks associated with de novo clonal abnormalities in the host cells was quite similar to that seen in the Ph positive relapse clones. The former most probably represent radiation damage sustained by some sequestered stem cells that expand in the posttransplant marrow space. Such a view is supported by several observations. In one patient these clones were detected as early as 2 weeks posttransplant. A second patient, a mixed chimera who exhibited host cell clonal abnormalities in the absence of a Ph chromosome, has had a prolonged disease-free survival, and eventually reverted to an all-donor karyotype. Finally, proliferation of clonally abnormal host cells in the immediate posttransplant phase of sublethally irradiated mice given xenogeneic marrow transplants has been reported during experiments aimed at identifying the biologic basis of radiation protection afforded by BMT.³¹ The fact that the breakage pattern in the de novo apparent radiation clones and in the newly arising clones in the relapsing Ph positive clones is strikingly similar points to their common origin. Such clonal evolution in the Ph positive clones probably does not represent progression to blastic phase; the majority of patients showing such additional changes at relapse had prolonged clinical courses in chronic phase.

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