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Aging Impairs Long-Term Hematopoietic Regeneration after Autologous Stem Cell Transplantation

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A B S T R A C T
Most of our knowledge of the effects of aging on the hematopoietic system comes from studies in animal models. In this study, to explore potential effects of aging on human hematopoietic stem and progenitor cells (HSPCs), we evaluated CD34+ cells derived from young (<35 years) and old (>60 years) adult bone marrow with respect to phenotype and in vitro function. We observed an increased frequency of myelo-biased output and an increased propensity for anemia [7,8]. The expanded primary leukemogenic potential of young HSPCs compared to old HSPCs [10-14] indicates that the age-related skewing toward a more myeloid-biased output [9-11] is a common phenomenon in healthy individuals. However, our findings do not suggest that age has only a limited impact on the phenotype and functional capacity of HSPCs under steady-state conditions, but can be important under conditions of chemotoxic and replicative stress.

INTRODUCTION
The bone marrow (BM) is one of the body’s most highly self-renewing tissues. Hematopoietic stem cells (HSCs) are capable of replenishing all blood cell types during the complete lifespan of an organism. The hematopoietic system does not escape the effects of aging, however. In humans, these effects are manifested clinically by an increased incidence of hematologic disorders, including leukemia [1-3], decreased adaptive immunity [4-6], and increased propensity for anemia [7,8].

Until recently, data on age-associated changes in human hematopoietic stem and progenitor cells (HSPCs) were limited. Two recent studies that addressed this issue suggested that, although some of the findings observed in the murine system could be confirmed in humans, increasing age seems to have only a limited impact on the phenotype and functional capacity in vivo and in vitro (as examined in xenotransplant models) of human HSPCs [15,16].

In this study, we investigated CD34+ cells derived from normal BM (NBM) of healthy young and elderly individuals with respect to phenotype and function. To study the impact of aging and stress response on human HSPCs in vivo, we analyzed the impact of age on HSPC mobilization and short-term and long-term regeneration after autologous stem cell transplantation (ASCT). A better understanding of HSPC aging will facilitate the development of more suitable treatment regimens for elderly patients undergoing ASCT.

MATERIALS AND METHODS

NBM
After achieving informed consent, BM aspirates were obtained from patients aged 60 years who underwent total hip replacement, volunteers aged 35 years, and young healthy potential donors for hematopoietic ASCT who underwent BM aspiration as part of a standard medical examination. NBM from 9 young healthy volunteers and 2 young potential donors was used for in vitro experiments. Young NBM samples for the microarray analysis came from young healthy volunteers and 1 young potential donor. All older NBM samples came from patients undergoing total hip replacement. The protocol for NBM collection was approved by the Institutional Review Board of the University Medical Center Groningen. All participants had normal general health and normal peripheral blood counts and an absence of hematologic disorders. Details of the experiments performed on the NBM samples are presented in Supplemental Table 1.

Flow Cytometry Analysis and Sorting Procedures
The flow cytometry and sorting procedures are described in detail in the Supplemental Materials.
In Vitro Culture Assays
A colony-forming cell (CFC) assay was performed in methylcellulose (MethoCult H4230; StemCell Technologies, Grenoble, France), supplemented with 20 ng/mL IL-3, 20 ng/mL IL-6, 20 ng/mL granulocyte colony-stimulating factor (G-CSF), 20 ng/mL e-Kit ligand, and 1 U/mL erythropoietin (Gigapharm, Brussels, Belgium) as described previously [17]. The assay was performed in duplicate. At 2 weeks after plating, colony-forming unit–granulocyte/macrophage (CFU-GM), burst-forming unit–erythroid (BFU-E), and colony-forming unit–granulocyte erythrocyte monocyte megakaryocyte (CFU-GEMM) numbers were measured and recorded.

For the long-term cultures, CD34+ cells were plated in bulk in 12-well plates or, for limiting dilution experiments, in a 96-well plate precoated with MS5 stromal cells. Cells were expanded in long-term culture (LTC) medium (α-minimum essential medium supplemented with heat-inactivated 12.5% FCS, Sigma-Aldrich, Zwijndrecht, The Netherlands), heat-inactivated 12.5% horse serum (Serum4, Sigma-Aldrich), penicillin and streptomycin, 2 mM glutamine, 572.2 μM β-mercaptoethanol (Sigma-Aldrich), and 1 μM hydrocortisone (Sigma-Aldrich), supplemented with 20 ng/mL IL-3, 20 ng/mL G-CSF (Rhone-Poulenc Rorer, Amstelveen, The Netherlands), and 20 ng/mL thrombopoietin (Kirin, Tokyo, Japan). Cultures were maintained at 37 °C in 5% CO2 and were demixed weekly for medium changes. In the MS5 cocultures, cells were counted weekly.

For the long-term culture initiating cell (LTC-IC) limiting dilution assay, 5 weeks of expansion was followed by the removal of suspension cells and addition of methylcellulose (StemCell Technologies) as described for the CFC assay. Two weeks later, wells containing CFCs were scored as positive, and the LTC-IC frequency was calculated using L-Calc software for limiting dilution analysis (StemCell Technologies). Liquid cultures were performed exactly as described for the cocultures, but without coculturing with MS5.

Transplantation Procedure
Details on the apheresis and transplantation procedures are provided in the Supplemental Material.

Clinical Cohort of ASCT Recipients
A cohort of 119 ASCT recipients was retrospectively studied. All subsequent patients with multiple myeloma (MM) or non-Hodgkin lymphoma (NHL) age <50 years or ≥60 years treated with high-dose chemotherapy followed by ASCT at the Department of Hematology, University Medical Center Groningen between June 1993 and June 2007 were included in this study. Patients who experienced progressive or relapsing disease within 1 year after ASCT were excluded from the analysis. Within the NHL group, only patients with diffuse large B cell lymphoma or mantle cell lymphoma were included. The younger age group (<50 years) comprised 64 patients (mean age, 44 ± 7 years), including 27 with NHL and 37 with MM. The older age group (≥60 years) included 55 patients (mean age, 63 ± 2 years), with 18 with NHL and 37 with MM. All patients with MM underwent ASCT upfront; after induction therapy with vincristine, Adriamycin, and dexamethasone (VAD) or with thalidomide, Adriamycin, and dexamethasone (TAD) [18-20]. All patients with NHL underwent ASCT as second-line treatment, after first-line treatment with 6 to 8 cycles of (Rituximab), Cyclophosphamide, Hydroxydaunorubicin, Vincristine (Oncovin), Prednisone [(R)-CHOP] chemotherapy. Reinduction treatment consisted of (R) Cisplatin, Cytarabine, Dexamethasone [(R)-DHAP] followed by (R) Etoposide, Ifosfamide, Methotrexate [(R)-VIM] and a second course [(R)-DHAP chemotherapy [21]. Patients with MM were conditioned with high-dose melphalan (200 mg/m2) (n = 70), cyclophosphamide + total body irradiation (n = 2), or Carmustine, Etoposide, Cytarabine, Melphalan (BEAM) (n = 2). Patients with NHL were conditioned with BEAM (n = 45).

After infusion of peripheral blood stem cells (PBSCs), the number of days needed to reach a granulocyte count of at least 0.5 × 109/L and to reach a thrombocyte count of at least 20 × 109/L was recorded for each patient. Moreover, cell counts were measured on day 28, day 40, and 1 year after ASCT. A leukocyte count of ≥ 4 × 109/L, thrombocyte count of ≥ 150 × 109/L, and hemoglobin (Hb) level of ≥ 7.5 mmol/L for women and ≥ 8.7 mmol/L for men were considered normal values in this analysis.

Statistical Analysis
The Mann-Whitney U test or χ2 test was used for analysis of individual group differences. Hematologic recovery was assessed using Kaplan-Meier probability curves, and statistical comparison of curves was performed using the log-rank test. The prognostic value of different variables for probability of hematologic recovery was assessed by univariate and multivariate analyses using a Cox multiple regression model. Differences with a P value ≤0.05 were considered statistically significant.

RESULTS
Age-Associated Phenotypic Changes Within the Human Hematopoietic System
To investigate whether the aging process has an effect on the composition of the different stem and progenitor cells populations within the total cell population, we performed phenotypic analyses of the total mononuclear cell fraction in young (age <35 years) and old (age ≥60 years) adult NBM. The percentages of total CD34+ cells, progenitor cell–containing CD34+CD38− cells, and multipotent CD34+CD38+ fractions increased significantly from young NBM (n = 5) to old NBM (n = 8) (Figure 1A). Subsequent analysis of the frequency of common myeloid progenitors, granulocyte macrophage progenitors, and megakaryocyte erythrocyte progenitors within the CD34+CD38− fraction revealed no significant differences between young NBM (n = 4) and old NBM (n = 4) (Figure 1B). In contrast, the percentage of lymphoid progenitors, defined as CD34+CD10+ cells within the total CD34+ cell population, was significantly lower in old NBM (P = .021) (Figure 1C).

Impact of Aging on HSPC Function In Vitro
We performed several in vitro assays to study the functional capacity of CD34+ cells derived from young and old adult NBM. The colony-forming potential of CD34+ cells for all included lineages was comparable in young NBM (n = 6) and old NBM (n = 5) (Figure 1D). In addition, the LTC-IC frequency, an established in vitro measurement for HSPCs, was comparable in young and old NBM-derived CD34+ cells (Figure 1E).

To further assess the impact of age on function and differentiation potential of CD34+ human cells, we cultured the cells in liquid and MS5 cocultures. As in the other in vitro assays, we found no significant difference in growth between CD34+ cells derived from young adult NBM (n = 4) and those derived from old adult NBM (n = 4) (Figure 1F). In addition, we observed no differences in serial replating capacity assessed in the MS5 cocultures between young NBM and old NBM (data not shown). Taken together, these findings indicate very similar in vitro functional capacities of young and old adult NBM, as measured in the different in vitro assays.

Our observation that young and old NBM CD34+ cells are quite comparable in steady-state conditions was confirmed by the results of our microarray-based gene expression analyses of CD34+ cells derived from young NBM (n = 10) and old NBM (n = 22). We found a very limited number of differentially expressed genes, with 18 up-regulated probes representing 14 annotated genes (P < .00001, Student t test) (Supplemental Table 2).

HSPCs In Vivo
To follow up on our in vitro research on the impact of aging on human HSPC function, we extended our research to an in vivo situation, studying ASCT. In this setting, we studied mobilization and apheresis of PBSCs and compared regeneration data in a group of 64 younger patients (age ≤50 years) and a group of 55 older patients (age ≥60 years) after ASCT. Patients with progressive or relapsing disease within 1 year after ASCT were excluded from our analysis. Patient characteristics are summarized in Table 1.

Mobilization and Apheresis
Different mobilizing therapy regimens were used, with the percentages of patients receiving any 1 of these therapies not differing significantly in the 2 age groups (Table 1). The target for cell harvest was 5 × 106 CD34+ cells/kg for patients.
with NHL and $10 \times 10^6$ CD34+ cells/kg for those with MM. The number of apheresis days necessary to achieve these targets was not significantly different in the 2 age groups (Table 2). In addition, there was no significant difference in the median number of CD34+ cells collected on the first day of apheresis in the 2 age groups. On the first day of apheresis, $11/2 \times 10^6$ CD34+ cells/kg were collected from the younger patients with MM, and $13/2 \times 10^6$ CD34+ cells/kg were collected from the older patients with MM. For patients with NHL, cell collection on the first day of apheresis was $11.6 \times 10^6$ CD34+ cells/kg in the younger patients and $9.6 \times 10^6$ CD34+ cells/kg in the older patients (Table 2).

**No Age-Related Effect on the Colony-Forming Potential of PBSC Material**

Routine CFU-GM assays were performed on a small aliquot of the collected autologous transplant material. CFU-GM data were available for all 119 patients in our cohort. The mean CFU-GM value was $34 \pm 29 \times 10^4$/kg (median, $28 \times 10^4$/kg) in the younger patients and $33 \pm 23 \times 10^4$/kg (median, $29 \times 10^4$/kg) in the older patients. CFU-GM numbers did not differ significantly between patients with MM and those with NHL in the age <50 years or age >60 groups. In addition, there was no inverse correlation between age and CFU-GM colony formation (data not shown).

**Regeneration of Peripheral Blood Cell Counts after ASCT**

To gain insight into the in vivo function of the CD34+ cells used for ASCT, we compared various peripheral blood cell parameters in the younger and older patients. The parameters studied are considered representative of short-term hematologic recovery after ASCT. The mean number of days after stem cell infusion needed to achieve a granulocyte count of at least $0.5 \times 10^9$/L was 18.5 ± 11.1 days in the younger patients and 22.8 ± 20.9 days in the older patients (Table 2), a nonsignificant difference. A significant difference was seen in the percentage of patients with a granulocyte count of $\geq 0.5 \times 10^9$/L at day 28 post-ASCT (18% of the older patients versus 4.9% of the younger patients; $P = .03$). For platelets, as for granulocytes, the mean number of days after
The effect of age on complete recovery of peripheral blood counts at 1 year after transplantation was most pronounced in the patients with NHL. In this group, 40% of the younger patients experienced complete recovery of peripheral blood counts by 1 year post-ASCT, compared with none of the older patients (Figure 2). Importantly, incomplete recovery was associated with statistically significantly lower platelet, leukocyte, and Hb levels (Supplemental Table 4).

**Independent Effect of Age on Regeneration after ASCT**

Other variables besides age found to be correlated with complete recovery of peripheral blood counts at 1 year post-ASCT on univariate analysis included number of infused CD34+ cells, diagnosis (either MM or NHL), short-term regeneration with granulocytes (days until a granulocyte count of >0.5 x 10^9/L) and short-term regeneration with platelets (days until a platelet count of >20 x 10^9/L). However, when these variables were evaluated in a multivariate analysis, only age at ASCT (P = .02; hazard ratio [HR], 2.4; 95% confidence interval [CI], 1.3 to 4.1) and diagnosis (P = .02; HR, 2.6; 95% CI, 1.2 to 4.0) were identified as independent prognostic variables for complete recovery of peripheral blood counts at 1 year post-ASCT.

**DISCUSSION**

Data on the impact of increasing age on the HSPC compartment in humans are limited. In agreement with previously reported data [9-11,15,16,22,23], we found a significant decrease in phenotypically defined lymphoid progenitors in older patients and a relative increase with aging in the percentages of both CD34+CD38− and CD34+CD38+ cells in old NBM compared with young NBM. These data do not provide information on the absolute number of HSPCs in younger and older human NBM, however, especially given that BM cellularity decreases with age [24,25]. The function of CD34+ cells derived from NBM of younger versus older individuals did not differ in various in vitro assays. Apparently

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**Table 1**

Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Age ≤50 y</th>
<th>Age ≥60 y</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, n</td>
<td>64</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>NHL, n</td>
<td>27</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>MM, n</td>
<td>37</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Age, yr, mean ± SD</td>
<td>44 ± 7</td>
<td>63 ± 2</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>65</td>
<td>57</td>
<td>.50</td>
</tr>
<tr>
<td>NHL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotherapy courses before mobilization, mean ± SD</td>
<td>7.2 ± 3.1</td>
<td>6.6 ± 2.9</td>
<td>.50</td>
</tr>
<tr>
<td>(R)-CHOP, %</td>
<td>100</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>Mobilizing chemotherapy, %</td>
<td>77</td>
<td>50</td>
<td>.20</td>
</tr>
<tr>
<td>(R)-DHAP</td>
<td>12</td>
<td>38</td>
<td>.06</td>
</tr>
<tr>
<td>High-dose ara-C</td>
<td>12</td>
<td>0</td>
<td>.50</td>
</tr>
<tr>
<td>CHOP</td>
<td>0</td>
<td>6</td>
<td>.50</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>0</td>
<td>6</td>
<td>.50</td>
</tr>
<tr>
<td>G-CSF alone</td>
<td>0</td>
<td>6</td>
<td>.50</td>
</tr>
<tr>
<td>Conditioning chemotherapy, %</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotherapy courses before mobilization, mean ± SD</td>
<td>3.3 ± 0.8</td>
<td>4 ± 3.4</td>
<td>.20</td>
</tr>
<tr>
<td>VAD, %</td>
<td>56</td>
<td>67</td>
<td>.50</td>
</tr>
<tr>
<td>PAD, %</td>
<td>25</td>
<td>18</td>
<td>.60</td>
</tr>
<tr>
<td>TAD, %</td>
<td>19</td>
<td>17</td>
<td>1.0</td>
</tr>
<tr>
<td>Mobilizing chemotherapy, %</td>
<td>62</td>
<td>75</td>
<td>.30</td>
</tr>
<tr>
<td>CAD</td>
<td>38</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>95</td>
<td>94</td>
<td>1.0</td>
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<tr>
<td>Conditioning chemotherapy, %</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>High-dose melphalan</td>
<td>95</td>
<td>94</td>
<td>1.0</td>
</tr>
<tr>
<td>Cyclophosphamide/full body irradiation</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

PAD, Bortezomib, Doxorubicin, Dexamethasone; CAD, Cyclophosphamide, Doxorubicin, Dexamethasone.

**Table 2**

Results of Apheresis and Regeneration of Peripheral Blood Counts after ASCT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age ≤50 yr</th>
<th>Age ≥60 yr</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apheresis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34+ cells collected at day 1, × 10^6/kg, median</td>
<td>11</td>
<td>13</td>
<td>.30</td>
</tr>
<tr>
<td>MM</td>
<td>11.6</td>
<td>9.6</td>
<td>.70</td>
</tr>
<tr>
<td>Patients achieving target CD34+ cell number by mobilization, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At day 1</td>
<td>59</td>
<td>64</td>
<td>.60</td>
</tr>
<tr>
<td>At day 2</td>
<td>30</td>
<td>23</td>
<td>.50</td>
</tr>
<tr>
<td>At day 3</td>
<td>8</td>
<td>7</td>
<td>.90</td>
</tr>
<tr>
<td>At day 4</td>
<td>3</td>
<td>4</td>
<td>.90</td>
</tr>
<tr>
<td>Transplantation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of infused CD34+ cells, × 10^6/kg, mean ± SD (median)</td>
<td>8.9 ± 9.7 (5.7)</td>
<td>6.2 ± 4.8 (5.0)</td>
<td>.10</td>
</tr>
<tr>
<td>Regeneration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days to granulocytes ≥0.5 x 10^9/L, mean ± SD (median)</td>
<td>18.5 ± 11.1 (16)</td>
<td>22.8 ± 20.9 (16)</td>
<td>.20</td>
</tr>
<tr>
<td>Patients with granulocytes ≥0.5 x 10^9/L at day 28, %</td>
<td>4.9</td>
<td>18</td>
<td>.03</td>
</tr>
<tr>
<td>Days to thrombocytes ≥20 x 10^9/L, mean ± SD (median)</td>
<td>28.2 ± 46.8 (17)</td>
<td>29.4 ± 25.5 (19)</td>
<td>.90</td>
</tr>
<tr>
<td>Patients with thrombocytes ≥20 x 10^9/L at day 40, %</td>
<td>11.5</td>
<td>20.4</td>
<td>.30</td>
</tr>
<tr>
<td>Patients with normal peripheral blood counts of Hb, leukocytes, and thrombocytes after 1 yr, %</td>
<td>56</td>
<td>29.6</td>
<td>.009</td>
</tr>
</tbody>
</table>
CD34+ NBM-derived cells from older and younger individuals are quite comparable under steady-state conditions. It also can be argued that the in vitro assays used in the present study are not sufficiently sensitive to detect potential age-related effects; however, only limited variation was seen between samples in 1 group in the in vitro readouts. Moreover, recent in vivo transplantation studies in NOD/SCID/IL-2Rγnull (NSG) mice with young and old human NBM yielded inconsistent results. One study did not reveal differences in the frequency of NSG-repopulating cells with increasing age [15], whereas another study reported a significant difference (after transplantation of 500 HSCs) in the frequency of NSG-repopulating cells with age, from approximately 2% to approximately 1% [16].

Various hypotheses can be proposed to explain the difference between murine and human studies with respect to the effect of age on HSCs. One possibility is related to limitations with the stringent purification of human HSCs compared with murine HSCs, which potentially could mask potential differences by contaminating progeny. Another hypothesis is that CD34+ BM-derived cells from older and younger individuals are comparable under steady-state conditions, and that potential differences will become apparent only in stressful conditions, for example, in serial transplantations with limited cell numbers or chemotherapy. From this perspective, we used ASCT to study the impact of age on human HSC function in vivo without xenogeneic immunologic or metabolic effects and in a clinically relevant setting.

At present, hematopoietic stem cell transplantation is the sole well-established clinically applied stem cell-based therapy. Elderly patients have the greatest need for this form of treatment. The clinical relevance is also illustrated by a previous study in a cohort of 98 patients with hematologic malignancies treated with high-dose chemotherapy followed by ASCT, which found that only 39% of patients had complete recovery of all 3 lineages after 1 year [26]. The disadvantage of our approach is that regeneration is dependent not only on the age of the transplanted HSPCs, but also on the effects of intensive chemotherapy on both the HSPCs and the BM microenvironment. Indeed, the ASCT procedure has significant effects on HSPC function, as we reported previously in a study of BM of patients at 1 year post-ASCT [27, 28]. Furthermore, as might be true for transplantation studies in NSG mice, age-related differences in homing capacity and microenvironmental changes could affect regeneration.

On the other hand, repetitive cycles of chemotherapy may serve as a model for the induction of accelerated aging, and the observed age-related effects might be enhanced and thus better appreciated. Indeed, we found a significant effect on long-term regeneration, but not on short-term regeneration, after ASCT, suggesting that older HSCs are more hampered than younger HSCs by the procedure of chemotherapy, mobilization, apheresis, transplantation, and regeneration. This effect seems more pronounced with more intensive chemotherapy. Patients with NHL received significantly more chemotherapy courses before mobilization of stem cells compared with patients with MM (younger patients, \( P < .0001 \); older patients, \( P = .007 \)) and showed a more significant effect of age on long-term regeneration.

Although peripheral blood cell counts at 1 year post-ASCT did not differ significantly between the younger and older patients at the group level, the negative effect of advanced age on hematopoietic regeneration is clearly shown by the much higher percentage of patients with incomplete recovery in the older age group compared with the younger age group. Importantly, incomplete recovery was associated with statistically significantly lower platelet, leukocyte, and Hb levels. This might lead to significant clinical problems; for example, incomplete recovery might limit treatment options for relapsing disease after ASCT owing to decreased hematologic tolerance to chemotherapy and radiation. Moreover, as a result of impaired regeneration after ASCT, infections in older patients might induce hematopoietic stress and (pan)
cytopenia [26, 29, 30]. The present study has a relatively small study cohort, and a (prospective) study including more patients is needed to confirm our data and further elucidate the clinical relevance. Nevertheless, our findings underscore the need for further research on underlying mechanisms and ways to improve the ASCT procedure, especially in older patients.

The upper age limit for ASCT is constantly increasing [31, 32]. One factor directly affecting the feasibility of ASCT is the mobilization capacity of CD34+ cells. The available data on the effects of increasing age on CD34+ cell mobilization are somewhat inconsistent. Several studies have reported poorer mobilization in elderly patients, but although fewer CD34+ cells were collected in these patients, these quantities did reach the standard target level for performing ASCT in most studies [33-35]. Thus, advanced age is not an obstacle for the collection of a stem cell product capable of restoring hematopoietic function. In our cohort, we found no quantitative differences in the numbers of mobilized and collected CD34+ cells afterapheresis. This discrepancy with previous studies might be related to our relatively small patient cohort, the attempted mobilization after a course of chemotherapy, or the volume of peripheral blood used forapheresis. Interestingly, murine studies have shown approximately 5-fold greater mobilization efficiency of primitive hematopoietic cells from aged mice, presumably related to reduced adhesion of hematopoietic progenitor cells to BM stroma [36].

The effects of age on homing of human primitive hematopoietic cells remain poorly understood. In murine models, a reduced seeding efficiency of old murine HSPCs compared with young HSPCs has been observed [14, 37]. Moreover, a time-lapse 2-photon microscopy study revealed that aged early hematopoietic progenitor cells localize more distantly from the endostemma, suggesting altered niche biology in the aged hematopoietic system [38].

Along with the aforementioned extrinsic mechanisms, the significantly lower percentage of older patients with normal trilineage hematopoiesis after ASCT might be related to intrinsic stem cell changes [39]. A potential cell intrinsic contributing factor is telomere shortening, a mechanism in intrinsic stem cell changes [39]. Along with the aforementioned extrinsic mechanisms, the significantly lower percentage of older patients with normal trilineage hematopoiesis after ASCT might be related to intrinsic stem cell changes [39]. A potential cell intrinsic contributing factor is telomere shortening, a mechanism in intrinsic stem cell changes [39].

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: C.M.W. performed experiments, analyzed data, and wrote the manuscript. N.M., R.N.V.-S., and A.Z.B.-V. performed experiments and analyzed data. J.J.S. and E.V. designed research, analyzed data, and contributed to the manuscript. J.T.M.d.W. designed research, collected and analyzed clinical data, performed statistical analyses, and contributed to the manuscript. G.H. designed research, analyzed data, and wrote the manuscript.

SUPPLEMENTARY DATA
Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.03.001.

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