RAPID COMMUNICATION

Polyclonal Primitive Hematopoietic Progenitors Can Be Detected in Mobilized Peripheral Blood From Patients With High-Risk Myelodysplastic Syndromes

By Michel Delforge, Hilde Demuynck, Peter Vandenbergh, Gregor Verhoef, Pierre Zachée, Victor Van Duppen, Peter Marijnen, Herman Van den Bergh, and Marc A. Boogaerts

Polyclonal primitive hematopoietic progenitors (HPPs) can be detected in mobilized peripheral blood (PBPC) from female patients with high-risk myelodysplastic syndrome (MDS). This finding is based on the analysis of clonal patterns of hematopoietic progenitors and their mature daughter cells in PBPC harvests of female patients with high-risk MDS. The clonal patterns were studied using assays such as the human androgen receptor (HUMARA) gene assay and the glucose-6-phosphate dehydrogenase (G6PD) isoenzyme patterns. The HUMARA assay benefits from a high heterozygosity frequency (\(-90\%\)) in the 5' located CAG repeat, stable methylation patterns, and polymerase chain reaction (PCR) accessibility. The HUMARA assay provides strong evidence for polyclonal immature hematopoietic progenitors in PBPC harvests of female patients with high-risk MDS. The study also indicates that X-chromosome inactivation patterns of female MDS patients can be distinguished by differences in methylation patterns. The HUMARA assay benefits from a high heterozygosity frequency (\(-90\%\)) in the 5' located CAG repeat, stable methylation patterns, and polymerase chain reaction (PCR) accessibility. Therefore, the study provides strong evidence that polyclonal immature hematopoietic progenitors can be mobilized and harvested in patients with high-risk MDS after treatment with high-dose chemotherapy.

© 1995 by The American Society of Hematology.
through 5) (ICE). Consolidation therapy included mitoxantrone (8 mg/m²/day on days 4 through 6) and Ara-C (2 x 500 mg/m²/day on days 1 through 6) (NOVIA). UPN 5 was refractory to the initial RI course and received a second intensified RI and consolidation course consisting of high-dose Ara-C (2 x 3 g/m²/day on days 1 through 6). Because of poor autologous marrow harvests (in UPN 2, 3, and 4) and lack of allogeneic donors, all patients underwent autologous PBPC collection in complete hematologic remission at a mean interval of 135 days (range, 63 to 237 days) after diagnosis. Patients were harvested after mobilization with growth factors alone (Granulocyte Colony-stimulating Factor [G-CSF], Amgen, Thousand Oaks, CA, and Rhône Poulenc, Paris, France) or the combination of chemotherapy (Ara-C and NOVIA) followed by growth factors. All patients had a normal karyotype at time of PBPC harvesting.

**Cell Isolation**

*Mature cells.* Ten milliliters of peripheral blood were drawn into EDTA-containing sterile tubes, at diagnosis (UPN 1, 4, and 5) and at the start of the first PBPC collection (all patients). Polymorphonuclear cells (PMN) and mononuclear cells (MNC) were separated by density gradient centrifugation at 400g using a combination of Mono-Poly Resolving Medium (ICN Biomedicals Inc, Costa Mesa, CA) and Lymphoprep (Nycomed Pharma AS, Oslo, Norway). Mature cell fractions were subsequently washed twice in phosphate-buffered saline (PBS) supplemented with 0.5% human serum albumin (HSA, Immuno AG, Vienna, Austria). The mean purity of the PMN fractions, as measured on an electronic cell counter (Cell Dyn 3500, Abbott, Wiesbaden, Germany), was 93% (range, 90% to 95%). MNC fractions were additionally incubated with fluorescein isothiocyanate (FITC), conjugated anti-CD3 (Leu-4; concentrations according to the manufacturer’s instructions) for isolation of CD3⁺ T lymphocytes.

**Progenitors.** Five milliliters of day-1 PBPC harvests were collected in RPMI 1640-medium (GIBCO BRL-Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (FCS) and 10 U/mL of preservative-free heparin. Cells were pelleted and subsequently cryopreserved at a concentration of 5 x 10⁶/mL in RPMI 1640 medium supplemented with dimethylsulfoxide 5% (DMSO; Merck, Darmstadt, Germany) and Hetastarch 6% (Plasmasteril; Fresenius AG, Fresenius Bad Homburg, Germany). Samples were stored at -80°C and analyzed within 3 to 6 months. At time of analysis, PBPC samples were rapidly thawed at 37°C and diluted 10-fold in PBS/0.5% HSA supplemented with DNase 1000 KU/mL (Boehringer Mannheim, Mannheim, Germany). After washing, CD34⁺ cells were selected by immunomagnetic adsorption on MiniMACS separation columns using a CD34 Progenitor Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Cell fractions enriched for CD34⁺ progenitors were subsequently resuspended in PBS/0.5% HSA and incubated during 30 minutes at 4°C with anti-CD34 FITC (HPCA-2), in combination with phycoerythrin (PE)-labeled anti-CD33 (Leu-M9) or anti-CD38 (Leu-17). All monoclonal antibodies were purchased from Becton Dickinson Immunocytometry Systems (BDIS; San Jose, CA). For flow cytometry analysis and FACSorting, the pellet of the immunofluorescent-labeled cells was resuspended in 0.5% paraformaldehyde in PBS.

**Buccal scrapes.** After thoroughly rinsing the mouth with tap water, buccal cells were collected by scraping the inner mouth mucosal surface with a blunt spatula. Cells were resuspended in PBS and, after centrifugation at 1,800g for 15 minutes, the cell pellet was dissolved in 200 μL of DNA-extraction buffer containing 178 μL of sodium-EDTA (SE), 18 μL of 10% sodium dodecyl sulfate (SDS), and 4 μL of protease K (10 mg/mL); (Promega, Leiden, The Netherlands). No granulocytes could be detected in buccal cell smears after a May-Grünewald-Giemsa staining.

**Short-term clonogenic assays.** PBPC fractions were plated in 24-well plates (Falcon, NJ) at a concentration of 2.5 x 10⁵/mL in 1 mL of MethoCult H4433 (Stem Cell Technologies Inc, Vancouver, British Columbia, Canada) and incubated at 37°C in a fully humidified atmosphere of 5% CO₂. After 14 days, if present, 65% of peripheral blood cells were mononuclear cells, and 35% were granulocyte-macrophage colonies (CFU-GM) and blast colony-forming units (BFU-E) colonies were randomly plucked with a drawn-out micropipette and directly transferred in 200 μL of DNA-extraction buffer.

**FACSorting**

Cell fractions were sorted on a FACSort (Becton Dickinson, San Jose, CA) with a CyAnics 15 mW air-cooled argon laser tuned at a wavelength of 488 nm equipped with Lysis II software and supplemented with a Cell Concentrator (Becton Dickinson, Erembodegem, Belgium) to enhance cell recovery. Thresholds were set on the forward versus right angle light scatter to exclude cellular debris and gating was performed using isotype controls (IgG1 FITC, IgG2aPE). Ten thousand CD3⁺ T lymphocytes and 10,000 CD34⁺ cells were sorted. Additionally, CD34⁺ subpopulations enriched for immature and more committed progenitors were obtained by drawing sort windows for CD34⁺CD38⁻, CD34⁺CD38⁺, and CD34⁺CD38hil fractions. At least 5,000 committed progenitors coexpressing CD38 or CD34 were sorted, whereas between 1,000 and 3,000 immature progenitors lacking coexpression of CD38 or CD33 could be obtained (Fig 1). All sorting experiments were performed in duplicate, with one half of the sorted sample being used for reanalysis of cell purity, which was at least 90%.

**Clonality Analysis**

DNA was extracted from granulocytic fractions using a standard proteinase K digestion. For all other cell specimens, 40 μg of glycogen (Boehringer Mannheim) was added as a carrier for high molecular weight DNA. After precipitation with isopropanol and

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age (yr)</th>
<th>MDS Subtype</th>
<th>Hgb (g/dL)</th>
<th>WBC (×10⁹/L)</th>
<th>Plt (×10⁹/L)</th>
<th>Blasts (%)</th>
<th>Blasts</th>
<th>Karyotype</th>
<th>Cellularity</th>
<th>Mobilization Regimen</th>
<th>Interval Between Diagnosis and Harvest (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>RAEB-1</td>
<td>9.7</td>
<td>2.1</td>
<td>17</td>
<td>10</td>
<td>27</td>
<td>NM</td>
<td>H</td>
<td>Filgrastim 5 µg/kg/d SC</td>
<td>114</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>RAEB-1</td>
<td>5.7</td>
<td>3.7</td>
<td>49</td>
<td>&lt;5</td>
<td>20</td>
<td>46,XX</td>
<td>H</td>
<td>Lenograstim 150 µg/m²/d SC</td>
<td>237</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>RAEB-1</td>
<td>9.0</td>
<td>60.5</td>
<td>72</td>
<td>14</td>
<td>21</td>
<td>46,XX</td>
<td>N</td>
<td>Filgrastim 5 µg/kg/d SC</td>
<td>115</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>RAEB-1</td>
<td>5.6</td>
<td>2.0</td>
<td>43</td>
<td>&lt;5</td>
<td>24</td>
<td>NM</td>
<td>H</td>
<td>NOVIA + filgrastim 5 µg/kg/d SC</td>
<td>63</td>
</tr>
<tr>
<td>5</td>
<td>49</td>
<td>RAEB-1</td>
<td>11.8</td>
<td>3.2</td>
<td>155</td>
<td>9</td>
<td>12</td>
<td>46,XX</td>
<td>H</td>
<td>Ara-C + filgrastim 5 µg/kg/d SC</td>
<td>146</td>
</tr>
</tbody>
</table>

**Abbreviations:** WBC, white blood cell count; Plt, platelet count; H, hypocellular; N, normocellular; SC, subcutaneously; NM, no mitoses.
cold ethanol wash, DNA was resuspended in 20 μL of Tris-HCl EDTA buffer (TE; pH 7.5). One half of this sample was digested overnight at 37°C in the addition of 40 U of Cfo I (Hha I) and 40 U of Hpa II (Boehringer Mannheim) for the digestion of unmethylated (or active) alleles. The other 10 μL was incubated without restriction enzymes in an equal reaction volume of 20 μL. For PMN fractions, 2 μg of DNA was used in the digestion procedure. The efficiency of these digestions was evaluated each time by a PMN fractions, 2 pg of DNA was used in the digestion procedure. After overnight incubation, restriction enzymes were inactivated by heating at 95°C for 10 minutes. For all cell fractions except granulocytes, clonality at the HUMARA locus was further assessed with a nested PCR using two sets of primers flanking the trimeric CAG repeat (outer primers: E1: 5′-CTG-GGAAGGGTCTACCCTCG-3′; T2: GCTGTGAAGGTTGCTGTTCCTCAT-3′). For nested PCR, 50 mmol/L KC1, 10 mmol/L Tris HCl (pH 9.0), 0.1% Triton X-100, 1 mmol/L MgC12, 2.5 U Taq Polymerase (Promega), 200 μmol/L of each dNTP, and 12 pmol/L of primer El and E2. DNA was amplified for 28 cycles (30 seconds at 95°C, 60 seconds at 60°C, and 90 seconds at 71°C) for all cell fractions except granulocytes, with 0.5 pmol of T1 labeled with 32P. Amplification was performed for 20 cycles (30 seconds at 95°C, 30 seconds at 65°C, and 30 seconds at 71°C) for all cell fractions except for granulocytic DNA, which was amplified for 29 cycles with no prior PCR1 procedure. Each PCR reaction was repeated in triplicate to avoid the selective amplification of one allele when only small amounts of DNA are present. Amplification of male DNA failed after all restriction enzyme incubation procedures, indicating that restriction enzyme digestions with Hpa II and Cfo I were complete. After DNA amplification, 5 μL of stop solution (95% formamide [GIBCO BRL, Life Technologies], 10 mmol/L EDTA, 0.01% Xylene Cyanol FF, and 0.01% Bromophenol blue) was added to 5 μL of PCR product and denaturing was performed 3 minutes at 95°C. Three microliters of this mixture was loaded on a 4% denaturing 19:1 acrylamide/bisacrylamide gel (8 mol/L Urea and 1 x TBE) followed by electrophoresis at 60 W for 150 minutes.

Gels were dried and exposed to an InstantImager (Packard Instruments Co, Meriden, CT). Autoradiographs were scored after 12 hours using the Instant Quant software package for allele quantification. For each sample, a corrected ratio (CrR) was calculated by dividing the ratio of the predigested sample (upper allele/lower allele) by the ratio of the nonpredigested sample. This CrR compensates for preferential amplification of one allele. The percentage of contaminating monoclonal cells was calculated using a standard curve of CrR values plotted against a known percentage of clonal cells (results not shown).

RESULTS

The sensitivity of the PCR-based HUMARA assay with real time phosphorimaging was determined by mixing various quantities of DNA from two male healthy volunteers with a different number of CAG repeats. These experiments have shown a sensitivity between 90% and 95%, which permits detection of a cell fraction contributing only 5% to 10% to a cell mixture. Cell populations were considered to be skewed when the expression of one of both alleles was higher than 75% after restriction enzyme incubation. This corresponds to allelic ratios less than 0.33 or greater than 3. CrR values of all analyzed cell fractions are listed in Table 2.

Mature Cells

In three patients (UPN 1, 4, and 5), PMN were available for clonality analysis at time of diagnosis. Allelic ratios of these PMN fractions have clearly shown the predominant expression of one allele after restriction enzyme incubation, which is due to the presence of a dominant myelodysplastic malignant clone. After intensive remission-induction and consolidation treatment, we have found polyclonal granulocytes in 4 of our 5 patients (UPN 1, 3, 4, and 5). This implies the restoration of a normal polyclonal myelopoiesis during complete hematologic remission (CHR; Fig 2). However, UPN 2 showed skewing in the myeloid fraction at time of PBPC harvesting. T lymphocytes were found to be polyclonal in all five patients at time of achieving a CHR.

Immature and Committed Progenitors

Because of insufficient cell numbers, we were not able to perform clonality studies by X-chromosome inactivation on hematopoietic progenitors at time of diagnosis. However, bone marrow immunophenotyping at that moment has shown the presence of high numbers (mean, 18%; range, 12% to 27%) of CD34+ myeloblasts in four patients (UPN 1, 3, 4, and 5). Therefore, it seems very unlikely that any remaining nonclonal hematopoietic progenitors could be easily detectable in these CD34+ blastic fractions.
At the time of CHR, the mean CD34 percentage in the PBPC fractions was 1.88% (range, 0.02% to 6.21%). After immunomagnetic adsorption with the Minimacs columns, CD34 purity increased to a mean of 52% (range, 14.5% to 76.8), with a mean enrichment factor of 368 times (range, 12.4 to 731 times).

These CD34+ cell fractions were found to be polyclonally derived in four of five patients (UPN 1, 3, 4, and 5), with CrR values ranging between 0.82 and 1.02 (Fig 3 and Table 2). UPN 2, with skewing towards one allele in the digested PMN fraction, also displayed a predominant inactivation of the same lower allele in her CD34+ population.

The low frequency of immature progenitors (CD34+38lo, CD34+33lo) precludes sorting of large cell numbers. However, we were still able to isolate at least 1,000 immature progenitors in every patient. An unequivocal polyclonal nature of these immature progenitor cell fractions was detectable in four patients (UPN 1, 3, 4, and 5; Fig 3). In UPN 5, less than 1,000 CD34+33lo progenitors could be sorted, which has resulted in dubious PCR patterns. However, CD34+38lo stem cells were clearly polyclonal in this patient. The lyonization patterns of more committed progenitors (CD34+38hi, CD34+33hi) were similar to those of the immature progenitors and the unfractionated CD34+ populations in four patients (UPN 1, 3, 4, and 5; Fig 3). The mean CrR of 0.47 in the CD34+38lo fraction of UPN 2 corresponds to the persistence of a small residual polyclonal fraction of about 30%, which is in contrast to the extremely skewed pattern in all committed progenitor fractions (Fig 4).

**Short-Term Clonogenic Assays**

UPN 1, 3, and 4, were characterized by an increased colony to cluster ratio. Five CFU-GM and BFU-E were randomly plucked for clonality analysis. Although each colony, being the progeny of a single (committed) stem cell, is monoclonal, the expression of different alleles in separate colonies gives indirect evidence for polyclonality in the PBPC fractions of these patients (Table 3 and Fig 5). However, in UPN 5, insufficient numbers of colonies could be grown for a reliable clonality analysis, whereas PBPC fractions of UPN 2 showed no colony growth but only cluster formation. Buccal scrapes were additionally obtained in all patients to compare constitutional lyonization patterns with those of hematopoietic tissues. Four patients were also polyclonal in their buccal scrapes (UPN 2, 3, 4, and 5). UPN 1 had a
Fig 3. Clonality analysis of immature hematopoietic progenitors with the HUMARA assay. PCR was performed before (−) and after (+) restriction enzyme incubation, as described in the Materials and Methods. Autoradiographs are visualized after overnight exposure to phosphorscreens. Results are shown for CD34⁺ cells, for CD34⁺ cells with low (CD34⁺38⁺low) or high CD38 coexpression (CD34⁺38⁺high), and for immature (CD34⁺33⁺low) and committed (CD34⁺33⁺high) myeloid progenitors. Shadow bands are a typical phenomenon of amplification of short-tandem repeats and are due to slippage of Taq polymerase. (A) UPN 5; (B) UPN 1; (C) UPN 3; (D) UPN 4.

Fig 4. Suppression of residual polyclonality along hematopoietic differentiation. PCR amplification of the HUMARA region and phosphorimaging were performed as described in the Materials and Methods. The upper allele remains visible in the immature CD34⁺38⁺low fraction, whereas it has completely disappeared in the more differentiated CD34⁺38⁺high fraction. −, no prior Cfo I or Hpa II digestion; +, prior Cfo I and Hpa II digestion.

skewed pattern in her mucosal tissue, whereas she was found to be polyclonal in all hematopoietic cell fractions at time of CHR. These results are in agreement with the recent findings of Gale et al., who failed to find a good correlation between lyonization patterns in blood and different somatic tissues.

DISCUSSION

In this study, we have analyzed the clonal nature of hematopoiesis in five newly diagnosed female MDS patients with RAEB-t at diagnosis and at the time of CHR after high-dose chemotherapy. The clonal origin of MDS has been clearly established by a variety of different techniques, including
Table 3. X-Inactivation Patterns of Individual Colonies

<table>
<thead>
<tr>
<th>UPN</th>
<th>CFU-GM Upper Allele</th>
<th>CFU-GM Lower Allele</th>
<th>BFU-E Upper Allele</th>
<th>BFU-E Lower Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2/5</td>
<td>3/5</td>
<td>2/5*</td>
<td>1/5*</td>
</tr>
<tr>
<td>3</td>
<td>4/5</td>
<td>1/5</td>
<td>3/5</td>
<td>2/5</td>
</tr>
<tr>
<td>4</td>
<td>2/5</td>
<td>3/5</td>
<td>3/5</td>
<td>2/5</td>
</tr>
</tbody>
</table>

Clonality analysis with the HUMARA assay performed on five CFU-GM and five BFU-E. Upper allele represents the number of CFU-GM or BFU-E with complete digestion of the lower allele, after incubation with Hpa II and Cfo I. Lower allele are those colonies in which the upper allele was completely digested. Insufficient colonies were grown in UPN 2 and 5 for a reliable clonality analysis. See also Fig 5.

• PCR results of two BFU-E were ambiguous due to contamination with other colonies.

Fig 5. Clonality analysis of individual colonies (CFU-GM). X-chromosome inactivation was performed with the HUMARA assay before (–) and after (+) restriction enzyme incubation. Only one allele is visible after restriction enzyme digestion, because each colony is derived from a single committed hematopoietic precursor. Colony 3 has the lower allele inactivated, whereas in colonies 1 and 2, the upper allele is methylated and therefore remains undigested. Results shown are for UPN 3.

Fractions from bone marrow or peripheral blood, with very little or no information on the clonal behavior of the stem cell fractions. One method of studying such hematopoietic progenitor cell involvement in MDS females is by analyzing the X-chromosome inactivation pattern of individual colonies derived from mononuclear cell fractions or from CD34+ cells grown in short-term clonogenic assays. However, this technique provides no information on the clonal nature of very immature progenitors and, moreover, clonal patterns can also become influenced by cytokines present in commercially available culture media.

Therefore, we have chosen to analyze MDS progenitor clonality more directly on cell populations reflecting different stages of hematopoietic development. Highly purified hematopoietic progenitors were obtained from mobilized peripheral blood by CD34 enrichment with immunomagnetic beads followed by FACSorting. Although cells expressing the CD34 glycoprotein at their surface are enriched for hematopoietic progenitors, they still form a heterogeneous population. For this reason, we have sorted different stem cell subfractions, including CD34+ cells with low CD38 expression (CD34+38low), being strongly enriched for the most immature and uncommitted progenitors, and CD34+ cells with high levels of CD38 expression (CD34+38high), particularly consisting of multilineage committed stem cells. To study myeloid lineage involvement, CD34+ cell fractions with low (CD34+38low) or high (CD34+38high) levels of coexpression of the myeloid-lineage-associated CD33 antigen were additionally obtained. Because no karyotypic abnormalities were detectable in our patients, clonality analysis was performed by X-chromosome inactivation, taking into account that X-inactivation is supposed to occur in one of the most primitive progenitors in MDS pathogenesis. We have used polymorphisms in the recently described X-linked HUMARA locus for detection of these X-chromosome inactivation patterns. Additionally, the use of a nested PCR procedure gives the opportunity to perform a reliable clonality analysis on very small cell populations.

In four of our patients, this assay has shown an unequivocal polyclonal pattern in the mature granulocytic and T-lymphoid cell fractions at the time of PBPC harvesting.
clearly implies the feasibility of restoring polyclonal myeloid hematopoiesis after high-dose chemotherapy. Because lymphocytes were not available for clonality analysis before starting chemotherapy treatment, it is not clear whether the lymphocytic compartment was initially also clonally involved. The same polyclonal nature was also found at the level of both immature (CD34\(^+\)38\(^-\)Io\(^-\)) and more committed (CD34\(^+\)38\(^-\)Io\(^-\), CD34\(^-\)33\(^+\)) stem cells. As can be calculated from the allelic ratios, the detectable residual monoclonal population for all these individual progenitor cell fractions was at the sensitivity limit of our assay, being 5% to 10%. X-inactivation patterns of individual colonies grown in short-term clonogenic assays gave supplementary evidence for the polyclonal nature of the stem cell compartment in three of these patients. Although no X-inactivation studies could be performed on CD34\(^+\) fractions at time of diagnosis, the presence of a dominant CD34 \(^+\) blastic fraction at that time was clearly shown by immunophenotyping.

In one patient, allelic ratios of mature and progenitor fractions remained skewed despite the achievement of a CHR after high-dose chemotherapy. Although the allelic ratio in the CD34\(^+\)38\(^-\)Io\(^-\) fraction corresponds to a calculated residual polyclonal immature stem cell compartment of about 30%, this normal hematopoiesis is completely suppressed by a malignant monoclonal population along the maturation and differentiation pathway. Additionally, no colonies could be grown in short-term clonogenic assays, further suggesting overgrowth of the polyclonal early hematopoietic progenitors by a malignant clonal population. This monoclonal predominance together with the absence of polyclonal hematopoietic restoration could be, at least partially, explained by the longer interval between diagnosis and PBPC harvesting in this particular patient. In contrast, the polyclonal nature of buccal scrapes and especially T lymphocytes makes it very unlikely that skewed patterns could be due to unbalanced lyonization, having occurred early in embryogenesis. Finally, the theoretical possibility still remains that the combination of hematopoietic reconstitution from a low number of residual stem cells, together with the analysis of low progenitor cell numbers, has made us unable to detect polyclonality in this particular MDS patient. To date, this clonal remission state still persists at 500 days after chemotherapy treatment, without any evidence of clinical or morphologic relapse.

In conclusion, this study has clearly shown the feasibility of restoring and harvesting a polyclonal immature progenitor cell compartment in high-risk MDS patients treated with intensive chemotherapy in combination with growth factors. Our findings imply that it is possible, at least in a subpopulation of MDS patients, to suppress selectively the malignant clone and provide growth advantage to the residual normal polyclonal progenitors. It is our impression that this polyclonal remission is particularly due to the administration of intensive combination chemotherapy, although the effect of the additional short-term administration of G-CSF on X-chromosome inactivation patterns still needs to be elucidated. In parallel with the observations made in another clonal disease, ie, chronic myelogenous leukemia,\(^{14}\) we believe that restoration and harvesting of a normal polyclonal stem cell compartment in MDS is a particular feature of the early disease course, before the normal residual stem cell compartment has become completely replaced by a malignant monoclonal population. Until now, three of our patients with polyclonal PBPC fractions have received transplants, resulting in rapid engraftment rates.\(^6\) The fourth patient with polyclonal PBPC fractions has not yet received a transplant, but the remission state persists at 150 days after remission-induction chemotherapy. Although promising, it still needs to be shown that autologous transplantation with polyclonal stem cell fractions can improve long-term disease-free survival in patients with MDS.

ACKNOWLEDGMENT

The authors thank Dr Catherine Verfaille (University of St Paul, Minneapolis, MN) for reading the manuscript.

REFERENCES

14. Busque L, Gilliland DG, Pritch JT, Steif CA, Weinstein HJ,


Polyclonal primitive hematopoietic progenitors can be detected in mobilized peripheral blood from patients with high-risk myelodysplastic syndromes

M Delforge, H Demuynck, P Vandenberghhe, G Verhoef, P Zachee, V van Duppen, P Marijnen, H Van den Berghe and MA Boogaerts