Normal and clonal hematopoietic progenitor cells have been demonstrated to coexist in chronic-phase chronic myelogenous leukemia (CML), but few data are available on the presence of non neoplastic hematopoiesis during the blast transformation phase. We used reverse transcription-polymerase chain reaction (RT-PCR) to investigate expression of the BCR/ABL transcript of individual hematopoietic progenitors from a CML patient in blast phase. We demonstrate that non clonal hematopoiesis is induced to re-emerge by conventional chemotherapy containing fludarabine®. In addition, we confirm that some pluripotent CD34+/CD33−/DR− cells circulating in the peripheral blood are not clonal. Our data provide an encouraging basis for further studies of in vitro purification of normal hematopoietic stem cells in advanced stage CML and of their use in the context of autologous bone marrow transplantation.

**ABSTRACT**
Normal and clonal hematopoietic progenitor cells have been demonstrated to coexist in chronic-phase chronic myelogenous leukemia (CML). Recent studies have shown that the expansion of the leukemic clone occurs at the level of intermediate-late progenitor cells, whereas residual normal precursors have been found within the CD34+/DR−/lin− cell compartment. The size, however, of the Ph1−cell population appears to be highly heterogeneous in early phase CML and it apparently declines as the time since diagnosis lengthens.

Therefore, it is still unclear whether non clonal hematopoietic progenitors are present in the more advanced stages of the disease (i.e. accelerated and blastic or acute phase), and whether they can be separated from their malignant counterparts.

Here we report on a CML patient in blastic phase whose hematopoietic progenitor cells were purified, and in whom individual colonies, cloned from those populations, were analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) to detect expression of the BCR/ABL transcript.

**Materials and Methods**

**Patients**
DM is a 23-year-old female. She was admitted to the leukemic unit of our institution in October 1992, 6 months after a diagnosis of accelerated phase PH1−CML. At the time of admission the patient presented fever, bone pain, lymphadenomegaly, metrorrhagia. Bone marrow aspirate and peripheral blood (PB) smears were consistent with a diagnosis of acute
phase CML. Blast cells showed a myeloid immunophenotype. Because of the rapid progression of the disease, she was offered an aggressive chemotherapy program consisting of 4 courses of fludarabine® and ARA-C followed by granulocyte colony-stimulating factor (G-CSF). The fludarabine® (FA) and ARA-C schedule protocol was: G-CSF 400 μg/m² day 1 before and during administration of FA (fludarabine® 30 mg/m² daily × 5 + ARA-C 2 g/m² daily × 5). G-CSF was continued in this FLAG regimen until the patient was in CR. The procedure was started when the white blood cell (WBC) count was 2.5×10⁹/L, and the platelet count 30×10⁹/L, and it was continued until 2×10⁶ CD34+ cells/kg had been collected.6,7 Circulating hematopoietic stem cells were collected with multiple leukaphereses during the recovery period following the 2nd chemotherapy course. At the time of harvest the patient showed a complete hematological response and a partial karyotypic conversion in the bone marrow (> 80% metaphases were Ph1+).

**CD34+ cell purification**

CD34+ cells were purified from the PB mononuclear cell (MNC) fraction by the avidin-biotin immunoabsorption technique.6,7 Subsequently, the cell population enriched with hematopoietic progenitor cells was depleted of CD33+/DR− cells by immunomagnetic separation.6,7 Table 1 details the enrichment steps. MNCs recovered from apereesis and purified progenitors (i.e. CD34+ and CD34+ CD33−/DR− cells) were seeded at concentrations of 50×10³ cells/mL and 5×10³/mL, respectively, in conventional methylcellulose cultures at 37°C in a fully humidified CO₂ atmosphere.6 After 14 days, hematopoietic colonies were scored, individually plucked and analyzed by PCR for the presence of the BCR/ABL transcript.

**bcr-abl chimeric transcript analysis by reverse transcription polymerase chain reaction**

Total cellular RNA was isolated from individual hematopoietic colonies using the method described by Chomczynski et al.8 RNA samples were resuspended in 20 μL of annealing buffer (10 mmol/L Tris 7.5; 1 mmol/L EDTA) and divided equally into two PCR reaction tubes for cDNA synthesis. RT-PCR was performed. An aliquot of 20 μL of the PCR products was added to 4 μL of loading buffer (LB) and was run on 8% polyacrylamide (acylamide-bisacrylamide 38:2) (PAGE) gels containing 1×TBE buffer (45 mM Tris-borate, 1 mM EDTA). Electrophoresis was performed at 250 W and 4°C with a refrigerated buffer system for 3-4 hours. The gels were soaked with ethidium bromide, photographed (data not shown), stained with silver (Silver Stain Kit, BioRad, Italy) and then photographed again. An approximately 345 bp fragment corresponding to the b2-a2 junction was present in positive samples (Figure 1). As expected, no Bcr-Abl product was detected in the negative controls (Figure 1). All the samples were positive for the Abl-Abl amplified fragment, used as c-DNA positive internal control (data not shown).

**Results**

Three aphereses procedures were performed. The CFU-GM and CD34+ cell yield is reported in Table 2. Stem cell purification was carried out using a cellular sample derived from the second apheresis product. Table 1 shows the recovery and the clonogenic efficiency of purified CD34+ and CD34+ CD33− DR− cells.

<table>
<thead>
<tr>
<th>no. cells</th>
<th>CD34+ cells %</th>
<th>CD34+ DR+</th>
<th>CE*</th>
<th>Ph+/Ph colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting population</td>
<td>1×10⁶</td>
<td>1.6</td>
<td>78</td>
<td>0.25</td>
</tr>
<tr>
<td>CD34− enriched</td>
<td>2×10⁷</td>
<td>95</td>
<td>80</td>
<td>5.2</td>
</tr>
<tr>
<td>CD33/DR− depleted</td>
<td>1.5×10⁶</td>
<td>96</td>
<td>3</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Circulating CD34+/CD33−/DR− cells were collected and purified after high-dose chemotherapy. *Clonogenic efficiency in semisolid medium. Colonies were scored, individually plucked and analyzed by PCR for the presence of the bcr/abl transcript at day 14 of culture.
RNA analysis demonstrated that 3 colonies out of 6 derived from non-purified PB cells and 3 colonies out of 5 derived from CD34+ cells were BCR/ABL(–). The selection of CD34+/CD33+/DR+ and CD34+/CD33–/DR– colonies resulted in the enrichment of BCR/ABL(–) progenitors (4 out of 5) (Figure 1).

**Discussion**

Despite the marked expansion of leukemic hematopoiesis, several studies have suggested that non clonal stem cells may be present within CML marrow. Here we show that bcr-abl negative hematopoietic progenitor cells coexist with leukemic stem cells in blastic crisis CML, and that Ph1- hematopoiesis can be restored by high-dose chemotherapy. In addition, we confirm that pluripotent CD34+/CD33–/DR– cells are enriched in non leukemic precursors. In vitro selection of benign progenitors in early phase CML and their use in the context of autologous bone marrow transplantation have been successfully attempted. Therefore, we believe that our data provide an encouraging basis for further studies of the purification and use of normal hematopoietic stem cells in advanced stage CML.

**References**