Successful autologous stem cell collection in patients with chronic myeloid leukemia in complete cytogenetic response, with quantitative measurement of BCR-ABL expression in blood, marrow, and apheresis products

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Abstract
Imatinib mesylate is the initial therapy of choice for chronic myeloid leukemia in chronic phase (CML-CP), but in some patients, the disease becomes resistant to imatinib. Autologous stem cell transplantation using cells collected while in complete cytogenetic response (CCyR) may represent a therapeutic option for these patients. We mobilized and collected autologous CD34+ stem cells from 20 CML-CP patients in CCyR, 19 of whom were taking imatinib, and measured BCR-ABL expression in the apheresis products, blood and bone marrow using real-time quantitative PCR (RQ-PCR). Stem cells were mobilized with G-CSF 10 μg/kg daily for 5 days. In patients whose initial collection was <2 × 10⁶ CD34+ cells/kg, G-CSF dose was increased to 10 μg/kg twice daily on the second attempt, and imatinib was held for 14 days if a third attempt was necessary. All 20 patients successfully mobilized the target yield of 2 to 5 × 10⁶ CD34+ cells/kg; 16 reached target yield with the first mobilization. The median number of CD34+ cells collected was 4.4 (range, 2.0 – 8.4) × 10⁶/kg in a median of 3 (range, 2 – 6) apheresis days. Of 17 patients whose stem cell products were evaluable by RQ-PCR, 11 (65%) had undetectable BCR-ABL in the CD34+ product with undetectable BCR-ABL; 4 of these (24%) had no detectable BCR-ABL in any apheresis products. BCR-ABL expression in apheresis products was correlated with levels of expression in the blood and marrow prior to mobilization. No patient has yet required transplantation. With median follow-up of 18 months, all patients remain in CCyR and 9 of 16 (54%) have undetectable BCR-ABL in the most recent blood and marrow sample.

Keywords: Chronic myeloid leukemia, imatinib mesylate, autologous stem cell mobilization, BCR-ABL, minimal residual disease

Introduction
Imatinib mesylate, an oral inhibitor of the BCR-ABL tyrosine kinase, is the initial therapy of choice for most patients with newly diagnosed chronic myeloid leukemia (CML), due to its superior efficacy, convenience, and reduced toxicity compared to other therapies such as interferon and cytarabine [1,2]. Imatinib produces major cytogenetic responses in most patients with CML in chronic phase (CML-CP), and complete cytogenetic responses (CCyRs) in many [1 – 3]. Although most of these responses have been durable, a minority of patients have progressive disease after several years. Molecular monitoring with real-time quantitative PCR (RQ-PCR) for BCR-ABL is a sensitive tool for monitoring the...
disease status of patients in CCyR on imatinib, and the magnitude of molecular response has been shown to predict the duration of cytogenetic response [4].

Allogeneic stem cell transplantation, the only known curative therapy for CML, is not a suitable option for many patients, due to lack of HLA-matched donors, age, comorbidities, or patient preferences. High dose chemotherapy followed by autologous stem cell transplantation (autoSCT), using CD34+ stem cells collected while in complete cytogenetic remission (CCyR), may represent a therapeutic option for patients whose disease becomes resistant to imatinib and other drug therapies. Prior to the imatinib era, autoSCT was shown to induce hematologic and cytogenetic responses in CML patients, but was associated with a high rate of relapse, likely due to contamination of the stem cell products with BCR-ABL+ cells [5–7].

Previously, we have reported preliminary success in mobilizing stem cells with G-CSF from patients in CCyR on imatinib, and measuring BCR-ABL expression in the stem cell products as well as blood and bone marrow [8]. Here, we report our completed data from 20 patients as well as quantitative molecular monitoring of disease status after mobilization. We show that in some patients it is possible to collect stem cell products in which BCR-ABL is undetectable by sensitive RQ-PCR.

Patients and methods

Patient eligibility

Twenty patients with histologically confirmed CML-CP in CCyR (absence of Ph+ cells in 20 metaphase cells analyzed from the bone marrow) within 6 weeks of first apheresis were enrolled. All had adequate organ function and performance status to eventually undergo high dose chemotherapy, but were not candidates for allogeneic SCT due to lack of a suitable donor or patient preference. All patients signed informed consent in accordance with Institutional Review Board guidelines.

Stem cell mobilization and collection

Target stem cell yield was 2 to 5 × 10^6 CD34+ cells/kg. Patients were mobilized using a 3-step protocol as follows; if <2 × 10^6 CD34+ cells/kg were collected after step 1, patients were remobilized with step 2, and then step 3 if needed.

Step 1. Filgrastim (G-CSF), 10 μg/kg/day (rounded to vial size) subcutaneously; either once daily or in two divided doses. Patients taking imatinib continued during mobilization; one patient taking interferon discontinued treatment 2 weeks prior to mobilization.

Step 2. G-CSF dose was doubled to 10 μg/kg subcutaneously twice daily.

Step 3. Imatinib was held for 2 weeks, then G-CSF was given 10 μg/kg subcutaneously twice daily.

Peripheral blood CD34+ cell counts were measured daily beginning on day 5 of G-CSF treatment. Leukapheresis began when CD34+ counts exceeded 10 000/μL in the blood, and continued daily until the target yield was reached, or an inadequate collection with declining yield was noted. G-CSF was continued until completion of apheresis. Stem cells were cryopreserved in DMSO and stored in liquid nitrogen, per standard apheresis laboratory and blood bank procedures.

Quantitative real-time PCR analysis for BCR-ABL expression

Samples of blood, bone marrow, and daily apheresis products were prepared and analyzed for BCR-ABL expression using previously described methods [9]. BCR-ABL expression was measured in blood and bone marrow within 6 weeks prior to the first mobilization, in each daily apheresis product, and in blood (every 2–3 months) and bone marrow (every 6–12 months) following stem cell collection. RQ-PCR of patient specimens, standard dilutions, and negative controls were analyzed in triplicate using a LightCycler instrument (Roche Diagnostics, Indianapolis, IN). BCR-ABL transcripts were amplified in 20 μL reactions containing 1.0 μL of cDNA; 10 mM Tris-HCl, pH 8.3; 50 mM of KCl; 4 mM MgCl2; 0.2 mM of each dNTP; 5 μg BSA; 1.25 U AmpliTaq Gold DNA polymerase (PE Biosystems, Foster City, CA); 300 nM of each primer; and 100 nM of probe. Similarly, ABL transcripts were amplified in order to control for variation between specimens in RNA integrity and cDNA synthesis efficiency. The final concentrations of primer and probe were 400 and 100 nM, respectively. The BCR-ABL p210 transcripts were amplified using previously published primer and probe sequences for the b3a2 and b2a2 splice variants [10]. The p190 and ABL transcripts were detected using the following primer and probe sequences: (p190 5’) GCAGATCTGGCCCAAC GAT, (p190 3’) TCAGACCTCTGA-GGCTCAA A GTC, (p190 probe) 6FAM – CATGGAGACGCAG AA GCCTTCAGG-TAMRA; (ABL 5’) AAAAA T AAGCCCAACCTTITTCG; (ABL 3’) CCATTC CCATTGTGA TTATAGC; (ABL probe) 6FAM- TCTAAGCATACTAAGGGTAAAAGCTCCG
GGTCTT-TAMRA. Standard TaqMan™ PCR parameters (ABI PRISM 7700 SDS) were applied to all BCR-ABL and ABL amplifications.

Quantification and normalization of BCR-ABL
The absolute concentrations of BCR-ABL and ABL transcripts in patient specimens were determined by reference to standard curves. All standard curves were generated from fivefold serial dilutions of cDNA (ranging from 80 to 250 ng) from a CML cell line containing the appropriate BCR-ABL transcript. Real-time RT-PCR results are reported as a ratio, or normalized quotient (NQ), of BCR-ABL/ABL. NQ values \( \leq 1 \times 10^{-5} \) are below the limit of detection with our assay. The sensitivity of each assay was approximately \( 10^{-6} \) for the p190 transcript and between \( 10^{-5} \) and \( 10^{-6} \) for both p210 transcripts.

Statistical analysis
Descriptive statistics, including median and range, were calculated for each continuous variable. Since the distributions of BCR-ABL expression levels in the blood, bone marrow, and apheresis products were highly non-normal, Spearman rank correlation coefficients (denoted \( r_s \)) were derived to assess the correlation among these quantities. A two-sample \( t \)-test was performed for comparisons of group means. \( p \) values \( \leq 0.05 \) were regarded as statistically significant.

Results
Patient characteristics
Baseline characteristics of the patients are shown in Table I. Twenty patients (10 male and 10 female) with a median age of 49 years (range, 17–71) were enrolled. The median time from CML diagnosis to enrollment was 31 months (range, 8–112 months). All patients were in CCyR at the time of enrollment; the median times from diagnosis to CCyR and CCyR to stem cell collection were 18 months (range, 3–92) and 9 months (range, 1–40), respectively. Nineteen patients were taking imatinib (median dose, 400 mg; range, 400–800 mg daily) for a median of 23 months (range, 9–30); eight of these had prior therapy with interferon. One patient in CCyR on interferon maintenance therapy never received imatinib. None had previously undergone high-dose chemotherapy and SCT. Of the 19 patients taking imatinib, the median time from imatinib initiation to documentation of CCyR was 8 months (range, 2–26).

Stem cell mobilization and collection
Stem cell mobilization and collection results for each patient are shown in Table I. All patients achieved total CD34\(^+\) cell yields of \( >2 \times 10^6/\text{kg} \). Seventeen patients (85%) achieved target yield with Step 1 of the mobilization protocol; one of these had the G-CSF dose doubled the evening prior to the last day of collection. One patient (5%) achieved the target yield with Step 2 (doubled G-CSF dose). Two patients (10%) required a discontinuation of imatinib use (Step 3) 2 weeks prior to mobilization with G-CSF 10 \( \mu \)g/kg daily. The median total CD34\(^+\) cells collected for all patients was \( 4.4 \times 10^6/\text{kg} \), in a median of 3 (2–6) apheresis days. No significant correlation was observed between stem cell yield and age (\( r_s = 0.22, p = 0.36 \)), duration of imatinib use (\( r_s = 0.27, p = 0.27 \)), nor whether patients had other therapies prior to imatinib (\( t = 0.46, p = 0.65 \)).

BCR-ABL expression in bone marrow, blood, and apheresis products
Samples were evaluable for 17 patients (2 patients had aberrant BCR-ABL transcripts that were not detectable by our probes, and one patient’s samples had unacceptable RNA quality).

BCR-ABL expression in bone marrow and blood prior to apheresis. Baseline BCR-ABL expression measured by RQ-PCR is shown in Figure 1. Median normalized ratios of BCR-ABL/ABL in the bone marrow (evaluable for 13 patients) and peripheral blood (15 patients) were \( 5.0 \times 10^{-4} \) (undetectable, \( 6.4 \times 10^{-2} \)) and \( 9.8 \times 10^{-4} \) (undetectable, \( 4.6 \times 10^{-2} \)), respectively. Prior to apheresis, five patients had undetectable MRD in the bone marrow, six had undetectable MRD in the peripheral blood, and three had undetectable BCR-ABL expression in both blood and bone marrow.

BCR-ABL expression in apheresis products. Fifty-two daily apheresis products from 17 patients were analyzed by RQ-PCR; results are shown in Figure 1. The median NQ of BCR-ABL/ABL was \( 1.1 \times 10^{-3} \) (undetectable, \( 9.2 \times 10^{-2} \)). Twenty-three of 52 apheresis products (44%) had undetectable BCR-ABL. Eleven of 17 patients (65%) had at least one daily apheresis product with undetectable BCR-ABL; four of these (24%) had undetectable BCR-ABL in all apheresis products. The median BCR-ABL/ABL normalized quotient (NQ) for each patient was correlated with premobilization NQ in the bone marrow (\( r_s = 0.64, p = 0.019 \)) and peripheral blood (\( r_s = 0.84, p = 0.0001 \)), as shown in Figure 2. There
Table I. Patient characteristics and results of CD34\(^+\) stem cell collection.\*  

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age and sex</th>
<th>Months since CML diagnosis</th>
<th>Therapy prior to imatinib</th>
<th>Therapy at enrollment</th>
<th>Months from CCyR to 1st mobilization</th>
<th>Mobilization attempts</th>
<th>Apheresis days</th>
<th>Total CD34(^+) cell yield ((\times 10^6/kg))</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>17, F</td>
<td>11</td>
<td>–</td>
<td>IM 400 mg</td>
<td>1</td>
<td>1(^\dagger)</td>
<td>3</td>
<td>3.3</td>
</tr>
<tr>
<td>2</td>
<td>40, F</td>
<td>25</td>
<td>–</td>
<td>IM 800 mg</td>
<td>6</td>
<td>1</td>
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<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>42, F</td>
<td>51</td>
<td>HHT/Ara-C/IFN</td>
<td>IFN</td>
<td>40</td>
<td>1</td>
<td>2</td>
<td>5.6</td>
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<tr>
<td>4</td>
<td>40, F</td>
<td>17</td>
<td>–</td>
<td>IM 600 mg</td>
<td>8</td>
<td>3</td>
<td>5 (3, 0, 2)</td>
<td>7.7 (1.7, 0, 6.0)</td>
</tr>
<tr>
<td>5</td>
<td>24, M</td>
<td>18</td>
<td>IFN</td>
<td>IM 400 mg</td>
<td>4</td>
<td>2</td>
<td>6 (3, 3)</td>
<td>4.9 (1.5, 3.4)</td>
</tr>
<tr>
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<td>58</td>
<td>–</td>
<td>IM 600 mg</td>
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<td>1</td>
<td>3</td>
<td>4.4</td>
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<td>70</td>
<td>IFN</td>
<td>IM 400 mg</td>
<td>10</td>
<td>3</td>
<td>6 (2, 0, 4)</td>
<td>3.4 (0.9, 0, 2.5)</td>
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<tr>
<td>8</td>
<td>34, F</td>
<td>33</td>
<td>IFN/Ara-C</td>
<td>IM 400 mg</td>
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<td>1</td>
<td>3</td>
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<tr>
<td>9</td>
<td>51, F</td>
<td>8</td>
<td>–</td>
<td>IM 400 mg</td>
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<td>IFN/Ara-C</td>
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<td>2</td>
<td>8.4</td>
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<tr>
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<td>2</td>
<td>5.4</td>
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<td>2</td>
<td>4.0</td>
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<tr>
<td>16</td>
<td>41, F</td>
<td>34</td>
<td>–</td>
<td>IM 800 mg</td>
<td>22</td>
<td>1</td>
<td>3</td>
<td>4.5</td>
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<tr>
<td>17</td>
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<td>IM 600 mg</td>
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<td>3</td>
<td>3.7</td>
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<tr>
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<td>71, F</td>
<td>43</td>
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<td>3</td>
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<tr>
<td>19</td>
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<td>IM 400 mg</td>
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<td>3</td>
<td>3.8</td>
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<tr>
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<td>27, F</td>
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<td>–</td>
<td>IM 400 mg</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>7.1</td>
</tr>
<tr>
<td>Median (range)</td>
<td>49 years (17 – 71)</td>
<td>31 months (8 – 112)</td>
<td>–</td>
<td>400 mg (400 – 800)</td>
<td>7 (1 – 40)</td>
<td>1</td>
<td>3 (2 – 6)</td>
<td>4.4 (\times 10^6)/kg (2.0 – 8.4)</td>
</tr>
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</table>

*Patient characteristics at study entry and outcomes of CD34\(^+\) stem cell mobilization, and daily doses of imatinib are given. For patients who required >1 mobilization, total number of days that stem cells were collected and stored and total CD34\(^+\) yields from all collections are given, followed by data in parentheses from the individual mobilization attempts. Zeroes indicate that peripheral blood CD34\(^+\) counts were inadequate for apheresis to proceed.

\(^{\dagger}\)G-CSF dose was doubled prior to the day of collection due to declining peripheral blood CD34\(^+\) cell count.

HHT, homoharringtonine; IFN, interferon; Ara-C, cytarabine; RA, retinoic acid; IM, imatinib mesylate (daily doses are listed).
was no correlation observed between duration of imatinib therapy or CCyR and BCR-ABL expression in apheresis products ($r_s = 0.01$, $p = 0.98$ and $r_s = -0.06$, $p = 0.80$, respectively).

**CML disease status and BCR-ABL expression following stem cell collection**

Median follow-up after stem cell collection is now 18 months; longest follow-up is 27 months. To date, all of the patients remain in CCyR and continue on imatinib. None have required autoSCT. Figure 3 shows BCR-ABL expression measured by RQ-PCR in peripheral blood for 16 evaluable patients, from the time of study enrollment to most recent follow-up. There was considerable variation in BCR-ABL expression over the course of the study. Of five patients who had undetectable BCR-ABL expression prior to stem cell collection, two continued to have undetectable BCR-ABL throughout the study, two have detectable BCR-ABL at most recent follow-up, and one has had fluctuating values. Of 11 patients who had detectable BCR-ABL expression prior to stem cell collection, six now have persistently undetectable BCR-ABL, two never achieved undetectable BCR-ABL, and three have had fluctuating values.

**Discussion**

Autologous stem cell collection is feasible in CML-CP patients in CCyR on imatinib. Seventeen of the 19 patients (89%) taking imatinib in our sample achieved target yields without discontinuing imatinib (Step 1 or Step 2), and the other two patients mobilized successfully with a brief discontinuation of imatinib, with an overall 100% success rate. Although imatinib has been shown to suppress the circulation of hematopoietic progenitor cells [11], our results suggest that G-CSF can overcome this suppression.

Several other groups have reported their experiences with stem cell mobilization in CML patients in CCyR after imatinib therapy; successful mobilization rates ranged from 40% to 80% [12 – 14]. The reasons for our greater success in stem cell mobilization are not entirely clear. Although we did not observe an association between prior therapy and CD34$^+$ cell yield, it is notable that in our sample, unlike all prior published studies, the majority of patients were treated with imatinib as initial therapy. Moreover, our stepwise mobilization protocol used both an increased dose of G-CSF and discontinuation of imatinib, which may have increased its efficacy in patients who did not successfully mobilize on the first attempt.

This is the first study of stem cell mobilization in imatinib-treated CML patients to analyze BCR-ABL expression comprehensively in all apheresis products. We demonstrated that a substantial fraction of

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**Figure 1.** BCR-ABL expression measured by RQ-PCR in bone marrow, blood and apheresis products. Normalized quotients of BCR-ABL/ABL were measured in bone marrow and blood at the time of study enrollment, and in all stem cell apheresis products.

**Figure 2.** Correlation between baseline BCR-ABL expression in bone marrow and blood with BCR-ABL expression in apheresis products. The median BCR-ABL/ABL NQ from each patient’s apheresis products is plotted against the same patient’s BCR-ABL/ABL NQ from bone marrow (A) and blood (B) at the time of study enrollment. Spearman rank correlation coefficients are shown.
products (those from 24% of patients) had undetectable BCR-ABL by sensitive RQ-PCR. Compared with prior studies [12], we had a greater proportion of products with undetectable BCR-ABL, but our study population was limited to chronic phase patients in CCyR and included fewer heavily pretreated patients, likely representing patients in earlier stage of disease. Interestingly, Martinelli et al. observed declining levels of BCR-ABL in successive aphereses from 9 patients treated with interferon-α [7]; we did not observe this pattern in our study (data not shown). The clinical significance of BCR-ABL expression in apheresis products is unknown, but it would be logical to expect that clinical outcomes with autoSCT could be improved (compared with the pre-imatinib era) if stem cell products with undetectable levels of BCR-ABL were used for transplantation.

Consistent with others’ findings, and with longer follow-up, we saw no worsening of cytogenetic or molecular responses following stem cell mobilization [12]. Although our small sample size limits conclusions, perhaps optimal timing of stem cell collection can be further refined by monitoring the degree of molecular response in the peripheral blood. Since BCR-ABL expression in the apheresis products is correlated with expression in the blood and bone marrow, stem cell collection could be performed when BCR-ABL expression becomes undetectable or stable. Although most studies have defined major molecular responses in terms of log-reduction of pretreatment levels of BCR-ABL, achievement of undetectable BCR-ABL expression may confer additional prognostic value [15]. Comparison with prior studies is further complicated by a lack of uniform techniques in qualitative and quantitative PCR techniques; standardization of quantitative PCR techniques, probes, and normalization methods for BCR-ABL will facilitate comparison and collaboration among research groups [16,17].

In the era of imatinib and other tyrosine kinase inhibitors (TKIs), it remains to be seen whether a role exists for autoSCT in CML. New TKIs for imatinib-resistant disease are now available commercially and in clinical trials [18,19]. However, long-term therapy with TKIs may not be feasible for some patients due to toxicity [20] or the emergence of disease resistance. Although none of our patients have required SCT, transplantation with molecularly negative autologous stem cells collected in CCyR has been shown to be feasible in Ph+ acute lymphoblastic leukemia and acute promyelocytic leukemia [21,22]. Autologous transplantation with Ph-negative stem
cells collected while in CCyR may represent a therapeutic option for CML patients intolerant or resistant to TKIs. In addition, these stem cells could serve as a “back-up” source of hematopoietic progenitor cells for allogeneic SCT transplant recipients whose donor cells fail to engraft.

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