Loss of quiescence and impaired function of CD34+/CD38low cells one year following autologous stem cell transplantation

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ABSTRACT

Patients who have undergone autologous stem cell transplantation are subsequently more susceptible to chemotherapy-induced bone marrow toxicity. In the present study, bone marrow primitive progenitor cells were examined one year after autologous stem cell transplantation and compared with normal bone marrow and mobilized peripheral blood stem cells. Post-transplantation bone marrow contained a significantly lower percentage of quiescent cells in the CD34+/CD38low fraction compared to normal bone marrow. In addition, we observed a strong decrease in stem cell/primitive progenitor frequency in post-transplantation CD34+ cells as defined by long-term culture assays. Measurement of the levels of reactive oxygen species by flow cytometry revealed comparable levels in post-transplantation and normal bone marrow CD34+/CD38low cells, while significantly higher levels of reactive oxygen species were observed in CD34+/CD38high cells following autologous stem cell transplantation compared to normal bone marrow. Moreover, post-transplantation CD34+ bone marrow cells demonstrated an increased sensitivity to buthionine sulfoximine, a trigger for endogenous production of reactive oxygen species. Gene expression analysis on CD34+ cells revealed a set of 195 genes, including HMOX1, EGR1, FOS and SIRPA that are persistently down-regulated in mobilized peripheral blood cells and post-transplantation bone marrow compared to normal bone marrow. In conclusion, our data indicate that the diminished regenerative capacity of bone marrow following autologous stem cell transplantation is possibly related to a loss of quiescence and a reduced tolerability to oxidative stress.

Introduction

Autologous stem cell transplantation (ASCT) allows the application of high-dose chemotherapy and this is included in the standard treatment regimens for multiple myeloma and relapsing lymphoma.[1,2] This strategy results in a considerably improved treatment outcome, but in 30-50% of the patients, the underlying malignant disorder relapses.[3-5] In these cases, the treatment options are limited, in part due to a diminished capacity of the transplanted cells to recover from a subsequent course of chemotherapy. Apparently, the applied chemotherapy and ASCT have resulted in an impaired chemotoxic stress response of the bone marrow cells.[6,7] These findings are in line with our recent observations demonstrating a shift within the CD34+ progenitor cell compartment post-ASCT towards phenotypically defined granulocyte/macrophage progenitors (GMPs), which coincided with a reduced clonogenic potential and enhanced cell cycle activity.[8] After allogeneic stem cell transplantation, a higher cycling activity of CD34+CD90+ primitive bone marrow cells was observed.[9] Moreover, regeneration after ASCT has been associated with increased proliferation and a significant reduction in primitive progenitors.[10,11]

Mobilized peripheral blood stem cells (PBSC) have become the standard cell source for ASCT. During the growth factor-induced stem cell mobilization, the hematopoietic stem cells (HSCs) egress from the bone marrow to the peripheral blood and are exposed to significantly higher oxygen levels compared to those in the bone marrow.[12-14] This change in oxygen levels might affect several cellular functions and can be a trigger to increase the production of reactive oxygen species (ROS).[15] Experiments in mice have clearly demonstrated that higher ROS levels in the HSC fraction hamper stem cell function and promote differentiation to a more mature phenotype, associated with changes in cell cycle.[16] In turn, cell cycle changes were demonstrated to affect long-term engraftment.[17-19]

It has still not been clarified whether the infused PBSC can re-install their normal cellular programming following engraftment in the bone marrow, a process that might be required for proper stem cell function. Therefore, quiescent cell cycle status and stem cell/primitive progenitor frequency together with ROS production of CD34+ cells from post-ASCT bone marrow (one year after transplantation) were studied and compared to normal bone marrow cells and PBSC. In addition, gene expression profiling was performed to obtain greater insight into the underlying molecular mechanisms. The results indicate that the diminished regenerative capacity of bone marrow post-ASCT might be related to a loss of quiescence of stem cells and primitive progenitors and enhanced ROS production by progenitor cells. In addition, micro-array studies demonstrated that changes in gene

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expression induced by mobilization are only partly restored in CD34+ bone marrow cells post-ASCT.

Methods

Patient material

Bone marrow aspirates from patients one year after ASCT and normal controls were obtained after informed consent according to institutional guidelines. Potential donors for allogeneic bone marrow transplantation and patients who underwent elective total hip replacement served as normal controls. PBSC material was obtained from patients who underwent apheresis for ASCT. The study was approved by the Medical Ethical Committee of the University Medical Center Groningen, The Netherlands.

Flow cytometry analysis and sorting procedures

The mononuclear cell (MNC) fraction from bone marrow was isolated by density gradient centrifugation using lymphoprep (PAA, Cölbe, Germany). CD34+ cells were isolated by EasySep immunomagnetic cell selection (StemCell Technologies, Vancouver, Canada) according to the manufacturer's instructions. Sorting of CD34+ bone marrow cells for long-term colony initiating cell (LTC-IC) experiments was performed by MoFLo sorting (Dako Cytomation, Carpinteria, CA, USA) using a CD34 PE-labeled antibody (Clone 8G12, BD Biosciences, San Jose, California, USA). The fluorescence activated cell sorting (FACS) analyses were performed on an LSR II flow cytometer (Becton Dickinson (BD), Alpen a/d Rijn, The Netherlands). Antibodies were obtained from BD. Data were analyzed using FlowJo (Tri Star, Inc., Ashland, OR, USA) software.

Hoechst and Pyronin Y staining

Cells were washed and re-suspended in hematopoietic progenitor cell growth medium (HPGM) (Lonza, Leusden, The Netherlands). The staining was performed in this solution with 5 µg/mL Hoechst 33342 (Invitrogen) at 37°C for 30 min, then 1.0 µg/mL Pyronin Y (Sigma) was added at 37°C for an additional 45 min. Cells were washed in the solution containing Hoechst and Pyronin Y, followed by FcR blocking at 4°C for 10 min. After staining with CD34-APC and CD38-Alexa700 at 4°C for 20 min, cells were washed and analyzed.

Long-term culture initiating cell assay

For long-term culture initiating cell (LTC-IC) assays, CD34+ cells were plated in limiting dilution in a 96-well plate pre-coated with MS5 stromal cells and cultured for five weeks after which methylcellulose was added. Detailed information can be found in the *Online Supplementary Methods*. Wells containing CFCs were scored as positive and the LTC-IC frequency was calculated using L-Calc Limiting Dilution Software (StemCell Technologies).

Measurement of intracellular ROS levels

Intracellular ROS levels were determined by staining cells with the probe 5- (and 6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H2DCFDA; Invitrogen) followed by flow cytometry analysis. A detailed description of this method is provided in the *Online Supplementary Methods*.

In vitro treatment with buthionine sulfoximine

To determine the sensitivity to buthionine sulfoximine (BSO), CD34+ normal bone marrow, post-ASCT bone marrow or PBSC cells were isolated and cultured in HPGM supplemented with 20 ng/mL IL3 and increasing concentrations of BSO followed by colony forming cell (CFC) assays. Details are provided in the *Online Supplementary Methods*.

Gene expression profiling

Details on genome-wide expression analysis, performed on Illumina (Illumina, Inc., San Diego, CA, USA) BeadChip Arrays Sentrix Human-12 v3 (46k probesets), are provided in the *Online Supplementary Methods*.

Statistical analysis

The Mann-Whitney U test was used for analysis of individual group differences. $P \leq 0.05$ was considered statistically significant.

Results

Patients' characteristics

In order to obtain an insight into the effects of the stem cell transplantation procedure on hematopoietic stem cell function, bone marrow cells from patients one year after ASCT (post-ASCT) were compared with normal bone marrow and PBSC. This study included post-ASCT patients with relapsing lymphoma treated with intensive chemotherapy and ASCT using BEAM as conditioning regimen and multiple myeloma (MM) patients treated with chemotherapy and ASCT using high-dose melphalan as conditioning regimen. Four patients were treated with ASCT for AL-amyloidosis (n=2), POEMS syndrome, and scleromyxedema. These patients were treated like MM patients with high-dose melphalan as conditioning regime. Full patients' characteristics of included patients (n=37) are provided in the *Online Supplementary Appendix*; median age was 54 years (range 44-68 years). The infused autologous stem cell transplant consisted of at least $4.0x10^6$ CD34+ cells/kg (range 4.0-25.0x10^6). The peripheral blood cell counts at the time of study demonstrated a mean hemoglobin level of 8.2 mmol/L (range 6.2-10.0 mmol/L), a mean leukocyte count of $4.6x10^9$/L (2.4-8.2x10^9/L), a mean granulocyte count of $2.9x10^9$/L (1.3-5.6x10^9/L) and a mean platelet count of $139x10^9$/L (32-239x10^9/L).

Reduced percentage of quiescent CD34+/CD38low cells in bone marrow post-ASCT

An important characteristic of hematopoietic stem cells (HSCs) is their quiescent cell cycle status. To examine the effects of the ASCT procedure on quiescence 1-year post-ASCT, the percentage of cells in the G0 phase was measured by staining the cells with Hoechst and Pyronin Y followed by flow cytometric analysis. Both the CD34+/CD38high and CD34+/CD38low fraction were analyzed. No significant difference was observed in percentages of CD34+/CD38low between normal bone marrow (n=9) and post-ASCT bone marrow (n=6) (mean percentage 11.5%, 95%CI: 4.3-18.7% *vs.* 4.1%, 95%CI: 0.6-7.6%), respectively, (P=0.77). For quiescence analyses, post-ASCT bone marrow cells (n=6) were compared with normal bone marrow cells (n=9) and mobilized peripheral blood stem cells (PBSC) (n=7). A representative sample of each group is shown in Figure 1A. Interestingly, post-ASCT bone marrow contained a significantly lower percentage of quiescent cells in the CD34+/CD38low fraction compared to normal bone marrow (mean percentage 23.6%, 95%CI: 6.5-40.8% *vs.* 48.6, 95%CI: 31.4-65.9%; P=0.045) (Figure 1B). Also CD34+/CD38low PBSC cells demonstrated a lower percentage of quiescent cells (mean percentage 26.3%, 95%CI: 8.4-44.2%) compared to normal bone marrow, but this difference did not reach statis-

tical significance (P=0.08). No significant differences in the percentage of quiescence cells were observed in the CD34$^+$/CD38^{high} fraction between the three groups (Figure 1B).

In line with our previously published results,[8] CD34$^+$/CD38^{low} cells of post-ASCT bone marrow demonstrated an increased percentage of cells in S/G2/M phase compared to CD34$^+$/CD38^{low} cells of normal bone marrow (mean percentage 6.3, 95%CI: 0.25-12.4% *vs.* 0.9%, 95%CI: 0.17-1.64%), P=0.001).

Diminished hematopoietic stem cell/primitive progenitor frequency in CD34$^+$ post-ASCT bone marrow cells

The reduced quiescence might imply a loss of stem cell function. Therefore, functional capacity of CD34$^+$ cells from post-ASCT (n=5), PBSC cells (n=8) and normal bone marrow (n=11), was examined using *in vitro* long-term culture initiating cell (LTC-IC) assays. This analysis revealed a strong decrease in hematopoietic stem cell/primitive progenitor (HSPC) frequency in the CD34$^+$ compartment of bone marrow post-ASCT compared to normal CD34$^+$ bone marrow cells (mean frequency 0.0016, 95%CI: 0.0003-0.0028 *vs*. 0.0206, 95%CI: 0.0162-0.0250; P=0.002) (Figure 2). The HSPC frequency of CD34$^+$ PBSC cells (0.0624, 95%CI: 0.0173-0.1075) was not significantly different from the frequency observed in normal bone marrow (P=0.12), suggesting that the decrease in HSPC frequency observed in post-ASCT bone marrow can not only be explained by the previous chemotherapy and the mobilization procedure, but might rather be induced by the transplantation procedure.

Increased ROS levels in CD34$^+$/CD38^{high} but not in CD34$^+$/CD38^{low} post-ASCT bone marrow cells

The mobilization procedure prior to ASCT implies the egression of hematopoietic stem and progenitor cells from the bone marrow to the peripheral blood. One of the factors that might contribute to the impaired function of post-ASCT bone marrow is an enhanced ROS production triggered by the changes in oxygen levels upon mobilization. To examine this in more detail, levels of reactive oxygen species (ROS) were measured by flow cytometric analysis in post-ASCT bone marrow (n=12), PBSC (n=7) and normal bone marrow (n=13). ROS levels in CD34$^+$/CD38^{low} post-ASCT bone marrow (mean MFI 8078, 95%CI: 2823-13334) were not significantly different from the levels observed in normal bone marrow cells. On the contrary, CD34$^+$/CD38^{high} post-ASCT bone marrow cells demon-

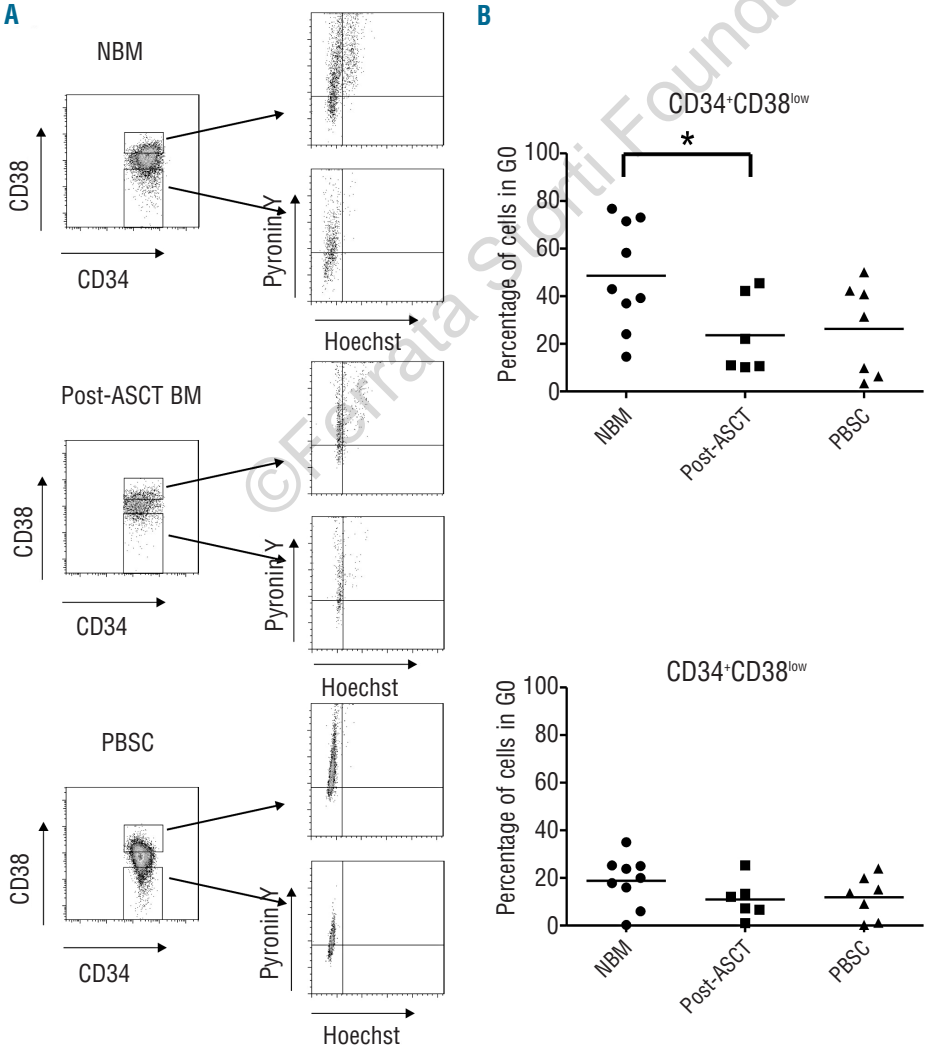

Figure 1. Reduced percentage of quiescent CD34$^+$/CD38^{low} cells in bone marrow post-ASCT. (A) Quiescence analysis of representative samples of normal bone marrow (NBM), post-ASCT bone marrow and PBSC. MNC cells from bone marrow and PBSC were enriched for CD34$^+$ cells by EasySep immunomagnetic cell selection and analyzed on an LSR flow cytometer. Dot plots on the left show cell populations after gating for CD34$^+$ cells. Gating strategies as shown in the figure were based on negative staining controls. A wide space was kept between gates for CD38^{low} and CD38^{high} cells to analyze relative pure cell populations. On the right, gating strategy for Hoechst/Pyronin Y staining is shown. The cellular uptake of these dyes allows the distinction of cells in G0 (2N DNA with low levels of RNA, so Hoechst^{low} and Pyronin Y^{low}), G1 (2N DNA with higher levels of RNA, so Hoechst^{low} and Pyronin Y$^{int/high}$) and S/G2/M (incorporation of both Hoechst and Pyronin Y). (B) Percentage of cells in G0 phase of cell cycle as analyzed for the CD34$^+$/CD38^{low} fraction (upper graph) and CD34$^+$/CD38^{high} fraction (lower graph). Horizontal lines indicate mean percentages per group. *P<0.05.

strated higher ROS levels compared to normal bone marrow (mean MFI 12467, 95%CI: 7606-17328 *vs.* 6430, 95%CI: 4062-8799; *P*=0.014). In addition, significantly higher ROS levels were observed in CD34$^+$/CD38^{low} PBSC compared to normal bone marrow (mean MFI 10440, 95%CI: 5153-15728 *vs.* 5194, 95%CI: 2530-7857; *P*=0.043). ROS levels of both the CD34$^+$/CD38^{low} and CD34$^+$/CD38^{high} fractions of PBSC were not significantly different from those of post-ASCT bone marrow (Figure 3).

Increased sensitivity of CD34$^+$ post-ASCT bone marrow cells to BSO treatment

To functionally test the effects of reactive oxygen stress on colony forming potential, CD34$^+$ normal bone marrow, post-ASCT bone marrow and PBSC cells were treated with increasing concentrations of BSO followed by the CFC assay. BSO inhibits glutathione synthetase and thereby increases intracellular ROS levels.[20,21] Upon treatment with 20 μM BSO, CD34$^+$ post-ASCT bone marrow cells demonstrated a significantly reduced ability to form CFU-GM colonies compared to normal bone marrow (*P*=0.013), indicating increased sensitivity to oxidative stress. Although not statistically significant, comparable results were observed for 50 μM BSO treatment (Figure 4). When compared with CD34$^+$ PBSC, a significant decrease in CFC potential of post-ASCT CD34$^+$ cells was observed for all tested concentrations of BSO (Figure 4).

Gene expression profiling

To define additional pathways affected by the ASCT procedure, micro-array analysis was performed comparing CD34$^+$ cells from post-ASCT bone marrow (n=7), normal bone marrow (n=31) and PBSC (n=9). To identify gene expression changes that are induced by the mobilization procedure, we first compared CD34$^+$ cells derived from normal bone marrow *versus* CD34$^+$ PBSC. This analysis revealed 1355 genes down-regulated (fold change <0.75 and *P*<0.0001) and 1508 genes up-regulated (fold change >1.5 and *P*<0.0001) in PBSC compared to normal bone marrow.

We were particularly interested in those gene expression changes that are associated with the mobilization procedure and remain affected in post-ASCT bone marrow. More especially, those gene expression changes that appear to be irreversibly changed upon mobilization and transplantation are likely to contribute to the diminished stem cell frequency of post-ASCT CD34$^+$ bone marrow. Analysis of the overlap of genes found to be down-regulated in PBSC compared to normal bone marrow and in post-ASCT compared to normal bone marrow (551 genes, fold change <0.75; *P*<0.0001) revealed 195 genes to be down-regulated in both PBSC and post-ASCT bone marrow compared to normal bone marrow (Figure 5A). The

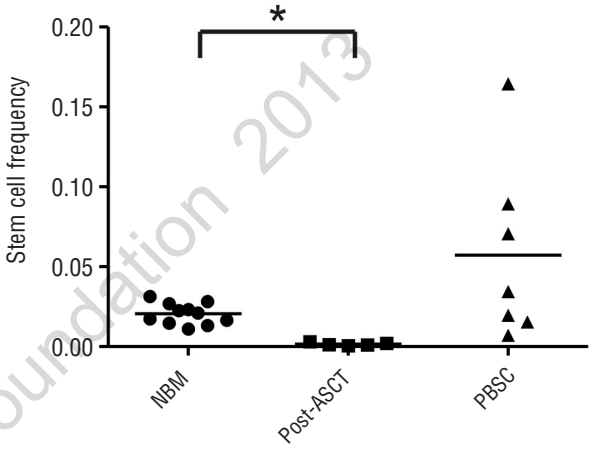

Figure 2. Strongly decreased HSPC frequency of CD34$^+$ post-ASCT bone marrow. *In vitro* HSPC frequency was tested using the long-term culture initiating cell assay and results indicate the frequency in proportion as calculated by L-CALC in which for example a proportion of 0.025 indicates a stem cell frequency of 1 in 40. Horizontal lines indicate mean frequency per group. *P<0.01.

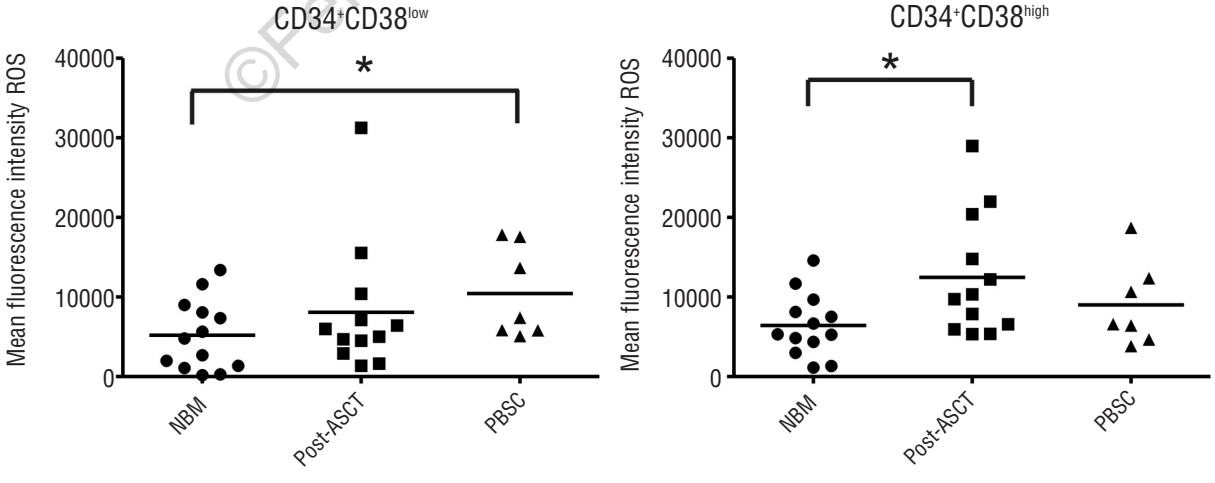

Figure 3. Increased ROS levels in CD34$^+$CD38^{high} post-ASCT bone marrow cells. Intracellular reactive oxygen species (ROS) levels were determined by staining with the probe 5- (and 6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H2DCFDA). ROS levels were measured by flow cytometric analysis and mean fluorescence intensity (MFI) of CM-H2DCFDA was calculated per sample using FlowJo. Analyses were performed for both the CD34$^+$/CD38^{low} fraction (left graph) and CD34$^+$/CD38^{high} fraction (right graph). Horizontal lines indicate the mean in MFI per group. *P<0.05.

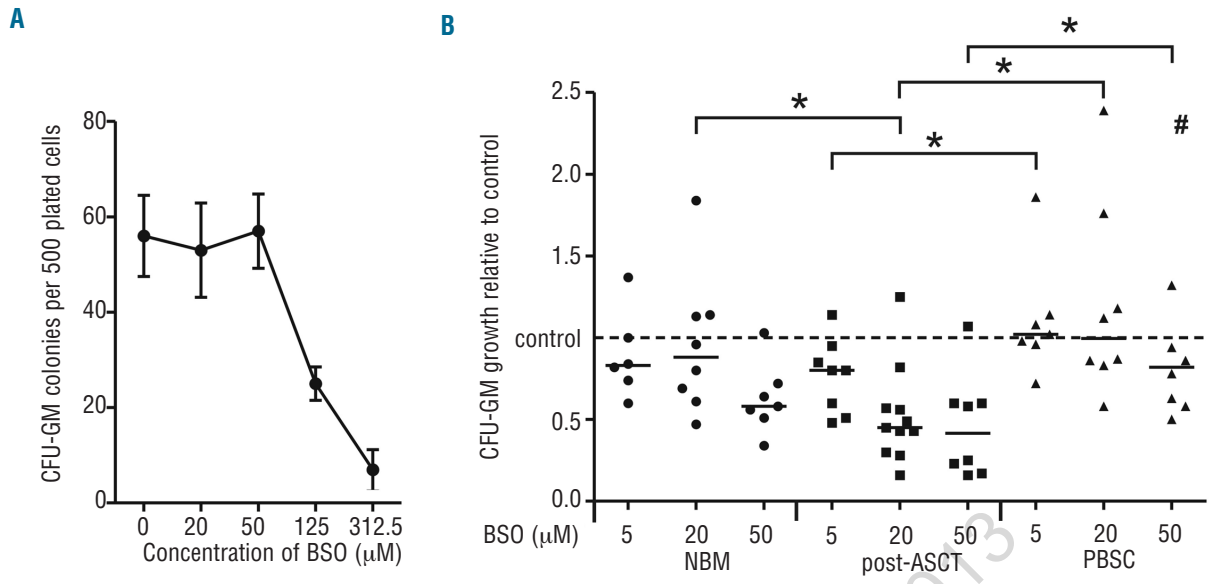

Figure 4. Increased sensitivity of CD34$^+$ post-ASCT bone marrow to BSO. (A) Cord blood CD34$^+$ cells were incubated with BSO in increasing concentrations followed by CFC-GM assay to determine the sensitivity of human hematopoietic CD34$^+$ cells for BSO. Results of 2 experiments. (B) CD34$^+$ normal bone marrow (NBM), post-ASCT bone marrow and PBSC cells were incubated with increasing concentrations of BSO and CFC-GM assays were performed. Results were corrected for the percentage of dead cells after treatment and before plating cells for the CFC assays. Results are normalized for the results for no treatment per group, set to 1 (dashed horizontal line). *P<0.05, # outlier result for PBSC 50 μM (3.36) not shown in the graph. Horizontal lines indicate the median for each group.

complete list of 195 genes is provided in the *Online Supplementary Table S1*. A number of genes are of particular interest based on their known involvement in stem cell maintenance, stem cell niche interactions and oxidative stress response, including HMOX1, EGR1, FOS and SIRPA (Figure 5B and D). GO analysis examining the list of 195 genes revealed that these genes may be involved, among other things, in inflammatory and defense responses and cell adhesion (Figure 5C). Based on our previous observations, we next performed a specific search for enrichment of genes involved in cell cycle or oxidative stress responses. However, although some genes, including HMOX1, GPX3 and FOS, were associated with these processes, no statistically significant enrichment for these GO terms was revealed within the set of 195 genes (*Online Supplementary Appendix*). The decrease in HMOX1 expression in PBSC and post-ASCT bone marrow compared to normal bone marrow was confirmed by qRT-PCR (Figure 5E).

Discussion

The results of our study reveal impairments in the HSPC compartment 1-year post-ASCT. These data might provide a basis for explaining the increased vulnerability to chemotherapy, an important clinical concern in patients post-ASCT. *In vitro* HSPC frequencies were reduced in CD34$^+$ bone marrow cells 1-year post-ASCT. Moreover, the percentage of quiescent CD34$^+$CD38^{low} cells was reduced. ROS levels were increased in the CD34$^+$CD38^{high} cells post-ASCT, coinciding with an increased sensitivity to BSO of the total CD34$^+$ cell fraction of post-ASCT bone marrow.

The data that were obtained by comparing normal bone marrow and post-ASCT bone marrow with PBSC indicate that some of the effects observed in post-ASCT bone marrow could also be observed in PBSC. Nevertheless, our data also indicate that while CD34$^+$/CD38^{low} PBSC do demonstrate signs of increased oxidative stress, these cells are not functionally impaired. So apparently, unlike the post-ASCT bone marrow cells, PBSC are able to overcome the negative effects of oxidative stress. These data suggest that the transplantation procedure has a major impact on the observed functional impairment of post-ASCT bone marrow, and that this impairment can not be solely attributed to the effects of mobilization.

Our findings are in line with the concept that the hematopoietic compartment post-ASCT must be rebuilt and maintained by a limited number of HSCs. The nearly normal peripheral blood cell counts of patients post-ASCT indicate that the HSCs are able to maintain a stable supply of progenitors and differentiated hematopoietic cells. However, since stem cell frequencies were reduced post-ASCT, it appears that these peripheral blood cell counts can only be generated via a higher cycling activity of the stem cell pool. We, indeed, observed that most CD34$^+$/CD38^{low} cells post-ASCT have lost their quiescent cell cycle status. Although the underlying molecular mechanisms still need to be clarified, it is possible that HSCs do not home properly to their quiescent niches in the bone marrow post-ASCT. Alternatively, it is also possible that intrinsic cell changes that are induced during the process of mobilization are not reverted to their original state once HSCs are returned in the post-ASCT bone marrow. Indeed, our gene array data on CD34$^+$ cells from normal bone marrow, from mobilized PBSC and post-ASCT bone marrow indicate that a number of gene expression

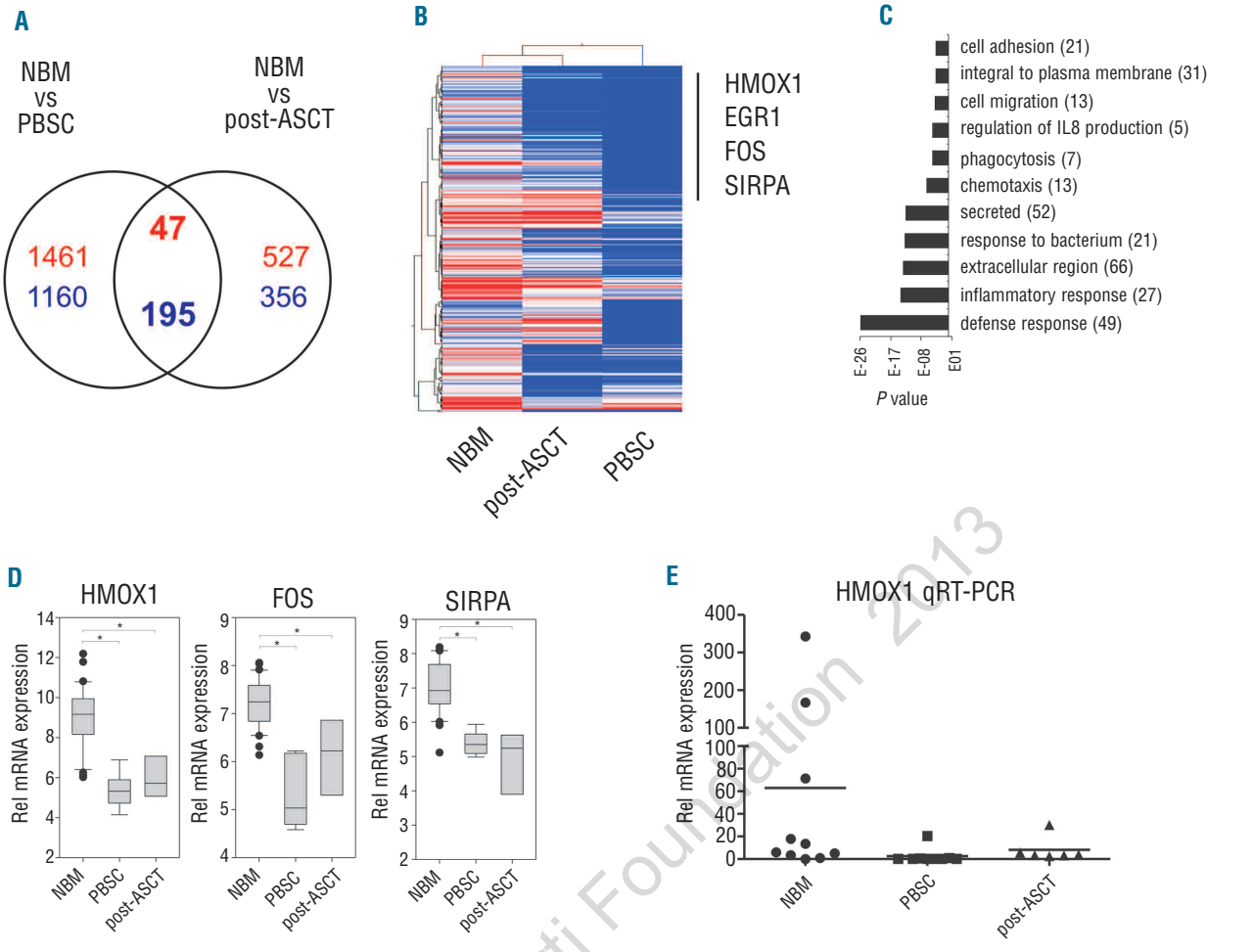

Figure 5. Gene expression changes in PBSC compared to normal bone marrow are not fully restored in post-ASCT bone marrow. (A) VENN diagram showing overlap in differentially expressed genes in PBSC *versus* normal bone marrow (left) and post-ASCT bone marrow *versus* normal bone marrow (right). Red indicates up-regulated genes and blue down-regulated genes. (B) Heat map indicating the gene expression changes wherein red color indicates relative high expression and blue color relative low expression. (C) GO analysis of 195 genes indicated in (A). (D) Gene expression changes as assessed by the micro-array analysis shown in detail for HMOX1, FOS and SIRPA. Statistically significant differences are indicated by an asterisk. (E) Results of an independent qRT-PCR analysis for HMOX1. Please note the two segments of the y-axis with a different scale.

changes that were induced by the process of mobilization were not reversible. In particular, for a number of genes that were down-regulated upon mobilization, we observed that expression remained low in post-ASCT CD34$^+$ cells. Interestingly, this included genes such as HMOX1 and EGR1. EGR1 is an early response gene involved in cytokine regulation, in particular of IL8.[22] Mice that are haploinsuffcient for Egr1 demonstrate an increased susceptibility to chemotherapy-related leukemia.[23] Although loss of Egr1 does not impair reconstitution capacity in primary recipient mice, Egr1$^{-/-}$ HSCs exhibit premature loss of function during serial transplantation,[22,24] suggesting a protective function of EGR1 in the case of replicative stress. Also HMOX1 (HO-1) has been implicated in the stress response of HSCs. In line with the situation seen in patients post-ASCT, HO-1$^{+/-}$ mice demonstrate normal steady hematopoiesis but a blunted hematopoietic recovery following several courses of 5-FU treatment and a limited HSC reserve during long-term hematopoietic stress.[25] Other observed gene expression differences in post-ASCT bone marrow suggest a reduced interaction of CD34$^+$ cells post-ASCT with the bone marrow niche. The expression of genes from the group of integrins (ITGB2, ITGB5) as well as SIRPA are down-regulated in PBSC and post-ASCT CD34$^+$ cells. The interaction of ITGB2 with ICAM1 was suggested to be important in the process of HSC engraftment in the bone marrow niche.[26] Also SIRPA was described as an important regulator of interactions between HSCs and the bone marrow niche.[27] The downregulation of these genes is probably necessary for the process of mobilization, but the down-regulation in post-ASCT bone marrow might have negative effects on HSC function through an altered interaction with the micro-environment.

Oxidative stress has been reported to have diverse and sometimes detrimental effects on HSCs. Loss of stem cell quiescence has frequently been associated with increased levels of ROS production, which in turn triggers various cell biological effects ranging from DNA damage, p53-mediated apoptosis to enhanced cell cycle progression and

differentiation of hematopoietic progenitors. Various key players have been identified that regulate oxidative stress in HSCs, including FoxO transcription factors,[15] BMI1[28] and HMOX.[25] While loss of FoxO resulted in increased cell cycling and apoptosis of HSCs,[15] Gfi1b$^{-/-}$ mice demonstrate elevated ROS levels but an expansion of the hematopoietic stem cell compartment.[29] So it seems that the context in which elevated ROS levels are present, will finally determine the consequences for the stem cell compartment. We also determined the ROS levels in stem and progenitor cells in normal BM, mobilized PBSC and in BM 1-year post-ASCT. While we observed a significant increase in ROS levels in the CD34^{+}/CD38^{low} compartment upon mobilization, no significant differences in ROS levels were observed in post-ASCT CD34^{+}/CD38^{low} cells compared to normal bone marrow. Interestingly, we did observe increased ROS levels in the CD34^{+}/CD38^{high} progenitor compartment post-ASCT. Further studies will be needed to determine the exact consequences of these increased ROS levels for post-ASCT progenitor cells.

Besides the strongly impaired capacity to recover from a second course of chemotherapy in case of relapsing disease, another major complication of ASCT is the occurrence of therapy-related myelodysplasia or acute myeloid leukemia (t-MDS/AML). Interestingly, Li *et al.* observed altered gene expression of genes related to mitochondria, oxidative phosphorylation and oxidative stress response in CD34^{+} PBSC cells from patients who develop t-MDS/AML. Moreover, these cells were characterized by increased ROS generation, reduced ROS detoxification and enhanced DNA damage after therapeutic exposure.[30] Although examined from a different perspective, these data can be seen to be in line with ours and clearly support a theory in which the ASCT procedure induces an impairment of hematopoietic stem cell function characterized by oxidative stress defects.

In conclusion, our data indicate that the diminished regenerative capacity of bone marrow post-ASCT might be related to a loss of quiescence in the CD34^{+}/CD38^{low} compartment and enhanced ROS production by progenitor cells. Gene expression profiling revealed potential target genes of which the re-activation might improve the stress recovery capacity of post-ASCT bone marrow. Further studies aimed at identifying the molecular mechanisms contributing to the susceptibility of post-ASCT bone marrow may help in the development of therapeutic interventions and improve this widely used treatment strategy.

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Authorship and Disclosures

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