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)+ as compared with 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− hematopoiesis 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 but 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− hematopoiesis for some patients with CML.

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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 (i.e., \( \geq 20 \times 10^3 \) initial marrow cells) and, at the same time, that Ph+ LTC-ICs be undetectable (which usually meant less than 1 Ph+ LTC-IC per \( 10^5 \) 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 standard conditions to detect these latter cell types. Later, when conditions for measuring normal and Ph+ LTC-ICs had been better defined, cells from the primary 10-day-old cultures were transferred to secondary feeder-containing LTC-IC assay cultures for 5 weeks and then finally transferred to colony assay cultures as described.

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. 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. 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.

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

### Table 1. Details of Patients at Autografting, Subsequent Outcome, and Survival

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age/ Sex</th>
<th>Previous Diagnosis to Autografting</th>
<th>Previous Splenectomy</th>
<th>Previous Disease Status</th>
<th>Outcome</th>
<th>From Diagnosis</th>
<th>From Autografting</th>
</tr>
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<tr>
<td>208</td>
<td>44/F</td>
<td>Hu</td>
<td>Yes</td>
<td>CP-1 Alive, in CCR off therapy*</td>
<td>132+</td>
<td>68+</td>
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<td>232</td>
<td>53/M</td>
<td>Vc, Pred, Dox; 6-Mp, Mtx</td>
<td>No</td>
<td>CP-3 Died of BP disease</td>
<td>10</td>
<td>4</td>
<td></td>
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<tr>
<td>240</td>
<td>41/M</td>
<td>Hu; Vc, Pred, Dox</td>
<td>No</td>
<td>CP-2 Died of TRT</td>
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<tr>
<td>341</td>
<td>39/M</td>
<td>Plica, Hu; Ara-C, Vb</td>
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<td>51+</td>
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<td>49+</td>
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<td>No</td>
<td>CP-1 Alive, in CCR on IFNa</td>
<td>57+</td>
<td>40+</td>
<td></td>
</tr>
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<td>59/M</td>
<td>Hu</td>
<td>No</td>
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<td>12</td>
<td>2</td>
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<td>Hu</td>
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<td>CP-1 Alive, in BP on Ara-C</td>
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<td>33+</td>
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<td>545</td>
<td>52/F</td>
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<td>32+</td>
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<tr>
<td>557</td>
<td>37/F</td>
<td>IFNa</td>
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<td>CP-1 Alive, in HR on IFNa</td>
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<td>30+</td>
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<td>Hu</td>
<td>No</td>
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<td>29+</td>
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<td>59/F</td>
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<tr>
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<td></td>
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<td>58/F</td>
<td>Hu</td>
<td>No</td>
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<td>1</td>
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<td>51/M</td>
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<td>22+</td>
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<tr>
<td>715</td>
<td>55/M</td>
<td>Hu; IFNa</td>
<td>No</td>
<td>BP-1 Died of BP disease</td>
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<td>7</td>
<td></td>
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<tr>
<td>748</td>
<td>22/M</td>
<td>Hu</td>
<td>No</td>
<td>CP-1 Alive, in CP on IFNa</td>
<td>22+</td>
<td>12+</td>
<td></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; Plica, plicamycin; Pred, prednisone; TRT, therapy-related toxicity; Vb, vinblastine; Vc, vinristine.

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

Supportive Care

anaesthesia and collected directly into transfer packs (Travenol) containing heparinized medium. A buffy-coat fraction was then prepared on a COBE 2991 Blood Cell Processor (COBE Canada Limited, Scarborough, Ontario), and aliquots of \(2 \times 10^8\) nucleated cells in \(\approx 2\) mL were dispensed into 175-cm\(^2\) tissue culture flasks (Falcon; Becton Dickinson Canada Inc, Mississauga, Ontario), each containing 60 mL of \(\alpha\)-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\(^{-5}\) mol/L 2-mercaptoethanol, and 10\(^{-6}\) mol/L hydrocortisone sodium succinate. Thus, the final cell concentration in the culture flasks was \(\approx 3 \times 10^6\) 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% CO\(_2\) 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 TC199 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 TC199 medium, loaded into a 600-mL transfer pack (Travenol), and 10 mL of autologous serum added to give a final serum concentration of \(\approx 2\)%.

This cell suspension was then infused rapidly into the central venous catheter of the patient via a line without a filter. In three cases (UPNs 573, 715, and 748), the marrow cells collected and infused 10 days later ranged from 0.9 to \(3 \times 10^6\) nucleated cells 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, and clinical response modifier program (BRMP) U/mg protein) subcutaneously once daily 3 to 7 days after the Autograft 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), \(2.5 \times 10^7\) U (=10\(^7\) Biological Response Modifier Program [BRMP] U/mg protein) subcutaneously, twice daily for 30 days starting on day 57 postautograft 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 \(\approx 1\) 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 \(2.6 \times 10^8\) (median 2.4) and the number recovered and infused 10 days later ranged from 0.9 to \(2.3 \times 10^8\) (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 3. Composition of the Autograft

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleated cells ((\times 10^8)/kg)</td>
<td>1.7</td>
<td>1.0-4.4</td>
</tr>
<tr>
<td>Clonogenic cells ((\times 10^7)/kg)</td>
<td>0.3</td>
<td>(=0.2^{*})</td>
</tr>
<tr>
<td>Ph</td>
<td></td>
<td>(=0.2^{*})</td>
</tr>
<tr>
<td>Ph'</td>
<td>2.4</td>
<td>(=0.7^{*})</td>
</tr>
<tr>
<td>Total</td>
<td>4.0</td>
<td>0.2-22</td>
</tr>
<tr>
<td>LTC-ICs (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ph</td>
<td>570</td>
<td>(=30^{*})</td>
</tr>
<tr>
<td>Ph'</td>
<td></td>
<td>(=6^{*})</td>
</tr>
<tr>
<td>Total</td>
<td>620</td>
<td>56-6300</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 × 10⁹/L occurred at a median of 29 (range 13 to 52) days (Fig 1), and recovery of platelets to 20 × 10⁹/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 × 10⁹/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 the 3 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 ≧0.5 × 10⁹/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).

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>
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<th>6-7</th>
<th>11-12</th>
<th>17-18</th>
<th>21-24</th>
<th>30</th>
<th>36</th>
<th>42</th>
<th>48</th>
<th>54</th>
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<td>100 (26)</td>
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<td>100 (25)</td>
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<td>0 (25)*</td>
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</table>

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

<table>
<thead>
<tr>
<th>Treatment, Days Delivered After Autografting</th>
<th>Reserve Cells</th>
<th>Neutrophil Recovery, Days After Autografting</th>
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<tr>
<td>UPN</td>
<td>GM-CSF</td>
<td>IL-3</td>
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<td>506</td>
<td>49-62</td>
<td>55*</td>
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<tr>
<td>534</td>
<td>35-48</td>
<td>55*</td>
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<tr>
<td>545</td>
<td>55-68</td>
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<td>105†</td>
</tr>
<tr>
<td>636</td>
<td>42-62</td>
<td>42†</td>
</tr>
</tbody>
</table>

Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor (Hoechst or Schering), 250 µg/m² or 5 µg/kg subcutaneously, daily; IL-3, interleukin-3 (Sandoz), 5 µg/kg subcutaneously, daily.

* Blood.
† Marrow.

Table 6. Outcome According to Disease Status at Autografting

<table>
<thead>
<tr>
<th>Remission</th>
<th>Graft Failure</th>
<th>Therapy-Related</th>
<th>Disease-Related</th>
</tr>
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<tbody>
<tr>
<td>Hematologic</td>
<td>Cytogenetic*</td>
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<tr>
<td>Group 1 (n = 16)</td>
<td>11</td>
<td>9/2</td>
<td>4</td>
</tr>
<tr>
<td>Group 2 (n = 6)</td>
<td>5</td>
<td>4/1</td>
<td>1</td>
</tr>
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</table>

Group 1, Sixteen patients in first chronic phase. Group 2, Three patients in accelerated phase, 1 in second chronic phase, 1 in third chronic phase, and 1 in first blast phase.

* Complete/partial.
† Two of these patients had graft failure.

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 IFNα. One (UPN 208) took IFNα 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 IFNα), 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.

Fig 2. Time to recurrence of ≥1% (A) and ≥10% (B) Ph+ cells after infusion of cultured marrow in 13 patients in whom complete cytogenetic remission was achieved. In A, 2 patients are censored: 1 at the time of death and the other at the time of commencement of IL-2 therapy. In B, 5 patients are censored: the 2 noted above and 3 others who were started on IFNα therapy when 4% to 8% Ph+ cells were detected.

Fig 3. Survival after autografting in group 1 (---t) (n = 16); group 2 (—–) (n = 6). Patients who remain in complete (A) or partial (A) cytogenetic remission are indicated.
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 hydroxyurea36,37 or IFNα.12 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.20

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.32,33 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.32

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 hematologic recovery been achieved earlier. There was no correlation between speed of recovery and the number of clonogenic 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 before34) 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.39 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 patients40,41 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.30 On the other hand, although the majority in this series continue with morphologic and/or significant cytogenetic evidence of disease (in accord with other studies32,33,39), 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" subgroup40 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.32

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 unmanipulated PB cells in two patients was reported in the Hammer- smith study.32 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.41 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,42 pharmacologic,43 biologic,44 and molecular45 ex vivo techniques for obtaining enriched populations of normal hematopoietic cells from CML marrow are now being ex-
explored 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.

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