Autografting With Cultured Marrow in Chronic Myeloid Leukemia: Results of a Pilot Study


Incubation of chronic myeloid leukemia (CML) marrow for 10 days in vitro causes a marked and selective loss of very primitive Philadelphia chromosome (Ph) progenitors. We have autografted 22 patients with CML (16 in first chronic phase [group 1] and 6 with more advanced disease [group 2]) with marrow treated in this way to facilitate restoration of Ph hemopoiesis after intensive therapy. Hematologic recovery to greater than 0.5 x 10^9/L neutrophils occurred in 16 patients, and to greater than 20 x 10^9/L platelets in 15 of 21 evaluable patients at a median of 29 and 48 days postautograft, respectively. Regenerating marrow cells were 100% Ph− in 13 patients and 75% to 94% Ph− in 3. Between 4 and 36 months (median 12) postautograft, Ph+ cells became detectable in all 1 (who died in remission) of the 13 patients who achieved complete cytogenetic remission. Four of 7 evaluable patients treated with low-dose interferon α were returned to complete cytogenetic remission. Thirteen group 1 patients (81%) are alive 1.0 to 6.7 years (median 2.6) after autografting: 4 in complete cytogenetic remission, 2 in hematologic remission, 6 in chronic phase, and 1 in myeloid blast phase. Three group 2 patients (50%) are alive at 2.6, 3.8, and 4.3 years after autografting: 1 in partial cytogenetic remission, 1 in chronic phase, and 1 in accelerated phase. Thus, autografts of cultured marrow can result in prolonged restoration of Ph− hemopoiesis for some patients with CML.

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CHRONIC MYELOID leukemia (CML) is a clonal malignancy that arises in a pluripotent hematopoietic stem cell.1 The neoplastic cells are characterized by a unique gene rearrangement that results in the creation of a BCR-ABL fusion gene2,3 and, in most cases, the formation of a shortened chromosome 22 known as the Philadelphia chromosome (Ph).4 More direct evidence that the BCR-ABL gene product plays a key role in the development of CML has been obtained recently from gene transfer experiments in mice.5

Intensive (“myeloablative”) therapy followed by transplantation of marrow from a histocompatible donor results in long-term disease-free survival in a significant proportion of patients with CML.6,7 However, most patients are not considered eligible for allogeneic bone marrow transplantation (BMT) because of either relatively advanced age or lack of a suitably matched donor.8 These constraints on the applicability of allogeneic BMT, together with the fact that Ph− hematopoietic stem cells persist in many patients,9–12 have provided a rationale for the development of autograft-based protocols. Evidence that the CML clone can be transplanted13,14 has also focused attention on the need to develop approaches that minimize the content of leukemic stem cells in the autograft while retaining sufficient Ph− hematopoietic cells to enable restoration of blood cell formation.

When CML marrow cells are placed in culture, the number of Ph− progenitors typically declines during the first few weeks and Ph− hematopoietic cells may then become detectable.15 A variety of studies have shown that at least some of these Ph− hematopoietic cells do not belong to the leukemic clone and are presumably normal.16–18 More recently, we have shown that very primitive normal progenitor cells, identified by their ability to produce colony-forming cells for 5 weeks or more in cultures containing a marrow feeder layer (and thus referred to as long-term culture-initiating cells [LTC-ICs]), can be maintained at input levels for the first 10 days in culture, whereas their leukemic counterparts (Ph+ LTC-ICs) decrease 30-fold under the same conditions.19 In addition, the initial concentration of normal LTC-ICs in unmanipulated CML marrow aspirates already often exceeds that of leukemic LTC-ICs, although both are usually present at a reduced concentration by comparison with LTC-ICs in normal marrow.20

On the basis of these observations, we began a study in 1987 to evaluate the feasibility of using cultured marrow cells as autografts to allow intensive treatment of patients with CML who were ineligible for allogeneic BMT.21 The results of this pilot study are now reported.

PATIENTS AND METHODS

Entry Criteria

Entry into the study was restricted to patients with Ph+ CML who were ≥60 years old, ineligible for allogeneic BMT, and in morphologic chronic phase (although not necessarily the first). Patients meeting these criteria were further selected on the basis of a laboratory assessment of their marrow after 10 days of incubation in vitro under conditions routinely used to initiate LTCs.22,23 This screening test was done to increase the likelihood of selecting patients in whom a subsequently harvested and similarly treated marrow would be likely to contain sufficient primitive normal hematopoietic cells to allow engraftment and might also have had its content

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of leukemic stem cells effectively minimized. The criteria used to try to meet these objectives were that the number of normal LTC-ICs present at the end of 10 days under LTC conditions be at least 2% of the average LTC-IC content of an equivalent input of fresh normal marrow cells (ie, ≥20 per 2.5 × 10^7 initial marrow cells) and, at the same time, that Ph+ LTC-ICs be undetectable (which usually meant less than 1 Ph+ LTC-IC per 10^7 initial cells). For the first 42 patients, this was done by maintaining the cells in the original cultures for a further 3 to 6 weeks and then determining the number and genotypes of granulopoietic (CFU-GM), erythroid (BFU-E), and multi-lineage (CFU-GEMM) colony-forming cells present. This involved harvesting all of the cells from the primary cultures and plating them into secondary methylcellulose cultures under standardized conditions to detect these latter cell types.\(^2\)\(^3\) Later, when conditions for measuring normal and Ph+ LTC-ICs had been better defined, cells from the primary day-0 cultures were transferred to secondary feeder-containing LTC-IC assay cultures for 5 weeks and then finally transferred to colony assay cultures as described.\(^1\)\(^9\)

Between April 1987 and February 1992, 87 candidates had their marrow assessed in this way. In 36 (41%) the progenitor findings met the criteria used for entry into the trial. Twenty-two of these 36 patients were subsequently (between November 1987 and July 1992) given intensive therapy and an autograft of marrow cells that had been cultured for 10 days (Table 1). More detailed analysis of the progenitor assays in the samples obtained from these patients will be presented elsewhere (manuscript in preparation).

Patients

The 22 patients were aged 22 to 59 years (median 43) at the time of autografting (Table 1). Sixteen were in first chronic phase (group 1) and the other 6 had more advanced disease (group 2). Group 2 included 1 patient who had myeloid blast-phase disease (unique patient number [UPN] 715), 2 who had been treated previously for lymphoid blast-phase disease and were in third and second chronic phase, respectively (UPNs 232 and 248), and 3 who had accelerated phase disease (UPNs 341, 399 and 545). These latter 3 patients had evidence of a cytogenetically evolved clone of cells (Table 2). One (UPN 341) had presented initially with marked lymphadenopathy, a biopsy of which showed infiltration with myeloid blast cells.

The treatment protocol was approved by the local review boards and patients gave informed consent before entry into the study.

Intensive Therapy

Patients were treated with one of two intensive therapy regimens. The first 3 patients (UPNs 208, 232, and 248) received etoposide 1.8 g/m² as a 26-hour intravenous (IV) infusion starting on day −7; cyclophosphamide 2.0 g/m² as a 2-hour IV infusion on days −6, −5, and −4; total body irradiation 1,200 cGy (UPNs 208 and 232) or 1,000 cGy (UPN 248) in twice daily fractions of 200 cGy on days −3, −2, and −1.\(^1\)\(^9\) The subsequent 19 patients received busulfan 1 mg/kg orally every 6 hours on days −7 to −4 (total of 16 doses); cyclophosphamide 60 mg/kg as a 2-hour IV infusion on days −3 and −2; and melphalan 90 mg/m² as a 15-minute IV infusion on day −1.\(^1\)\(^9\) Cyclophosphamide was omitted in one patient (UPN 506) because of known cardiomyopathy. All patients (except UPN 506) were given vigorous hydration as uroepithelial protection and those treated with the busulfan-based regimen received prophylactic phenytoin.\(^1\)\(^9\)

Autografting Procedures

Three days before the commencement of intensive therapy (day −10), ∼1 L of marrow was aspirated from the pelvis under general

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age/ Sex</th>
<th>Interval (mos.)</th>
<th>Previous Treatment</th>
<th>Splenectomy</th>
<th>Disease Status</th>
<th>Outcome</th>
<th>Survival (mos.)</th>
</tr>
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<tr>
<td>208</td>
<td>44/F</td>
<td>0.64</td>
<td>Hu</td>
<td>Yes</td>
<td>CP-1</td>
<td>Alive, in CCR off therapy*</td>
<td>132+</td>
</tr>
<tr>
<td>232</td>
<td>53/F</td>
<td>6.3</td>
<td>Vc, Pred, Dox; 6-Mp, Mtx</td>
<td>No</td>
<td>CP-3</td>
<td>Died of BP disease</td>
<td>10</td>
</tr>
<tr>
<td>248</td>
<td>41/F</td>
<td>5.0</td>
<td>Hu, Vc, Pred, Dox</td>
<td>No</td>
<td>CP-2</td>
<td>Died of TRT</td>
<td>6</td>
</tr>
<tr>
<td>341</td>
<td>39/M</td>
<td>6.6</td>
<td>Pica, Ha; Ara-C, Vb</td>
<td>No</td>
<td>AP</td>
<td>Alive, in CP off therapy</td>
<td>57+</td>
</tr>
<tr>
<td>365</td>
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<td>15.0</td>
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<td>No</td>
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<td>Alive, in CCR on IFNa</td>
<td>64+</td>
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<tr>
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<td>51+</td>
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<tr>
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<td>Alive, in CP on Hu</td>
<td>50+</td>
</tr>
<tr>
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<td>33/M</td>
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<td>Alive, in CCR on IFNa</td>
<td>57+</td>
</tr>
<tr>
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<td>59/M</td>
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<td>Died of TRT</td>
<td>12</td>
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<td>534</td>
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<td>Hu</td>
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<td>Alive, in BP on Ara-C</td>
<td>47+</td>
</tr>
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<td>545</td>
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<td>AP</td>
<td>Alive, in AP on IFNa</td>
<td>53+</td>
</tr>
<tr>
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<td>36/M</td>
<td>15.0</td>
<td>Hu</td>
<td>No</td>
<td>CP-1</td>
<td>Alive, in CP on Hu</td>
<td>46+</td>
</tr>
<tr>
<td>557</td>
<td>37/F</td>
<td>10.0</td>
<td>IFNa</td>
<td>No</td>
<td>CP-1</td>
<td>Alive, in HR on IFNa</td>
<td>40+</td>
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<tr>
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<td>8.0</td>
<td>Hu</td>
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<td>CP-1</td>
<td>Alive, in CP off therapy</td>
<td>37+</td>
</tr>
<tr>
<td>573</td>
<td>59/F</td>
<td>11.0</td>
<td>Hu, Bu</td>
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<td>CP-1</td>
<td>Alive, in CP on Bu</td>
<td>40+</td>
</tr>
<tr>
<td>575</td>
<td>31/M</td>
<td>8.0</td>
<td>Hu</td>
<td>No</td>
<td>CP-1</td>
<td>Alive, in HR on IFNa</td>
<td>37+</td>
</tr>
<tr>
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<td>36/F</td>
<td>64</td>
<td>Hu; IFNa</td>
<td>No</td>
<td>CP-1</td>
<td>Died of TRT</td>
<td>68</td>
</tr>
<tr>
<td>585</td>
<td>56/F</td>
<td>8.0</td>
<td>Hu</td>
<td>No</td>
<td>CP-1</td>
<td>Died of TRT</td>
<td>9</td>
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<tr>
<td>636</td>
<td>51/M</td>
<td>59.0</td>
<td>Hu</td>
<td>No</td>
<td>CP-1</td>
<td>Alive, in CP on IFNa</td>
<td>81</td>
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<tr>
<td>715</td>
<td>55/M</td>
<td>22.0</td>
<td>Hu; IFNa</td>
<td>No</td>
<td>BP-1</td>
<td>Died of BP disease</td>
<td>29</td>
</tr>
<tr>
<td>748</td>
<td>22/M</td>
<td>10.0</td>
<td>Hu</td>
<td>No</td>
<td>CP-1</td>
<td>Alive, in CP on IFNa</td>
<td>22+</td>
</tr>
</tbody>
</table>

Abbreviations: AP, accelerated phase; Ara-C, cytosine arabinoside; BP, blast phase; Bu, busulfan; CCR, complete cytogenetic remission; CP, chronic phase; Dox, doxorubicin; HR, hematologic remission; Hu, hydroxyurea; 6-Mp, 6-mercaptopurine; Mtx, methotrexate; PCR, partial cytogenetic remission; Pica, plicamycin; Pred, prednisone; TRT, therapy-related toxicity; Vb, vinblastine; Vc, vincristine.

* Cytogenetic analysis of marrow has shown the presence of a Ph- but cytogenetically altered clone (see Results).

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Assessment After Autografting

Supportive Care

anaesthesia and collected directly into transfer packs (Travenol) containing heparinized medium. Auffy-coat fraction was then prepared on a COBE 2991 Blood Cell Processor (COBE Canada Limited, Scarborough, Ontario), and aliquots of 2 × 10⁶ nucleated cells in media dispensed into 175-cm² tissue culture flasks (Falcon; Becton Dickinson Canada Inc, Mississauga, Ontario), each containing 60 mL of α-medium supplemented with 0.4 mg/mL glutamine, 0.04 mg/mL inositol, 0.01 mg/mL folic acid, 12.5% horse serum, 12.5% fetal calf serum, 10 U/mL heparin, 10⁻⁶ mol/L 2-mercaptoethanol, and 10⁻⁶ mol/L hydrocortisone sodium succinate. Thus, the final cell concentration in the culture flasks was ~3 × 10⁶ nucleated cells/mL. The flasks were then incubated without perturbation for the first 3 days at 37°C and then at 33°C for another 7 days in a humidified atmosphere of 5% CO₂ in air. At the end of 10 days (day 0), the nonadherent cells were poured into centrifuge bottles and the adherent cells detached and suspended using trypsin. Both the nonadherent and adherent cells were centrifuged at 260g for 20 minutes, combined, washed once in TCM-199 medium, and finally filtered through the coarse and fine filters of a marrow filtering set (Scientific Instruments Division, University of Washington, Seattle, WA). The final volume of cells was adjusted to 500 mL with TCM-199 medium, loaded into a 600-mL transfer pack (Travenol), and 10 mL of autologous serum added to give a final serum concentration of 60 mL of cy-medium supplemented with 0.4 mg/mL glutamine, 0.04 mg/mL inositol, 0.01 mg/mL folic acid, 12.5% horse serum, 12.5% fetal calf serum, 10 U/mL heparin, 10⁻⁶ mol/L 2-mercaptoethanol, and 10⁻⁶ mol/L hydrocortisone sodium succinate. Scarff et al. was defined as the presence of 100% (complete) or 66% to 99% (partial) Ph⁻ metaphases in Giemsa-banded preparations from 24-hour marrow cultures.

Treatment After Autografting

With one exception, patients in whom complete cytogenetic remission had been achieved were followed off therapy until ±10% (later in the study, ≥1%) Ph⁺ metaphases reappeared in marrow samples, at which time interferon α (IFNo) (recombinant IFNa2b; Schering Canada Inc, Pointe Claire, Quebec) 1 to 3 × 10⁶ U/m² subcutaneously once daily 3 to 7 d/wk was started. The exception was UPN 715, who was treated with a course of interleukin-2 (IL-2) (natural IL-2, Biotech Pharma, Dreieich, Germany), 2.5 × 10⁶ U (=10⁷ Biological Response Modifier Program [BRMP] U/mg protein) subcutaneously, twice daily for 30 days starting on day 57 postautograft while in complete remission.

Statistical Methods

Survival plots were developed according to the method of Kaplan and Meier. Surviving patients were censored on the last day of follow-up, which in all cases was ≥1 year postautograft. Statistical significance was determined by the log-rank method.

RESULTS

Composition and Infusion of the Autograft

The number of nucleated cells placed in culture ranged from 1.6 to 2.6 × 10⁹ (median 2.4) and the number recovered and infused 10 days later ranged from 0.9 to 2.3 × 10⁹ (median 1.2). The composition of the autograft in terms of nucleated cell, clonogenic cell and LTC-IC numbers per kg of patient body weight is shown in Table 3. During the infusion, most patients developed fever and chills despite premedication with diphenhydramine and hydrocortisone,

Table 2. Cytogenetic Analysis of Marrow in Patients With Accelerated Phase Disease

<table>
<thead>
<tr>
<th>UPN</th>
<th>At Diagnosis</th>
<th>At Autografting</th>
</tr>
</thead>
<tbody>
<tr>
<td>341</td>
<td>50,XY, +6,t(9;22)(q34;q11), +10, +19, + der(22)t(9;22)(q34;q11) [17]/</td>
<td>46,XY [17]/</td>
</tr>
<tr>
<td>399</td>
<td>48,XX,t(9;22)(q34;q11) [17]/</td>
<td>46,XY [17]/</td>
</tr>
<tr>
<td>545</td>
<td>46,XX,t(9;22)(q34;q11) [25]</td>
<td>46,XY [17]/</td>
</tr>
</tbody>
</table>

Table 3. Composition of the Autograft

<table>
<thead>
<tr>
<th>Ph⁻</th>
<th>Ph⁺</th>
<th>Ph⁺</th>
<th>Ph⁻</th>
<th>Ph⁻</th>
<th>Ph⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>2.4</td>
<td>0.2³⁻²⁰</td>
<td>0.7³⁻²²</td>
<td>4.0</td>
<td>0.2⁻²²</td>
</tr>
</tbody>
</table>

* No progenitors were detectable; the value shown represents the limit of sensitivity of the measurement.
† No value, as in 14 of 22 cases, no progenitors were detectable.
but no other untoward reaction occurred. There were no cases of bacteremia or fungemia that could be attributed to the infusion of the autograft.

**Hematopoietic Recovery**

In 16 patients, recovery of neutrophils to $0.5 \times 10^9/L$ occurred at a median of 29 (range 13 to 52) days (Fig 1), and recovery of platelets to $20 \times 10^9/L$ occurred in 15 at a median of 48 (range 16 to 337) days. The patient who recovered neutrophils but not platelets (UPN 248) died of therapy-related toxicity on day 28 postautograft. The 2 patients who had previously undergone splenectomy (UPNs 208 and 573) recovered neutrophils on days 17 and 13 and platelets on days 16 and 62 postautograft, respectively. One of the remaining 6 patients (UPN 585) died on day 35 in spite of early neutrophil recovery. The other 5 failed to show adequate neutrophil recovery within the first 6 weeks and were subsequently given thawed unmanipulated reserve cells (see below). For the analysis of hematopoietic recovery, these 6 patients were censored on the day of infusion of reserve cells or death (UPN 585).

During the initial phase of hematopoiesis after autografting, cytogenetic analysis showed 100% Ph- marrow cells in 13 of the 16 patients in whom neutrophil recovery occurred, with 75% to 94% Ph- cells in the other 3 (Table 4). Southern blot analyses of DNA from the same follow-up marrow samples and/or from simultaneously obtained PB granulocytes showed similar ratios of cells with and without the BCR-ABL rearrangement (data not shown).

Neutrophil recovery was significantly faster in patients who had previously undergone splenectomy than in those who had not ($P < .001$). There was no correlation between the speed of hematopoietic recovery (either neutrophil or platelet) and the number of nucleated cells, clonogenic cells, or LTC-ICs infused. The genotype of the regenerating hematopoietic cells (which were exclusively or predominantly Ph-) correlated with the predominant genotype of LTC-ICs infused (in most cases, Ph-), but not the predominant genotype of clonogenic cells infused (in most cases, Ph+). Once achieved, hematopoiesis was sustained and there were no cases of late cytopenia.

All patients except one (UPN 409, who continues to be mildly lymphopenic) recovered lymphocytes to $1.0 \times 10^9/L$ within 1 year of the autograft.

**Graft Failure**

In five patients (UPNs 506, 534, 545, 583, and 636), satisfactory neutrophil recovery did not occur during the first 6 weeks after autografting and clinical circumstances dictated that the unmanipulated reserve cells be infused (Table 5). However, in three of these patients (UPNs 545, 583, and 636), marrow samples taken before rescue with reserve cells had been sufficiently cellular to allow cytogenetic analysis. These showed 100% Ph- cells in two (UPNs 583 and 636) and 85% Ph- cells in the third (UPN 545) (Table 4). After infusion of the reserve cells, all five patients recovered neutrophils to $\geq 0.5 \times 10^9/L$ (Table 5). The marrow cells obtained during this period were found to be 100%, 80%, and 28% Ph+ in UPNs 534, 545, and 636, respectively. Thus, in the two patients (UPNs 545 and 636) for whom cytogenetic data were available before and after infusion of unmanipulated cells, this was associated with an increase (from 15% to 80% and from 0% to 28%) in Ph positivity. Cytogenetic data were not obtained after infusion of the reserve cells in the other two patients (UPNs 506 and 583), who died of therapy-related toxicity shortly after neutrophil recovery. There was no correlation between graft failure and the size of the spleen, marrow fibrosis, or the number of nucleated cells, clonogenic cells, or LTC-ICs infused.

**Later Cytogenetic Changes**

The results of the cytogenetic analysis of metaphases from all follow-up marrow samples obtained after autografting are shown in Table 4. In all but 1 of the 13 patients in whom a complete cytogenetic remission was achieved, Ph- cells became detectable between 4 and 36 months (median 12) postautograft (Fig 2). The remaining patient (UPN 248) died early in remission. Nine of these 12 patients were then treated with IFN-α. Four patients (UPNs 208, 365, 409, and 448) returned to complete cytogenetic remission. In one of these (UPN 208), a Ph-negative, but cytogenetically altered clone of cells [46,XX,t(1;18)(p13;q21), t(3;16)(q25;p13), t(7;14)(p22;q32), del(13)(q12q21)] was apparent in the marrow sample obtained 21 months postautograft (19% of cells) and was still detectable another 3 years later (35% of cells).20 In three patients (UPNs 341, 573, and 575), the proportion of Ph- cells continued to increase in spite of IFNα treatment. Two (UPNs 399 and 557) are still too early to evaluate, having been on IFNα for only 6 months. Three patients were not treated with IFNα. In two of these (UPN 423, who remained relatively thrombocytopenic, and UPN 572, who refused), the proportion of Ph- cells continued to increase; in the third (UPN 715), blast-phase disease recurred.

The three patients in whom partial cytogenetic remission was achieved (UPNs 232, 552, and 748) showed a continuously increasing proportion of Ph+ cells in the marrow, in-
Table 4. Cytogenetic Analysis of Marrow Before and After Autografting

<table>
<thead>
<tr>
<th>UPN</th>
<th>Preautograft</th>
<th>1-2</th>
<th>3-4</th>
<th>6-7</th>
<th>11-12</th>
<th>17-18</th>
<th>21-24</th>
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<th>54</th>
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<tr>
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<td>100 (30)</td>
<td>97 (75)</td>
<td>100 (76)</td>
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<td>100 (29)*</td>
<td>100 (30)</td>
<td>100 (26)</td>
<td></td>
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<tr>
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<td>10 (58)</td>
<td>94 (31)</td>
<td>7 (15)</td>
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<td>16 (25)</td>
<td>100 (5)</td>
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<td>34 (50)</td>
<td>100 (25)</td>
<td>100 (25)</td>
<td>100 (28)</td>
<td>90 (50)</td>
<td>32 (25)*</td>
<td>0 (15)*</td>
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<tr>
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<td>100 (33)</td>
<td>98 (50)</td>
<td>100 (50)</td>
<td>100 (25)</td>
<td>100 (25)</td>
<td>100 (25)</td>
<td>96 (25)</td>
<td>92 (25)*</td>
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Abbreviations: ED, early death (therapy related); GF, graft failure.

* Marrow sample was taken while the patient was receiving treatment with IFNo.
† Marrow sample was taken after infusion of unmanipulated reserve cells for graft failure.
‡ Estimate of cells carrying the rearrangement by molecular analysis.
Table 5. Treatment and Subsequent Neutrophil Recovery in Patients With Graft Failure

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<th>Treatment, Days Delivered After Autografting</th>
<th>Neutrophil Recovery, Days After Autografting</th>
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<td>UPN GM-CSF Reserve Cells IL-3</td>
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Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor (Hoechst or Schering), 250 pg/m² or 5 pg/kg subcutaneously, daily; IL-3, interleukin-3 (Sandoz), 5 pg/kg subcutaneously, daily.

* Blood.
† Marrow.

Including the one patient (UPN 748) in this group who received IFNa. Two patients who were rescued from graft failure by infusion of reserve cells (UPNs 545 and 636) also showed a continuously increasing proportion of Ph+ cells in the marrow.

Outcome

Four patients died of therapy-related toxicity: UPN 248 on day 28 of veno-occlusive disease of the liver, UPN 506 on day 67 of pneumonia, UPN 583 on day 131 of pulmonary hemorrhage, and UPN 585 on day 35 of sepsis. Two patients died of transformed CML: UPN 232 on day 125 of recurrent lymphoid blast-phase disease and UPN 715 on day 214 of recurrent myeloid blast-phase disease.

The status of each patient on the last day of follow-up is shown in Table 1. Outcome according to disease status at autografting is summarized in Table 6. Survival plots are shown in Fig 3.

Group 1. Thirteen patients are alive 1.0 to 5.7 years (median 2.6) after autografting (survival 81%; 95% confidence interval [CI], 53% to 94%). Four of these (UPNs 208, 365, 409, and 448) are in complete cytogenetic remission, two are in hematologic remission (but with greater than 33% Ph+ marrow cells), six are in chronic phase, and one (UPN 534) has developed myeloid blast-phase disease. Three of the four patients in complete cytogenetic remission remain on low-dose IFNa. One (UPN 208) took IFNa for 2 years (1 to 3 years postautograft), but then decided to stop, and has been followed off therapy for the last 2 years.

Group 2. Three patients (all treated in accelerated phase) remain alive 2.6, 3.8, and 4.3 years after autografting (survival 50%; 95% CI, 11% to 80%): UPN 399 is in partial cytogenetic remission (and remains on low-dose IFNa), UPN 341 is in chronic phase and UPN 545 is in accelerated phase. Two patients (UPNs 341 and 399) have not shown any evidence of the original (or any other) evolved clone of cells after autografting. One patient (UPN 545) has developed a new subclone [46,XX,t(9;22)(q34;q11),?del(6)(q23)] without further evidence of the evolved clone present before autografting.
All patients except two (UPN 208, who has unrelated medical problems and UPN 534, who has blast-phase disease) enjoy good health (Karnofsky performance status 90% to 100%), including those on low-dose IFNα.

DISCUSSION

There are clearly many patients with CML who would benefit from a treatment that could significantly prolong their possible life expectancy beyond the average of ~5 years with either hydroxyurea or IFNα. Several years ago we developed a protocol of intensive therapy supported by autografting with cultured marrow to address this need. For a pilot study of its feasibility, patients were selected on the basis of a prior laboratory assessment of their marrow progenitors. In retrospect, this strategy may have been prudent in view of more recent evidence documenting the extensive heterogeneity (greater than 2 log variability) in the marrow content of very primitive normal and leukemic progenitors among individual patients with CML irrespective of the white blood cell count. The results show that restoration of normal hematopoiesis was obtained in the majority of patients, indicating that marrow cells maintained in culture for 10 days can be used to support intensive therapy. The speed of the hematopoietic recovery was consistent with an autograft-derived origin of the predominantly (≥75%) Ph+ cells initially produced. Even taking into account the fact that patients were selected, this seems a better result than might have been achieved with unmanipulated PB or marrow cells by comparison with results reported by others for such autografts. In the two instances where it was possible to document the genotype of the cells in the marrow before and after infusion of unmanipulated reserve marrow cells in the series described here, it is of interest that the proportion of Ph+ cells was shown to increase. Moreover, once achieved, hematopoiesis was sustained with no cases of late graft failure, a complication that has been described after PB cell autografts in CML.

Four patients (18%) died as a consequence of the treatment; three of these deaths occurred after a prolonged period of cytopenia and thus might have been avoided had hemato poetic recovery been achieved earlier. There was no correlation between speed of recovery and the number of clonogenic cells in the marrow before and after infusion of unmanipulated PB cells or LTC-ICs infused (per kg body weight, normal and/or Ph+). The finding that neutrophil recovery was significantly faster in the two patients who had previously undergone splenectomy (an observation made before shows the likely importance of parameters other than progenitor numbers in the autograft to the speed of this process. Accordingly, elective splenectomy may be a useful maneuver to hasten hematologic recovery in patients with CML being considered for autograft-based treatments.

Despite many advances in the management of leukemia, the prognosis for patients with CML in blast phase has remained dismal, with a median survival of a few months. In the present study, all three patients who underwent treatment after progression to blast phase died early: two of recurrent disease and one of therapy-related toxicity. This is in keeping with the results from large series of such patients that indicate the inability of intensive therapy to eradicate the blast-phase subclone. However, the three patients treated for accelerated phase disease remain alive and well; two are without evidence of an evolved clone of cells, raising the possibility that they may have derived benefit from the treatment.

The general rationale for autografting in first chronic phase is that a reduction in the target population of leukemic stem cells available for secondary mutational events will delay emergence of a blast-phase subclone and thereby prolong survival. Therefore, it may be of significance that a cytogenetic remission could be achieved and sustained for at least a year in half of the patients. The fact that Ph+ cells subsequently became detectable is not surprising in view of experience with syngeneic transplants and evidence that immune effector cells in allografts can make an important contribution to the prevention of disease recurrence after allogeneic BMT. On the other hand, although the majority in this series continue with morphologic and/or significant cytogenetic evidence of disease (in accord with other studies), only one of the patients treated in first chronic phase has thus far developed blast-phase disease. However, the design of this study precludes assessment of the independent contribution of other variables such as patient selection with regard to this endpoint. It is likely that many of the patients selected here belonged to a "good-prognosis" subgroup and would have survived free of blast phase for a longer period than average regardless of the therapy they received. Similarly, although it is gratifying that a low dose of IFNα (typically 3 × 10^6 U, 3 d/wk) was effective in reducing the recurrent Ph+ population in half of the patients treated (in addition to being well tolerated), their original selection on the basis of a greater marrow content of primitive normal cells (Ph- LTC-ICs) raises the possibility that a similar result might have been achieved with IFNα in full dose as primary therapy.

These reservations notwithstanding, 5 of the 22 patients entered into this feasibility study continue with predominantly Ph+ hematopoiesis for greater than 3 years and for these, the natural history of the disease may have been altered. Durable Ph+ hematopoiesis after autografting unp manipulated PB cells in two patients was reported in the Hammer smith study. However, in both cases, hematopoiesis was Ph+ early after autografting, and Ph+ cells became predominant only later, suggestive of an endogenous rather than an autograft-derived origin. This pattern of late emergence of Ph+ hematopoiesis is reminiscent of the occasional cases of cytogenetic conversion reported in patients recovering from narrow hypoplasia after busulfan. Taken together, these findings provide additional support for the view that Ph+ stem cells with long-term repopulating potential are present, but suppressed in some (perhaps many) patients with CML. Thus, the challenge that remains is to develop sensitive and specific procedures for their enumeration and to devise strategies (or combinations of strategies) for their selective isolation to use in conjunction with improved treatment protocols. In addition to the culture-based procedure described here, physical, pharmacologic, biologic, and molecular ex vivo techniques for obtaining enriched populations of normal hematopoietic cells from CML marrow are now being ex-
plored in a number of centers. The possibility of exploiting in vivo treatments that bring about transient Ph- hematopoiesis has also shown promise. With the advent of clinically applicable gene-marking procedures, it should soon be possible to obtain definitive information about the relative roles of normal and leukemic cells in the autograft and their contributions to restoration of normal hematopoiesis and disease recurrence. Such approaches, in combination with strategies to enhance the normal stem cell content of the autograft as described here, should provide the type of information needed to define an improved treatment for CML.

ACKNOWLEDGMENT

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REFERENCES

26. Grieg AP, Shepherd JD & Phillips GL. Busulphan and phe-
Intern Med 112: 313, 1990 (letter))

27. Seabright M: A rapid banding technique for human chromo-

28. Kaplan EL, Meier P: Nonparametric estimation from incom-

SV, Mantel N, McPherson K, Peto J, Smith PG: Design and analysis
of randomized clinical trials requiring prolonged observation of each
patient. II. Analysis and examples. Br J Cancer 35:1, 1977

term oligoclonal hematopoiesis after transplantation of culture-
purged autologous bone marrow in chronic myeloid leukemia
(CML). Blood 80:67a, 1992 (suppl, abstr)

31. Heilmann R, Heimpel H, Hasford J, Kolb HJ, Pralle H, Hoss-
feldt DK, Queisser W, Loffler H, Heinze B, Georgii A, Wussow
PV, Bartram C, Greisshammer M, Bergmann L, Essers U, Falge C,
Hoehaus A, Queisser U, Sick C, Meyer P, Schnitz N, Verpoort
K, Eimermacher H, Walther F, Westerhausen M, Kleeberg UR, Heil-
ein A, Kabisch A, Barz C, Zimmermann R, Meuret G, Tichelli A,
Berdel WE, Kanz L, Anger A, Tichelli A, Brockhaus W, Zankovich
R, Schlafer U, Weissenfels I, Mainzer K, Tobler A, Per-
and hydroxyurea in chronic myeloid leukemia: Prolongation of
survival by hydroxyurea. Blood 82:398, 1993

32. Brito-Babapulle F, Bowcock SJ, Marcus RE, Appleby J,
Th'ng KH, Dowding C, Rassool F, Guo A-P, Catovsky D, Galton
DAG, McCarthy D, Goldman JM: Autografting for patients with
chronic myeloid leukaemia in chronic phase: Peripheral blood stem
cells may have a finite capacity for maintaining haemopoiesis. Br J
Haematol 73:76, 1990

33. Kantarjian HM, Talpaz M, LeMaistre CF, Spinolo J, Spitzer
G, Yau J, Dicke K, Jagannath S, Deisseroth AB: Intensive combina-
tion chemotherapy and autologous bone marrow transplantation
leads to the reappearance of Philadelphia chromosome-negative cells in

34. Goldman JM, Johnson SA, Islam A, Catovsky D, Galton
DAG: Haematological reconstitution after autografting for chronic
granulocytic leukemia in transformation: The influence of previous

TL, Fleetwood KA, McCredie KB, Freireich EJ: Chronic myelogenous
83:445, 1977

36. Haines ME, Goldman JM, Worsley AM, McCarthy DM, Wy-
att SE, Dowding C, Kameary L, Th'ng KH, Wathen NH, Pollock
A, Galvin MC, Samson D, Geary CG, Catovsky D, Galton DAG:
Chemotherapy and autografting for chronic granulocytic leukemia in
transformation: Probable prolongation of survival for some pa-

37. Reiffers J, Trosset R, Marti G, Montastruc M, Faberes C,
Cony-Makhoul P, David B, Bourdeau MJ, Bihou-Nabera C, La-
combe F, Fouillatre-Fabre F, Vezon G, Bernard P, Broustet A: Autol-
ogous bone stem cell transplantation for chronic granulocytic leu-
kaemia in transformation: A report of 47 cases. Br J Haematol
77:339, 1991

38. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey
J, Kolb HJ, Rimm AA, Ringden O, Rozman C, Speck B, Truitt
RL, Zwaan FE, Bortin MM: Graft-versus-leukemia reactions after

39. De Fabritiis P, Sandrelli A, Meloni G, Alimena G, Monte-
fusco E, Lo Coco F, De Felice L, Mandelli F: Prolonged suppression of
myeloid progenitor cell numbers after stopping interferon treat-
ment for CML may necessitate delay in harvesting marrow cells for
autografting. Bone Marrow Transplant 6:247, 1990

40. Sokal JE, Cox EB, Baccarani M, Tura S, Gomez GA, Robert-
son JE, Tso CY, Braun TJ, Clarkson BD, Cervantes F, Rozman C,
The Italian Cooperative CML. Study group: Prognostic discrimina-

41. Singer CRJ, McDonald GA, Douglas AS: Twenty-five year
survival of chronic granulocytic leukaemia with spontaneous karyo-

42. Verfaillie CM, Miller WJ, Boylan K, McGlave PB: Selection
of benign primitive hematopoietic progenitors in chronic myelogenous
leukemia on the basis of HLA-DR antigen expression. Blood
79:1003, 1992

43. Carlo-Stella C, Mangoni L, Piovani G, Almici C, Garau D,
Caramatti C, Rizzoli V: In vitro marrow purging in chronic myelogenous
leukemia: Effect of mafosfamide and recombinant granulocyte-
macrophage colony-stimulating factor. Bone Marrow Transplant
8:265, 1991

44. McGlave PB, Arthur D, Miller WJ, Lasky L, Kersey J: Auto-
logous transplantation for CML using marrow treated ex vivo with
recombinant human interferon gamma. Bone Marrow Transplant
9:115, 1990

45. Skorski T, Nieborowska-Skorska M, Barletta C, Malaguar-
era L, Szczylczik C, Chen S-T, Lange B, Calabretta B: Highly efficient
elimination of Philadelphia 'leukemic' cells by exposure to bcr/abl
antisense oligodeoxynucleotides combined with mafosfamide. J Clin
Invest 92:194, 1993

46. Korbling M, Burke P, Braine H, Elfenbein G, Santos G, Kaizer H:
Successful engraftment of blood derived normal hemopoietic
stem cells in chronic myelogenous leukemia. Exp Hematol
9:684, 1981

47. Carella AM, Podesta M, Frassoni F, Raffo MR, Pollicardo
N, Pungolino E, Vimercati R, Sessarego M, Parodi C, Rabitti C,
Ferrero R, Benvenuto F, Figari O, Carlier P, Levacis G, Valsbonesi
M, Vitale V, Giordano D, Pierlaigi D, Nati S, Guaraccio A, Rosso
C, Saglio G: Collection of normal blood repopulating cells during early
hemopoietic recovery after intensive conventional chemotherapy
in chronic myelogenous leukemia. Bone Marrow Transplant
12:267, 1993

48. Simonsson B, Oberg G, Killander A, Bjoromman M, Bjorholm
Wahlin A, Lofvenberg E, Carrenske J, Westin J: Intensive treatment
in order to minimize the Ph-positive clone in chronic myelogenous
leukemia. Stem Cells 11:73, 1993 (suppl 3)

49. Brenner MK, Rill DR, Holladay MS, Heslop HE, Moen RC,
Buschle M, Krance RA, Santana VM, Anderson WF, Ihle JN: Gene
marking to determine whether autologous marrow infusion restores

50. Deisseroth AB, Zu S, Claxton D, Hanania EG, Fu S, Ellerson
D, Goldberg L, Thomas M, Jaincek K, Anderson WF, Hester J,
H, Calvert L, Tibbitts P, Talpaz M, Kantarjian H, Champlin R, Reading
C: Genetic marking shows that Ph' cells present in autologous trans-
plants of chronic myelogenous leukemia (CML) contribute to relapse
after autologous bone marrow in CML. Blood 83:3069, 1994
Autografting with cultured marrow in chronic myeloid leukemia: results of a pilot study [see comments]