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### The biology of human hematopoietic stem and progenitor cells in acute myeloid leukemia, aging and autologous transplantation

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# CHAPTER 7

## **Autologous stem cell transplantation induces a phenotypical shift from CMP to GMP progenitors, reduces clonogenic potential and enhances *in vitro* and *in vivo* cycling activity defined by <sup>18</sup>F-FLT PET scanning**

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## Abstract

Autologous stem cell transplantation (ASCT) introduces a reduced tolerance to chemotherapy even in patients with adequate engraftment, suggesting long-term effects of the transplantation procedure on the bone marrow (BM) capacity. To study the hematopoietic cell compartment post-ASCT CD34<sup>+</sup> BM cells (n=16) from patients 6-9 months post-ASCT were studied with regard to the progenitor subsets, colony frequency and cell cycle status. The BM compartments were studied *in vivo* using PET tracer 3-fluoro-3-deoxy-L-thymidine (<sup>18</sup>F-FLT PET). BM CD34<sup>+</sup> cells post-ASCT were compared to normal CD34<sup>+</sup> cells and demonstrated a phenotypical shift from common myeloid progenitor (CMP mean percentage 3.7% vs. 19.4%, p=0.001) to granulocyte-macrophage progenitor (GMP mean percentage 51.8% vs. 27.6%, p=0.01). In addition a reduced clonogenic potential and higher cycling activity especially of the GMP fraction (41% ± 4 in G2/S phase vs. 19% ± 2, p=0.03) were observed in post-ASCT BM compared to normal. The enhanced cycling activity was confirmed *in vivo* by demonstrating a significant higher uptake of the <sup>18</sup>F-FLT PET tracer by the BM compartment. This study demonstrates that ASCT results in defects of the hematopoietic compartment at least 6 months post-ASCT characterized by changes in composition of progenitor subsets and enhanced *in vitro* and *in vivo* cycling activity.

## Introduction

Autologous stem cell transplantation (ASCT) is a frequently applied treatment modality for patients with multiple myeloma (MM) and relapsing lymphoma.<sup>1;2</sup> However, in 30-50% of the patients the underlying malignant disorder relapses within 2-5 years after ASCT. Treatment options are then limited due to drug resistance of the tumor, but also due to the severe concurrent chemotherapy-induced pancytopenia. Several *in vitro* studies have shown that the impaired bone marrow capacity might be related to intrinsic defects of the hematopoietic compartment reflected by a reduced clonogenic potential of the long-term initiating cells in conjunction with a shortening of telomeres.<sup>3-5</sup> Following allogeneic stem cell transplantation also a higher mitotic activity of bone marrow CD34<sup>+</sup>/CD90<sup>+</sup> cells has been demonstrated.<sup>6</sup>

Recent studies in mice and human have shown that the compartments of the hematopoietic stem cell (HSC) and progenitor cells (CMP, GMP, and megakaryocyte-erythroid progenitor, MEP) can be defined phenotypically with certain surface markers.<sup>7;8</sup> These phenotypically defined subpopulations are also verified by *in vitro* colony assays. In these assays the CMP fraction provided myeloid and erythroid colony formation while the GMP fraction generated exclusively myeloid colony formation.<sup>9</sup> In addition, the progenitors and more differentiated hematopoietic cells can be studied *in vivo*. Recent studies have demonstrated that the cycling activity of the bone marrow compartment can be visualized by using the PET tracer 3-fluoro-3-deoxy-L-thymidine (<sup>18</sup>F-FLT PET).<sup>10;11</sup> Distinct patterns of uptake have been demonstrated. In myelodysplasia a significant higher cycling activity of the bone marrow compartment was noticed while in aplastic anemia a significant reduction in cycling activity was shown.<sup>10;11</sup> Combining both *in vitro* and *in vivo* experiments, important information can be obtained regarding the functionality of the hematopoietic stem cell compartment following ASCT.

The results of this study demonstrate that bone marrow CD34<sup>+</sup> cells 6-9 months after ASCT show a shift from CMP to a more GMP-enriched phenotype, a reduced colony frequency and a higher cycling activity compared to normal CD34<sup>+</sup> cells. These results were validated *in vivo* by demonstrating a significant higher uptake of <sup>18</sup>F-FLT in the bone marrow compartment post-ASCT.

## Materials and Methods

### *Patients*

Bone marrow samples from patients and normal controls were obtained after informed consent according to institutional guidelines. CD34<sup>+</sup> cells were isolated by EasySep immunomagnetic cell selection (StemCell Technologies, Vancouver, Canada) according to manufacturer's instructions.

### *Flowcytometry analysis and sorting procedures*

Sorting of the CD34<sup>+</sup> cells into progenitor fractions was performed on the basis of the combinatorial expression of cell surface antigens as previously reported.<sup>9</sup> CMP were defined as CD34<sup>+</sup>CD38<sup>+</sup>CD110<sup>-</sup>CD45RA<sup>-</sup>, GMP as CD34<sup>+</sup>CD38<sup>+</sup>CD110<sup>-</sup>CD45RA<sup>+</sup> and megakaryocyte-erythroid progenitors (MEP) as CD34<sup>+</sup>CD38<sup>+</sup>CD110<sup>+</sup>CD45RA<sup>-</sup>. The fluorescence activated cell sorting analyses were performed on a FACS Calibur (Becton Dickinson (BD), Alpen a/d Rijn, The Netherlands). Antibodies were obtained from BD. Data were analyzed using WinList 3D (Verity Software House, Topsham, ME, USA) and FlowJo (Tri Star, Inc, Ashland, OR, USA) software.

### *CFC progenitors assay*

The colony forming cell (CFC) assay was performed in 1.2% methylcellulose containing 30% FCS, 57.2  $\mu$ M  $\beta$ -mercaptoethanol, and 2 mM Glutamine, supplemented with 20 ng/ml IL-3, 20 ng/ml IL-6, 20 ng/ml G-CSF, 20 ng/ml c-Kit ligand and 1 U/ml EPO (Cilag; Eprex; Brussels, Belgium) as previously described.<sup>12</sup> To define the *in vitro* cycling activity of the CFCs, CD34<sup>+</sup> cells were incubated with variable concentrations of cytarabin (Mainer Pharma, Belgium) i.e. 5 x 10<sup>-7</sup>M, 1 x 10<sup>-6</sup>M, 5 x 10<sup>-6</sup>M, 7.5 x 10<sup>-6</sup>M, 1 x 10<sup>-5</sup>M, 5 x 10<sup>-5</sup>M, 1 x 10<sup>-4</sup>M during 24 hrs and subsequently plated in the CFC assay. After 14 days the CFC colony number was counted with an inverted microscope.

### *Cell cycle analysis*

For cell cycle analysis Hoechst staining was performed. Briefly, cells were resuspended at 1 x 10<sup>6</sup>/ml in warm HPGM medium, 5  $\mu$ g/ml Hoechst 33342 (Sigma-Aldrich, Zwijndrecht, The Netherlands) was added and cells were incubated at 37°C for 45 minutes. Medium was removed and cells were stained with antibodies for progenitor analysis. Washed cells were kept on ice until FACS analysis.

### *<sup>18</sup>F-FLT PET*

<sup>18</sup>F-FLT was produced with a radiochemical purity of >95% and a specific activity of >10 TBq/mmol. <sup>18</sup>F-FLT in a dose of 400 MBq ( $\pm$ 10%) was administered intravenously as described.<sup>10,11</sup> To quantify bone marrow expansion, we developed a simple scoring system in which one point is given for expansion in every one third of the long bones, based on the well-known distribution of <sup>18</sup>F-FLT in the central skeleton and proximal part of the femoral and humeral bone. The <sup>18</sup>F-FLT uptake was also quantified using standardized uptake value (SUV) analysis in patients and control subjects. We selected several sites of the axial skeleton to sample <sup>18</sup>F-FLT activity. The regions of interests used in SUV analysis were based on the mean value within the 50% isocontour's boundaries using a Siemens Leonardo workstation. The control patients were patients with untreated non-small cell lung cancer or testicular cancer, who had undergone <sup>18</sup>F-FLT PET for other studies with normal blood hematology values and no signs of bone marrow metastases at presentation or during 6 months of follow-up. In addition, the patients were free of chemotherapeutic or radiotherapeutic treatment. The

protocols were approved by the institutional medical ethical committee of the University Medical Center Groningen.

### Statistics

For analysis of overall and individual group differences, we used Kruskal-Wallis and Mann-Whitney U tests. For correlations, we used Spearman. Differences with a P-value  $\leq 0.05$  were considered statistically significant.

## Results

### Patients

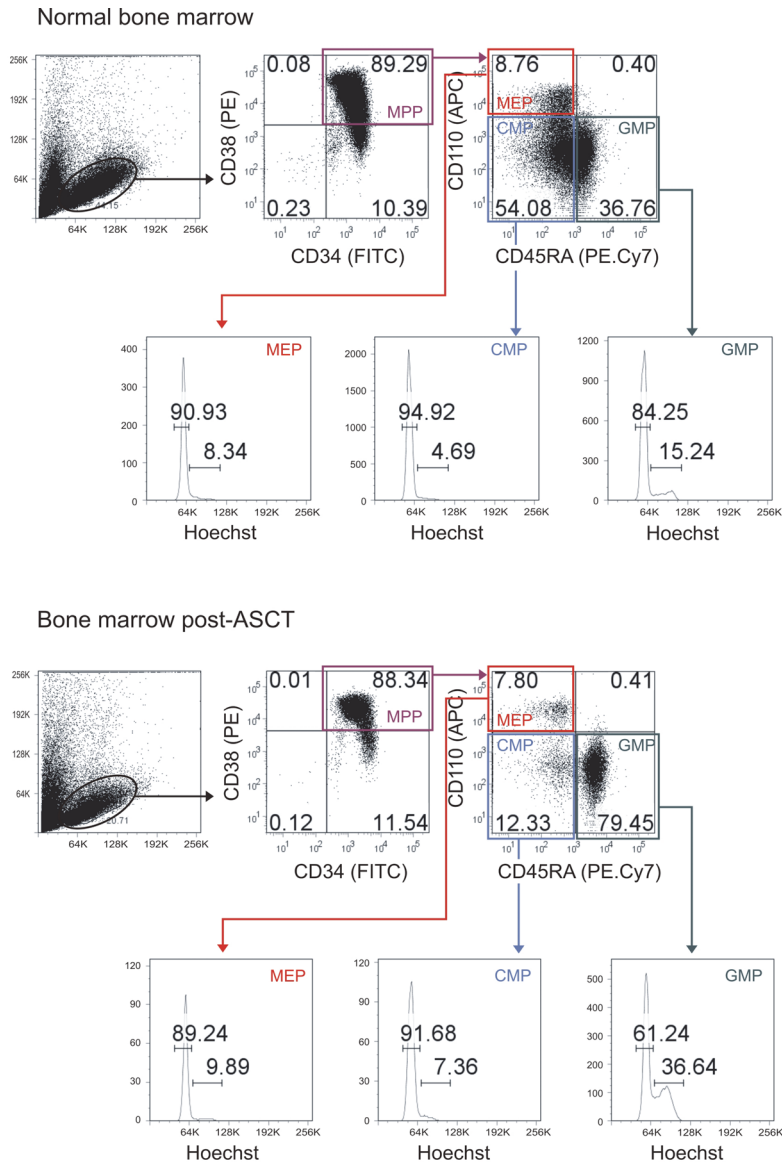
This study included patients with relapsing lymphoma (n=5) treated with intensive chemotherapy and ASCT using BEAM as conditioning regimen, and multiple myeloma (MM) patients (n=11) treated with chemotherapy and ASCT using high dose melphalan (200 mg/m<sup>2</sup>) as conditioning regimen. The infused autologous stem cell transplant consisted of at least  $4 \times 10^6$  CD34<sup>+</sup> cells/kg (range 4.3-12.5  $\times 10^6$ ) with only three patients receiving less than  $5 \times 10^6$  CD34<sup>+</sup> cells/kg. The studied patients were analyzed 6-9 months following ASCT. The peripheral blood cell counts demonstrated a mean haemoglobin level of 7.6 mmol/l (range 6.5-8.7), a mean leukocyte count of  $6.9 \times 10^9/l$  ( $3.4-15 \times 10^9/l$ ), a mean granulocyte count of  $4.4 \times 10^9/l$  ( $2.1-11.4 \times 10^9/l$ ) and a mean platelet count of 202  $\times 10^9/l$  ( $80-318 \times 10^9/l$ ). Patient characteristics are depicted in Table 1.

**Table 1** Patient characteristics and peripheral blood cell counts

Patient no	Clinical diagnosis	Age (yrs)	Hb (mmol/l)	Leucocytes ( $\times 10^9/l$ )	Granulocytes ( $\times 10^9/l$ )	Trombocytes ( $\times 10^9/l$ )
1	MM	62	7.1	5.5	3.3	179
2	MM	52	7.1	5.1	2.1	214
3	MM	42	8.4	7.3	5.5	147
4	MM	52	7.7	3.4	2.4	111
5	MM	48	8.3	7.0	3.5	211
6	MM	62	7.9	8.1	5.8	212
7	MM	61	7.6	7.4	4.5	312
8	MM	62	6.9	6.1	4.2	318
9	MM	52	6.5	9.0	4.5	313
10	MM	63	6.7	4.7	2.3	217
11	MM	52	7.3	5.8	2.9	170
12	NHL	59	8.1	6.7	4.7	199
13	NHL	59	7.7	4.3	2.15	126
14	HL	50	7.2	15	11.4	276
15	NHL	48	8.1	6.4	4.0	80
16	NHL	46	8.7	10.1	7.6	154

Abbreviations: MM = multiple myeloma; NHL = non-Hodgkin lymphoma; HL = Hodgkin lymphoma

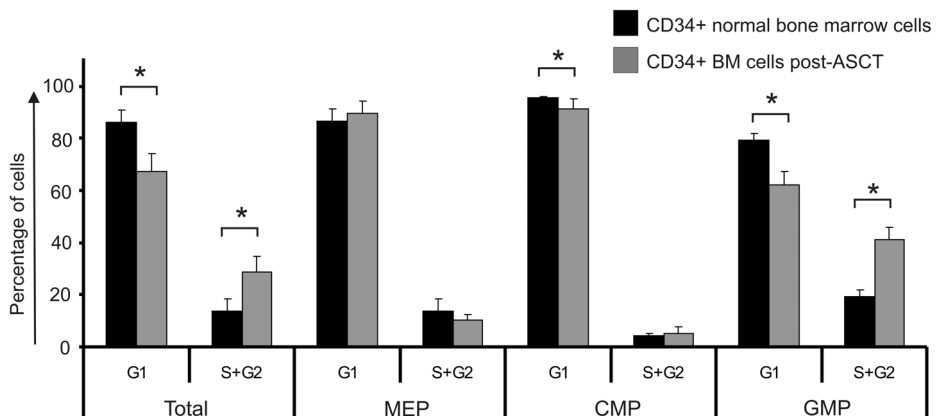
Bone marrow cytology demonstrated no abnormalities. There was no significant difference in age between patients post-ASCT (median 52 years (range 42-63) and controls (median 48 years (range 29-68) ( $p=0.1$ ).



**Figure 1 Shift to a GMP enriched phenotype in the bone marrow compartment following ASCT.** Representative FACS dot plots showing progenitor subsets of normal bone marrow and bone marrow 6-9 months post-ASCT. In post-ASCT bone marrow the percentage of phenotypically defined GMP was increased, whereas a reduced percentage was observed in the CMP fraction. With Hoechst staining a higher cycling activity was observed in the CD34<sup>+</sup>/CD38<sup>low</sup> and CMP subfractions of post-ASCT bone marrow compared to normal.

*Bone marrow CD34<sup>+</sup> progenitor subsets post-ASCT*

The isolated CD34<sup>+</sup> bone marrow cells (n=8) were phenotypically characterized for the percentage of CMP, GMP and MEP and compared with the distribution pattern of normal bone marrow CD34<sup>+</sup> cells (n=7). No significant difference was seen in the percentage of MEP in post-ASCT bone marrow vs. normal controls (mean percentage 4.3% (95%CI 2.1-6.5) versus 2.6% (95%CI 0.8-4.5), p=0.3). However, a significantly increased percentage of phenotypically defined GMP (mean percentage 51.8% (95%CI 39.6-64.2) versus 27.6% (95%CI 19.6-35.5), p=0.01) was observed, whereas a reduction in the phenotypically defined CMP fraction (mean percentage 3.7% (95%CI 0.7-6.9) vs. 19.4% (95%CI 11.3-27.6), p=0.001) was shown post-ASCT. A representative experiment is depicted in Figure 1. Comparable results were observed in a limited number of patients studied with a follow-up time of more than 3 years as demonstrated for a patient 10 years post-ASCT (Supplementary Figure). No distinct difference in progenitor subsets was observed between MM or lymphoma patients.

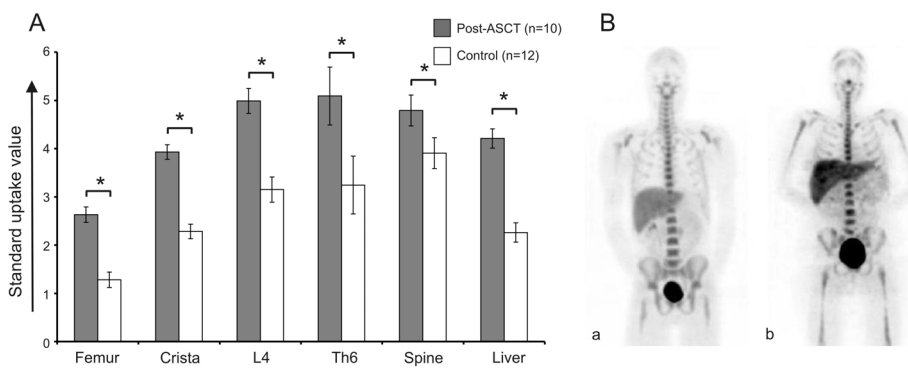


**Figure 2** Cell cycle analysis revealed a significantly higher fraction of CD34<sup>+</sup> bone marrow cells post-ASCT in S/G2 phase compared to normal bone marrow cells. Cell cycle analysis of CD34<sup>+</sup> cells of patients following ASCT (N=3) compared to normal CD34<sup>+</sup> bone marrow cells (N=3). Both the total CD34<sup>+</sup> cell fractions as well as the progenitor subsets (MEP, CMP and GMP) were analyzed. Post-ASCT bone marrow showed a significantly higher fraction of cells in S/G2 phase compared to normal bone marrow in both the total CD34<sup>+</sup> and the GMP fraction. Significant differences are marked by an asterisk.

To further characterize the CD34<sup>+</sup> cell fraction post-ASCT, progenitor frequency was tested by using the *in vitro* CFC assay. A decrease in CFC frequency per 10<sup>3</sup> plated CD34<sup>+</sup> cells was shown compared to normal CD34<sup>+</sup> bone marrow cells (109 ± 52 vs. 185 ± 56, p=0.008). In these CFC-assays no significant difference was found in the colony distribution of colony forming unit granulocyte/monocyte (CFU-GM) and erythroid burst forming units (BFU-E) in post-ASCT BM compared to normal BM. In CFC-assays from post-ASCT BM the percentage of CFU-GM colonies



was 60.4% (compared to 55.5% for normal BM,  $p=0.67$ ) and 39.1% for BFU-E colonies (compared to 41.4% for normal BM,  $p=0.78$ ). The decrease in CFC frequency from post-ASCT CD34<sup>+</sup> cells was not due to an impaired proliferative activity. Cell cycle analysis revealed a significantly higher fraction of CD34<sup>+</sup> cells in G2/S phase (mean 29% (95%CI 19.1-38.4) vs. 14% (95%CI 1.6-25.7),  $p=0.03$ ) and a reduced percentage of cells in G1 phase (mean 67% (95%CI 56.4-78.2) vs. 86% (95%CI 73.9-98.4),  $p=0.03$ ) compared to normal CD34<sup>+</sup> cells (Figure 2). It appeared that especially the GMP fraction of post-ASCT CD34<sup>+</sup> cells displayed a higher cycling activity (mean percentage 35% (95%CI 27.7-43.0) in G2/S phase vs. 19% (95%CI 13.0-25.7),  $p=0.03$ ) whereas no significant differences were observed for the MEP and CMP fractions. The higher cycling activity of the GMP fraction was also observed in bone marrow cells of patients with a longer follow-up time. Analysis of the GMP fraction in a patient 10 years post-ASCT demonstrated 36.7% of cells in G2/S phase. To verify whether the progenitor fraction within the CD34<sup>+</sup> fraction had an enhanced cycle activity and therefore would be more susceptible for chemotherapy-induced apoptosis, CD34<sup>+</sup> cells were *in vitro* incubated with variable concentrations of Ara-C (range  $5 \times 10^{-7}M$  to  $3 \times 10^{-3}M$ ) for 24 hrs and subsequently cultured in the CFC assay. Normal CD34<sup>+</sup> cells demonstrated a 45% reduction in CFC frequency at a concentration of  $5 \times 10^{-5} M$  ( $n=4$ ). Comparable results were observed with post-ASCT CD34<sup>+</sup> cells ( $n=3$ ) (reduction of 39%) (N.S.). No significant difference was observed in the percentage of CD34<sup>+</sup>/CD38<sup>low</sup> between patients post-ASCT and normal controls. However, also in the CD34<sup>+</sup>/CD38<sup>low</sup> fraction a higher cycling activity was observed post-ASCT compared to normal controls (Figure 1) ( $p=0.03$ ), suggesting that also this fraction is affected by the ASCT procedure.



**Figure 3 Standard uptake value (SUV) of <sup>18</sup>F-FLT-scans of normal individuals and patients following ASCT.** (A) The mean of the left and right SUV was calculated from femur, crista, lumbar 4 vertebral body (L4), thoracic 6 vertebral body (Th6), spine and liver demonstrating a significant increase ( $p<0.005$ ) of SUV for each location in patients following ASCT compared to normal individuals. Significant differences are marked by an asterisk. (B) <sup>18</sup>F-FLT PET scan of normal individual (a) and of patient post-ASCT (b).

### *<sup>18</sup>F-FLT PET scans in patients post-ASCT*

Recent studies have shown that <sup>18</sup>F-FLT PET scan can be used to visualize the cycling activity of the bone marrow compartment whereby the degree of uptake is a reflection of the rate of DNA synthesis.<sup>10,11</sup> The <sup>18</sup>F-FLT PET scans in 10 patients post-ASCT demonstrated a significant increase in standard uptake values (SUV) measured at different locations of the bone marrow compartment (Figure 3). The mean values of the left and right area were obtained in comparison to normal controls: femur  $3.6 \pm 0.7$  vs.  $1.2 \pm 0.5$ ,  $p < 0.001$ ; crista  $3.9 \pm 0.6$  vs.  $2.2 \pm 0.5$ ,  $p < 0.001$ ; lumbar 4 vertebral body  $4.9 \pm 1.3$  vs.  $3.1 \pm 0.9$ ,  $p < 0.005$ ; thoracic 6 vertebral body  $5.0 \pm 0.6$  vs.  $3.2 \pm 0.9$ ,  $p < 0.001$ ; spine  $4.7 \pm 2.5$  vs.  $3.9 \pm 0.7$ ,  $p < 0.005$ . Moreover a significant expansion of the bone marrow compartment was noticed (expansion factor of  $6 \pm 2.5$ ). Representative <sup>18</sup>F-FLT PET scans of a patient post-ASCT and of a normal individual are demonstrated in Figure 3B.

## Discussion

The present study demonstrates that the ASCT procedure results in defects of myeloid progenitors at different levels. Bone marrow CD34<sup>+</sup> cells post-ASCT show a phenotypical shift from CMP to GMP progenitors and have a reduced clonogenic potential. Moreover, these cells demonstrate an enhanced cycle activity *in vitro*, which could be confirmed *in vivo* by <sup>18</sup>F-FLT PET scanning.

It is remarkable that even though we observe enhanced proliferation of myeloid cells as determined by <sup>18</sup>F-FLT PET scanning as well as by cell cycle analysis of the GMP fraction, the steady state peripheral blood cell counts appear to be almost normal 6-9 months post-ASCT. Thus there seems to be a disbalance in the hematopoietic compartment, whereby under steady state conditions the peripheral blood cell counts can be maintained up to normal levels, but at the expense of an increased proliferation rate. Although molecular mechanisms remain to be determined, it is conceivable that cell intrinsic as well as extrinsic factors are contributing to the observed phenotypes post-ASCT. So far no enhanced apoptosis was observed in bone marrow cells post-ASCT as determined by electron microscopy studies (unpublished data E. Vellenga, 2009). Several studies have shown an enhanced decline of telomere length post-ASCT predominantly of the myeloid lineage.<sup>5,13</sup> In aging telomerase knockout mice, enhanced proliferation and cycling activity of myeloid progenitors have been demonstrated in conjunction with a preferential differentiation along the myeloid lineage with significant increase in the GMP fraction.<sup>14-16</sup> Therefore chemotherapy application and the ASCT procedure might have fastened the aging process in patients post-ASCT, resulting in the shift to a GMP phenotype.

The enhanced cycling activity by CD34<sup>+</sup> cells is not restricted to ASCT. Also patients 6 to 12 months following allogeneic stem cell transplantation have an enhanced cycling activity of the CD34<sup>+</sup> cells suggesting that the enhanced cycling activity is not only associated with the exposure of DNA-

damaging agents.<sup>6</sup> It is therefore more conceivable that the hematopoietic compartment undergoes a replicative stress response due to the infusion and engraftment of a limited amount of cells. It is of interest whether alternative transplantation strategies might improve this situation by increasing the number of hematopoietic stem cells in the bone marrow cavity by direct infusion of the stem cell transplant in the bone marrow. Indeed studies in mice have shown that direct infusion of the stem cell transplant in the bone marrow cavity has advantages compared to infusion intravenously.<sup>17;18</sup> Comparable results have been demonstrated with cord blood transplantation in the setting of allogeneic stem cell transplantation.<sup>19;20</sup> Whether this approach will also reduce the replicative stress response in the ASCT setting requires further study.

In summary, this study demonstrates that ASCT results in defects of the hematopoietic compartment post-ASCT characterized by a shift to a GMP-enriched phenotype, reduced clonogenic potential and enhanced *in vitro* and *in vivo* cycling activity.

### **Acknowledgements**

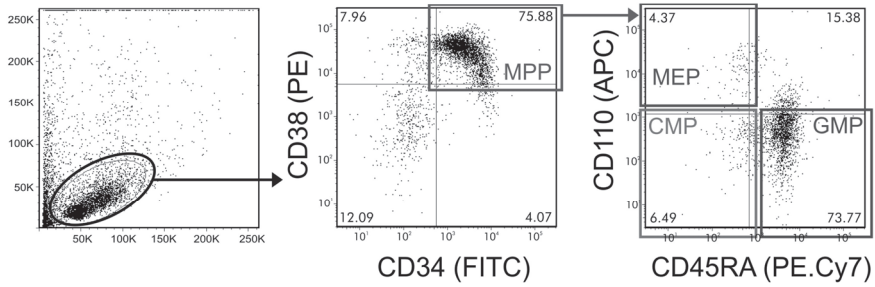
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## References

1. Lokhorst HM, Schmidt-Wolf I, Sonneveld P et al. Thalidomide in induction treatment increases the very good partial response rate before and after high-dose therapy in previously untreated multiple myeloma. *Haematologica* 2008;93:124-127.
2. Vellenga E, van Putten WL, van 't, V et al. Rituximab improves the treatment results of DHAP-VIM-DHAP and ASCT in relapsed/progressive aggressive CD20+ NHL: a prospective randomized HOVON trial. *Blood* 2008;111:537-543.
3. Domenech J, Linassier C, Gihana E et al. Prolonged impairment of hematopoiesis after high-dose therapy followed by autologous bone marrow transplantation. *Blood* 1995;85:3320-3327.
4. Noach EJ, Ausema A, van OR et al. Chemotherapy prior to autologous bone marrow transplantation impairs long-term engraftment in mice. *Exp.Hematol.* 2003;31:528-534.
5. Widmann T, Kneer H, Konig J, Herrmann M, Pfreundschuh M. Sustained telomere erosion due to increased stem cell turnover during triple autologous hematopoietic stem cell transplantation. *Exp.Hematol.* 2008;36:104-110.
6. Thornley I, Sutherland DR, Nayar R et al. Replicative stress after allogeneic bone marrow transplantation: changes in cycling of CD34+CD90+ and CD34+. *Blood* 2001;97:1876-1878.
7. Edvardsson L, Dykes J, Olofsson T. Isolation and characterization of human myeloid progenitor populations--TpoR as discriminator between common myeloid and megakaryocyte/erythroid progenitors. *Exp.Hematol.* 2006;34:599-609.
8. Manz MG, Miyamoto T, Akashi K, Weissman IL. Prospective isolation of human clonogenic common myeloid progenitors. *Proc.Natl.Acad.Sci.U.S.A* 2002;99:11872-11877.
9. Olthof SG, Fatrai S, Drayer AL et al. Downregulation of signal transducer and activator of transcription 5 (STAT5) in CD34+ cells promotes megakaryocytic development, whereas activation of STAT5 drives erythropoiesis. *Stem Cells* 2008;26:1732-1742.
10. Agool A, Schot BW, Jager PL, Vellenga E. 18F-FLT PET in hematologic disorders: a novel technique to analyze the bone marrow compartment. *J.Nucl.Med.* 2006;47:1592-1598.
11. Shields AF, Grierson JR, Dohmen BM et al. Imaging proliferation in vivo with [F-18]FLT and positron emission tomography. *Nat.Med.* 1998;4:1334-1336.
12. Schepers H, van GD, Wierenga AT et al. STAT5 is required for long-term maintenance of normal and leukemic human stem/progenitor cells. *Blood* 2007;110:2880-2888.

13. Bhatia R, Van HK, Palmer A et al. Longitudinal assessment of hematopoietic abnormalities after autologous hematopoietic cell transplantation for lymphoma. *J.Clin.Oncol.* 2005;23:6699-6711.
14. Ju Z, Jiang H, Jaworski M et al. Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and engraftment. *Nat.Med.* 2007;13:742-747.
15. Rossi DJ, Bryder D, Zahn JM et al. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc.Natl.Acad.Sci.U.S.A* 2005;102:9194-9199.
16. Rossi DJ, Bryder D, Seita J et al. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature* 2007;447:725-729.
17. Kimura T, Asada R, Wang J et al. Identification of long-term repopulating potential of human cord blood-derived CD34-flt3- severe combined immunodeficiency-repopulating cells by intra-bone marrow injection. *Stem Cells* 2007;25:1348-1355.
18. Li Q, Hisha H, Yasumizu R et al. Analyses of very early hemopoietic regeneration after bone marrow transplantation: comparison of intravenous and intrabone marrow routes. *Stem Cells* 2007;25:1186-1194.
19. Castello S, Podesta M, Menditto VG et al. Intra-bone marrow injection of bone marrow and cord blood cells: an alternative way of transplantation associated with a higher seeding efficiency. *Exp.Hematol.* 2004;32:782-787.
20. Frassoni F, Gualandi F, Podesta M et al. Direct intrabone transplant of unrelated cord-blood cells in acute leukaemia: a phase I/II study. *Lancet Oncol.* 2008;9:831-839.

Bone marrow 10 years post-ASCT



**Supplemental Figure** Progenitor FACS analysis of bone marrow of a patient ten years post-ASCT demonstrating a high percentage of phenotypically defined GMP cells as seen in the patients 6-9 months post-ASCT.

