Innovative Two-Step Negative Selection of Granulocyte Colony-Stimulating Factor–Mobilized Circulating Progenitor Cells: Adequacy for Autologous and Allogeneic Transplantation

By Alessandro Rambaldi, Gianmaria Borleri, Gianpietro Dotti, Piermario Bellavita, Ricardo Amaru, Andrea Biondi, and Tiziano Barbui

A major obstacle in purifying either autologous or allogeneic hematopoietic stem cells from granulocyte colony-stimulating factor (G-CSF) mobilized circulating progenitor cells (CPC) is represented by the huge cellularity present in each apheresis product. To obtain a significant debulking of unwanted cells from the leukapheresis, we developed a modified protocol of immune rosetting whereby human ABO-Rh-compatible red blood cells (RBCs) are treated with chromium chloride and then coated with murine monoclonal antibodies (MoAbs) against leukocyte antigens. When experiments were performed with leukaphereses obtained from normal donors or from T-cell acute lymphoblastic leukemia (T-ALL) patients, RBCs were coated with murine MoAbs against human mature myeloid cells (CD11b) and T cells (CD6); whereas, in the case of patients with B-precursor ALL, B-cell non-Hodgkin’s lymphoma (B-NHL), or multiple myeloma (MM), RBCs were coated with anti-CD11b only. After incubation with CPC, rosetting cells (myeloid precursor cells, granulocytes, monocytes, and T cells) were removed by Ficoll-Hypaque density gradient centrifugation with a blood cell processor apparatus, COBE (LakeWOOD, CO) 2991. After this step, a significant reduction of the initial cellularity was consistently obtained (range, 72% to 97%), whereas the median absolute recovery of the CD34+ cells was above 85% (range, 64 to 100), with a 10-fold relative enrichment ranging from 3% to 43%. In a second step, CPC can be further purged of contaminating T or B cells by incubation with lymphoid-specific magnetic microbeads (anti-CD2 and -CD7 to remove T cells; anti-CD19 to remove B cells) and elution through a type-D depletion column (composed of ferromagnetic fiber) inserted within a SuperMACS separator device (Miltenyi Biotec, Bergisch-Gladbach, Germany). By this approach, a highly effective (three to four logs) T-cell depletion was achieved in all experiments performed with normal donors or T-ALL patients (median loss of CD3+ cells: 99.8% [range 99.2 to 100]) and an equally efficient B-cell depletion was obtained from B-precursor ALL, B-NHL, or MM patients. At the end of the procedure the T- or B-cell depleted fraction retained a high proportion of the initial hematopoietic CD34+ stem cells, with a median recovery above 70% (range 48% to 100%) and an unmodified clonogenic potential. In five patients (two follicular NHL and three ALL) the purified fraction of stem cells was found disease free at the molecular level as assessed by polymerase chain reaction (PCR) analysis of the t(14;18) chromosome translocation or clono-specific DNA sequences of IgH or T-cell receptor γ and δ chain genes. Purified autologous and allogeneic CPCs were transplanted in three and six patients, respectively, who showed a prompt and sustained hematologic engraftment. In conclusion, this method represents a simple and reproducible two-step procedure to obtain a highly efficient purging of T or B cells from G-CSF expanded and mobilized CPCs. This approach might lead to the eradication of the neoplastic clone in the autologous stem cell inoculum as well as for T-cell depletion during allogeneic transplantation.

© 1998 by The American Society of Hematology.
attention, especially in the case of patients with nonhematologic malignancies. Moreover, most acute leukemia cell types express the CD34 antigen, thus preventing the use of this method of purification in the autologous setting.

The aim of this study was to develop an efficient, reproducible, and relatively inexpensive method for clinical scale preparation of CPC for transplantation procedures. Furthermore, the selective- and lineage-specific elimination of the neoplastic fraction from the autologous graft as well as the normal T cells from the allogeneic graft was the aim of the same procedure. This novel methodology consists of two distinct steps: in the first step, 85% of the initial cellularity (granulocytes, monocytes, and eventually T cells) is allowed to form rosettes with chromium chloride-treated human ABO-Rh-compatible red blood cells (RBGs) coated with murine monoclonal antibodies (MoAbs) antihuman CD11b (and CD6 when T-cell depletion is needed) and then removed by gradient sedimentation, without a significant loss of the CD34+ cells present in the input. Subsequently, in step two, this debulkedapheresis is very efficiently purged of unwanted B or T cells by the use of lineage-specific monoclonal microbeads.

MATERIALS AND METHODS

Mobilization and harvesting of autologous or allogeneic CPCs. Autologous CPCs were collected in patients with non-Hodgkin's lymphoma (NHL), acute lymphoblastic leukemia (ALL), and MM, after different consolidation protocols of high-dose chemotherapy followed by rhG-CSF (Filgrastim; Roche, Milan, Italy) administration (5 µg/kg/day) as previously described.20 Leukapheresis was performed as soon as white blood cells (WBCs) were at least 3.0 x 10^9/L and peripheral blood CD34+ cells were ≥ 0.5%. Ten liters of blood were processed daily through an indwelling central venous catheter (Groshong CV Catheter; Bard Inc, Salt Lake City, UT) by using a cell separator COBE spectra (COBE, Lakewood, CO). Allogeneic CPCs were collected from HLA-identical, MLC-negative normal siblings upon treatment with 2 × 5 µg/kg/day of rhG-CSF administered subcutaneously every 12 hours for 5 or 6 consecutive days. The procedure of leukapheresis was started on day 5 (after the ninth dose of G-CSF) by using a COBE spectra cell separator. Ten liters of blood were processed daily by using the cubital vein in all donors, as previously described.20 The absolute number of CD34+ cells, lymphoid cells (T, B, and NK cells), and mature myeloid cells (granulocytes and monocytes) were evaluated daily in the peripheral blood by flow cytometry with fluorescein isothiocyanate conjugated (FITC) murine MoAbs against human CD34, CD3, CD19, CD56, CD11b, CD14, CD16, or negative control and a FACScan analyzer (Becton Dickinson, Mountain View, CA). In vitro colony assay for erythroid and myeloid colony forming units was performed by one operator and the estimated cost (including production and purification of antibodies, chemical reagents, tissue culture media, two blood cell separation sets, and 1 U of filtered, irradiated, RBCs) was 400 US dollars.

Immunomagnetic purging of T or B cells. To obtain a high degree of T- or B-cell depletion, partially purified hematopoietic progenitors (0.5 to 13 x 10^6 cells) obtained by immune rosetting were labelled with primary unconjugated MoAbs reacting against T cells (anti-CD2 antigen, clone 35.1; IgG2a, ATCC CRL 8026) reactive with human granulocytes, monocytes, NK cells and committed myeloid precursor cells, IgM, clone 3F12B8; ATCC HB8136) reacting with human T cells and some B cells (CD6 antigen). Partially purified preparations of these MoAbs were obtained by ammonium sulphate precipitation of spent culture supernatants of each hybridoma. ABO-Rh-compatible, irradiated (25 Gy), and filtered human red blood cells (HRBCs) were obtained from the Blood Bank. For coating with MoAbs, 150 mL of packed HRBCs were washed three times in normal saline (centrifugation at 3000 RPM for 5 minutes at room temperature) with a COBE 2991 apparatus and a blood cell processor set. After the third wash, 30 mL (3 mg/mL) of partially purified MoAbs (CD11b and eventually CD6 in the case of normal donors and T-ALL patients) were added at the same blood processor set. Thereafter, and under continuous agitation, 250 mL of a 0.1% solution of chromium chloride (CrCl3.6H2O; Sigma, St.Louis, MO) prepared in normal saline from a 1% [W/V] stock solution with the pH adjusted to 5.0 with 10 N NaOH) were added dropwise over a 15-minute time period. After incubation for 5 additional minutes at room temperature the reaction was stopped by the addition of 300 mL of phosphate-buffered saline (PBS) supplemented with 2.5% Human Serum Albumin (PBS-HSA). Appearance of the isotype of the antibodies (IgM or IgG) does not affect the coupling process to RBGs. MoAb-coated HRBCs were washed twice, resuspended in 100 mL of PBS 2.5% HSA, and mixed with 11 to 58 x 10^9 white blood cells (200 mL final volume, hematocrit less than 5%) obtained from leukophereses of G-CSF-treated individuals. Rosette formation was performed within the same blood cell processor set by centrifugation steps at 3000 RPM for 30 minutes. At the end cells were resuspended in 400 mL of PBS 2.5% HSA, transferred and layered onto the top of 200 mL Ficoll Hypaque (by using a second blood cell processor set), and centrifuged for 45 minutes at 3000 rpm. Nonrosetting cells were harvested at the Ficoll interface and washed twice with PBS-HSA. After 5 minutes of incubation with hypotonic NH4Cl buffer (NH4Cl 8.99 gr/L, KHCO3 1 gr/L, Na2EDTA 0.037 gr/L, pH 7.3) to lyse residual erythrocytes, cells were washed with PBS-HSA, resuspended in RPMI 1640 10% FCS, counted, and stained with MoAbs for FACS analysis. The described procedure required approximately 3 hours of work performed by one operator and the estimated cost (including production and purification of antibodies, chemical reagents, tissue culture media, two blood cell separation sets, and 1 U of filtered, irradiated, RBCs) was 400 US dollars.

Immune rosettes. The method is based on a previously published procedure based on the ability to couple murine MoAbs to RBCs by chromium chloride.21 The following mouse hybridoma cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD): OKM1 (IgG2b, ATCC CRL 8026) reactive with human granulocytes, monocytes, monocytes, NK cells and committed myeloid precursor cells (CD11b antigen), and T12 (IgM, clone 3F12B8; ATCC HB8136) reacting with human T cells and some B cells (CD6 antigen). Partially purified preparations of these MoAbs were obtained by ammonium sulphate precipitation of spent culture supernatants of each hybridoma. ABO-Rh-compatible, irradiated (25 Gy), and filtered human red blood cells (HRBCs) were obtained from the Blood Bank. For coating with MoAbs, 150 mL of packed HRBCs were washed three times in normal saline (centrifugation at 3000 RPM for 5 minutes at room temperature) with a COBE 2991 apparatus and a blood cell processor set. After the third wash, 30 mL (3 mg/mL) of partially purified MoAbs (CD11b and eventually CD6 in the case of normal donors and T-ALL patients) were added at the same blood processor set. Thereafter, and under continuous agitation, 250 mL of a 0.1% solution of chromium chloride (CrCl3.6H2O; Sigma, St.Louis, MO) prepared in normal saline from a 1% [W/V] stock solution with the pH adjusted to 5.0 with 10 N NaOH) were added dropwise over a 15-minute time period. After incubation for 5 additional minutes at room temperature the reaction was stopped by the addition of 300 mL of phosphate-buffered saline (PBS) supplemented with 2.5% Human Serum Albumin (PBS-HSA). Appearance of the isotype of the antibodies (IgM or IgG) does not affect the coupling process to RBGs. MoAb-coated HRBCs were washed twice, resuspended in 100 mL of PBS 2.5% HSA, and mixed with 11 to 58 x 10^9 white blood cells (200 mL final volume, hematocrit less than 5%) obtained from leukophereses of G-CSF-treated individuals. Rosette formation was performed within the same blood cell processor set by centrifugation steps at 3000 RPM for 30 minutes. At the end cells were resuspended in 400 mL of PBS 2.5% HSA, transferred and layered onto the top of 200 mL Ficoll Hypaque (by using a second blood cell processor set), and centrifuged for 45 minutes at 3000 rpm. Nonrosetting cells were harvested at the Ficoll interface and washed twice with PBS-HSA. After 5 minutes of incubation with hypotonic NH4Cl buffer (NH4Cl 8.99 gr/L, KHCO3 1 gr/L, Na2EDTA 0.037 gr/L, pH 7.3) to lyse residual erythrocytes, cells were washed with PBS-HSA, resuspended in RPMI 1640 10% FCS, counted, and stained with MoAbs for FACS analysis. The described procedure required approximately 3 hours of work performed by one operator and the estimated cost (including production and purification of antibodies, chemical reagents, tissue culture media, two blood cell separation sets, and 1 U of filtered, irradiated, RBCs) was 400 US dollars.

From www.bloodjournal.org by guest on October 31, 2017. For personal use only.
Molecular evaluation of minimal residual disease (MRD). In patients with ALL, MRD was evaluated by molecular analysis of junctional regions of rearranged T-cell receptor (TCR) γ or δ genes. Specific patterns of recombination of TCR γ or δ genes were identified at diagnosis by Southern blot analysis, according to standard techniques.23 The TCR δ and γ gene rearrangements were subsequently amplified by polymerase chain reaction (PCR) with V δ or V γ family primers and J δ or J γ general primers, respectively. The PCR products were cloned in pMos vector (Amersham, Buckinghamshire, UK) and -CD6 antibodies. The experimental procedure of immune rosetting was always carried out on the second or third harvest after a sufficient amount of unmodified CPCs were used or stored in liquid nitrogen. As shown in Table 1, 18 experiments were performed with a median starting cellularity of 41.3 × 10^9 (range, 29.7 to 58.2) nucleated cells. After immune rosetting, the median reduction of the initial cellularity was 91% (range, 72 to 97) and the cell loss was mainly due to a marked (more than 85%) depletion of myeloid cells and T lymphocytes. A parallel enrichment of CD34+ cells was obtained (more than 95%).

### RESULTS

Effect of immune rosetting on overall recovery and composition of G-CSF mobilized CPC. In normal donors receiving G-CSF (10 µg/kg/d) for 5 to 6 days, the procedure of CPC mobilization and collection was safe and always well tolerated. The apheresic products were debulked of committed myeloid cells and T lymphocytes by immune rosetting with anti-CD11b and -CD6 antibodies. The experimental procedure of immune rosetting was always carried out on the second or third harvest after a sufficient amount of unmodified CPCs were used or stored in liquid nitrogen. As shown in Table 2, 18 experiments were performed with a median starting cellularity of 41.3 × 10^9 (range, 29.7 to 58.2) nucleated cells. After immune rosetting, the median reduction of the initial cellularity was 91% (range, 72 to 97) and the cell loss was mainly due to a marked (more than 85%) depletion of myeloid cells and T lymphocytes.
To purge the contaminating normal or neoplastic T lymphocytes still present in the apheresic products after the debulking procedure with immune rosettes, partially purified allogeneic (9 experiments from normal donors) or autologous (7 experiments from T-ALL patients) CPCs were incubated with a mixture of anti-CD2 and -CD7 MoAbs and indirectly stained with goat antimouse magnetic microbeads. After loading onto a D-type depletion column, normal or leukemic contaminating T cells were significantly removed as judged by staining with anti-CD3 MoAbs (Table 2) and other T-cell–specific antigens like CD5, CD4, and CD8 (data not shown). The overall T-cell depletion obtained by the two combined procedures allowed a final 3 to 4 logs reduction of the T-cell content. Despite such aggressive removal of T cells, the median overall recovery of CD34+ cells was above 70% in both autologous and allogeneic CPCs (Table 2). Similar experiments were performed to remove B cells in CPCs obtained from patients with B-precursor ALL, B-NHL, and MM. As shown in Table 3, the percent and the absolute number of CD19+ cells detectable after this purification approach were very limited. Again, the absolute recovery of CD34+ cells from the initial leukapheresis was excellent, with a mean value above 80%.

**Evaluation of MRD.** A PCR-based quantitation of MRD was performed on CPC samples obtained from five patients before and after depletion of contaminating tumor B or T lymphocytes by purging with lineage-specific immunomagnets. Molecular analysis was performed by demonstration of chimeric genomic products generated by the t(14;18) chromosomal translocation or by analysis of leukemia-specific DNA sequence, amplified from the rearranged TCR γ, δ, or IgH chain genes. As shown in Table 4, after the purification procedure, CD19-purged HPCs obtained from two follicular NHLs and two B-precursor ALLs were judged as PCR negative within the sensitivity limits of our assays (between 10^−4 to 10^−3). Similarly, after purging with T-cell–specific microbeads the PCR evaluation of DNA samples from a T-ALL patient showed the absence of the leukemic-specific clone (Table 4 and Fig 1). Interestingly, in this case the amount of MRD was very limited and not detectable if searched within the whole unmodified apheresis product (Fig 1). However, the leukemic contamination was clearly shown in the unwanted, wasted, T-cell fraction retained within the magnetic column. Similar results were obtained in all the analyzed cases in which the neoplastic B-lymphoid cells were similarly trapped within the depletion column (Table 4).

## Table 3. B-Cell Depletion by Immune Rosettes and B-Cell–Specific Magnetic Microbeads (CD19) of Leukaphereses Obtained From B-NHL, B-precursor ALL, or MM Patients

<table>
<thead>
<tr>
<th>Antibody Used</th>
<th>Apheresis (%)</th>
<th>Immune Rosettes (CD11b+)</th>
<th>D-column (CD34+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell (×10^9)</td>
<td>19.4 (8.6-41.4)</td>
<td>2.4 (1.1-4.4)</td>
<td>2 (0.9-3.6)</td>
</tr>
<tr>
<td>% overall cell loss</td>
<td>85 (52-97)</td>
<td>87 (59-98)</td>
<td></td>
</tr>
<tr>
<td>CD34+ cells (×10^9)</td>
<td>0.3 (0.07-1.3)</td>
<td>0.3 (0.06-1)</td>
<td>0.2 (0.05-1)</td>
</tr>
<tr>
<td>%CD34+ cells</td>
<td>1.8 (0.6-5.4)</td>
<td>12 (1.5-28)</td>
<td>14 (1.5-37)</td>
</tr>
<tr>
<td>%CD34 recovery</td>
<td>88 (53-100)</td>
<td>80 (48-100)</td>
<td></td>
</tr>
<tr>
<td>%CD34 enrichment fold</td>
<td>9 (2-35)</td>
<td>10 (2-42)</td>
<td></td>
</tr>
<tr>
<td>CD11b+ cells (×10^9)</td>
<td>15 (3.1-39.6)</td>
<td>0.4 (0.01-1.2)</td>
<td>0.2 (0.005-0.9)</td>
</tr>
<tr>
<td>%CD11b+ cells</td>
<td>72 (37-98)</td>
<td>19 (0.6-38)</td>
<td>11 (0.4-28)</td>
</tr>
<tr>
<td>CD11b+ cell loss</td>
<td>96 (83-100)</td>
<td>97 (87-100)</td>
<td></td>
</tr>
<tr>
<td>CD3+ cells (×10^9)</td>
<td>3.3 (0.5-8.4)</td>
<td>1.4 (0.4-3)</td>
<td>1.3 (0.4-2.6)</td>
</tr>
<tr>
<td>%CD3+ cells</td>
<td>22 (1.4-55)</td>
<td>58 (34-90)</td>
<td>62 (42-92)</td>
</tr>
<tr>
<td>CD3+ cell loss</td>
<td>48 (11-78)</td>
<td>52 (16-79)</td>
<td></td>
</tr>
<tr>
<td>CD19+ cells (×10^9)</td>
<td>0.06 (0-0.2)</td>
<td>0.02 (0-0.09)</td>
<td>0.0004 (0-0.004)</td>
</tr>
<tr>
<td>%CD19+ cells</td>
<td>0.4 (0-2)</td>
<td>0.6 (0-2.3)</td>
<td>0.02 (0-0.1)</td>
</tr>
<tr>
<td>CD19+ cell loss</td>
<td>72 (2-2.97)</td>
<td>99 (94-100)</td>
<td></td>
</tr>
</tbody>
</table>

*In two MM patients purging experiments were performed by using anti-CD19 and anti-CD56 magnetic microbeads.

### Table 4. Molecular Evaluation of Minimal Residual Disease After Lineage-Specific Purging

<table>
<thead>
<tr>
<th>Patients</th>
<th>Disease</th>
<th>Apheresis</th>
<th>Wasted Cells*</th>
<th>Purified Stem Cells</th>
<th>Sensitivity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NHL</td>
<td>t(14;18) BCL2/IgH</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>NHL</td>
<td>t(14;18)BCL2/IgH</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>preB-ALL</td>
<td>Telg (Vγ4 Jγ1.3)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>T-ALL</td>
<td>Telg (Vγ6 Jγ6)</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>preB-ALL</td>
<td>IgH</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

*Wasted cells were eluted after removal of the D column from the magnetic field of the SuperMACS apparatus.
†The sensitivity of the PCR reaction was determined by log dilution of the patient’s DNA obtained at diagnosis with DNA from a normal donor.
Because the use of unmodified allogeneic CPC has been possibly associated with increased chronic GVHD\(^{28}\) in six acute leukemia patients undergoing transplantation from HLA-identical siblings, allogeneic CPCs were extensively T-cell depleted by immune rosetting and immunomagnetic purging. However, to prevent an increased rate of graft failure and leukemia relapse,\(^{29}\) escalating moderate\(^{30}\) amounts of donor T lymphocytes (from a minimum of 2.5 to a maximum of 50 \(\times\) 10\(^6\)/kg) were rescued from the rosetted cells (by hypotonic lysis with NH\(_4\) Cl buffer, as described in Materials and Methods), enumerated by immunophenotyping with anti-CD3 antibody, and added back to the stem cell fraction just before cryopreservation. Only two apheresic procedures were necessary to infuse more than 5 \(\times\) 10\(^6\) /kg CD34\(^+\) cells and despite the in vitro manipulation, a prompt hematologic reconstitution was observed in each patient (Table 6). According to previously published experience,\(^{16,17}\) a conventional GVHD prophylaxis with a combination of Methotrexate and Cyclosporine A was performed\(^{20,31}\) and neither acute (more than grade I) or chronic GVHD was observed in these patients.

**DISCUSSION**

The manipulation of specific cell subpopulations of marrow or peripheral blood origin has become an interesting way to increase the applicability and reduce the toxicity of hematopoietic transplantation. However, the manipulation of CPCs obtained from G-CSF treated normal donors or patients is hampered by the huge cellularity present in the apheretic products, which often exceeds the absolute number of 50 \(\times\) 10\(^6\) cells, and by the fact that under the stimulatory effect of G-CSF, mature myeloid cells (mostly granulocytes) acquire different characteristics of cell density preventing their sedimentation on normal Ficoll gradients. Therefore, two main approaches to the CD34\(^+\) purification have been taken either by positive or by negative selection. Indeed, in the perspective of genetic manipulation, positive selection of CD34\(^+\) cells is likely to be the ideal option to ensure that the selected gene is transduced only in the small target population of pluripotent progenitor cells.\(^{32}\) Although transplantation of positively selected CD34\(^+\) cells purified by immunoaffinity columns or immunomagnetic bead adsorption has been shown as a feasible procedure,\(^ {4,9,11-15}\) the specific depletion of unwanted cells seems preferable for several reasons including the preservation of the manipulation of CD34\(^+\) cells from binding with murine MoAbs and the need of their subsequent detachment by using chemical or physical methods. Moreover, most acute leukemias of both myeloid and lymphoid origin are positive for CD34 antigen expression thus reducing in this setting the clinical applicability of methods that rely only on the positive selection of the stem cell fraction. On
the contrary, the selective elimination of residual neoplastic cells detectable in the stem cell fraction obtained from some patients with MM, follicular lymphoma, and breast cancer could be achieved by the use of MoAbs either in association with complement, conjugated to toxic compounds, or by the use of magnetic microbeads proven to eradicate the neoplastic clone at the molecular level. However, it has to be mentioned that positive selection of CD34+ cells, combined with negative depletion steps, could also achieve high levels of purity, even though purging strategies based only on depletion techniques could avoid an extensive removal of T cells from the autograft of patients with B-lymphoproliferative disorders and solid tumors, thus reducing the risk of long-lasting immunodeficiency.

In this manuscript, we described a simple and reproducible method based on a two-step negative selection of cytokine mobilized circulating progenitor cells. With the first step of immune rosetting and subsequent Ficoll gradients we were able to obtain a drastic reduction of the massive starting cellularity of the leukapheresis product. The use of human RBCs suitable for autograft of patients with B-lymphoproliferative disorders and solid tumors, thus confirming the lack of any toxicity associated with the purification procedure.

The infusion of sibling-matched allogeneic hematopoietic stem cells purified according to this method, along with the add back of escalating amounts of T cells, was followed by a rapid hematologic engraftment, thus confirming the lack of any toxicity associated with the purification procedure.

In the setting of autologous transplantation, our data showed that an efficient purification of neoplastic B- or T-lymphoid cells could be reached and in the five cases analyzed at the molecular level, a disappearance of the neoplastic clone could be shown. Several obvious advantages are potentially associated with the use of purified stem cells for autologous hematopoietic transplantation in acute leukemia and lymphoma. We know that many patients can eventually relapse and die from their disease because of the infusion of tumor cells along with the autologous graft. Indeed, MRD persistence after autologous transplantation in NHL patients is associated with poor outcome, thus suggesting that neoplastic contamination of the graft should be eradicated for cure. The possibility of eradicating the residual leukemic cells in the apheresis product represents an important result because very little data about effective immunologic purging of ALL are available so far. Obviously, whereas these data represent a clear demonstration of the technical feasibility of a rapid and reproducible two-step purging procedure of cytokine-mobilized HPCs, the clinical outcome of autologous and allogeneic transplants in the diverse proposed clinical settings waits for future validation.

REFERENCES


38. Bertolini F, Thomas T, Battaglia M, Gibelli N, Pedrazzoli P,


Innovative Two-Step Negative Selection of Granulocyte Colony-Stimulating Factor–Mobilized Circulating Progenitor Cells: Adequacy for Autologous and Allogeneic Transplantation

Alessandro Rambaldi, Gianmaria Borleri, Gianpietro Dotti, Piermarco Bellavita, Ricardo Amaru, Andrea Biondi and Tiziano Barbui