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Chemotherapy prior to autologous bone marrow transplantation impairs long-term engraftment in mice

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Objective. **Autologous bone marrow transplantation in cancer patients is often preceded by multiple cycles of chemotherapy. In this study, we assessed in a mouse model whether stem cells were affected by prior chemotherapy.**

Methods. **Donor mice were treated with three consecutive injections of 150 mg/kg 5-fluorouracil (5-FU). Peripheral blood counts were allowed to recover before the subsequent dose of 5-FU was given. Mice recovered from three doses of 5-FU and showed normal steady-state hematopoiesis. Bone marrow cells from these mice were mixed with congenic competitor cells and transplanted into lethally irradiated recipients.**

Results. **Although in vivo homing of cells from these mice was not impaired, donor leukocyte contribution steadily decreased posttransplantation. In contrast to in vivo homing, both in vitro migration toward stromal-derived factor (SDF)-1 and the average CXC chemokine receptor-4 (CXCR4) expression were lower in 5-FU–treated cells. Moderate reductions in L-selectin and CD11a expression were observed on stem cells of 5-FU–treated mice. CD43, CD44, CD49d, and CD49e were normally expressed and could thus not explain the reduced engraftment of these cells.**

Conclusion. **We therefore conclude that 5-FU either directly damages stem cells or that the replicative stress induced by 5-FU causes a decline in stem cell reconstitution potential resulting in lower chimerism levels posttransplantation, that declines in time.** **© 2003 International Society for Experimental Hematology. Published by Elsevier Inc.**

Autologous bone marrow transplantation is increasingly applied to treat hematological malignancy [1]. The rate of hematopoietic reconstitution posttransplantation strongly depends on the quality of the infused graft, which is partly determined by the severity of chemotherapy pretreatment [2–5]. It has been reported that lymphoma patients treated with intensified cycles of cyclophosphamide, doxorubicin, vincristine, and prednisone, followed by autologous bone marrow transplantation, demonstrated a poor hematopoietic reconstitution despite normal bone marrow cellularity at the time of harvest [4,6]. Reduced numbers of primitive stem cells may have caused delayed engraftment in this study [2].

Studies in mice have identified only few chemotherapeutic agents that directly damage hematopoietic stem cells [3,7–10]. The main reason for this is probably that the majority of hematopoietic stem cells is normally quiescent and consequently is no direct target for most cytotoxic drugs. A cytotoxic agent often used in murine studies of stem cell sensitivity to chemotherapy, 5-fluorouracil (5-FU), was also shown not to damage stem cells directly [11,12], but stem cells start proliferating rapidly after chemotherapy and can become very sensitive to subsequent treatments [13]. In addition, repeated courses of chemotherapy may also have an effect on the quality of stem cells, i.e., the inherent potential of individual stem cells to contribute to the process of hematopoiesis [14–16].

The mechanisms causing the reduced repopulation potential of bone marrow grafts exposed to chemotherapy have remained obscure so far. In theory, several not mutually exclusive models can be proposed, in which chemotherapy might affect the quantity or the "quality" of transplantable stem cells [17]. Replicative stress imposed upon stem cells by multiple rounds of chemotherapy could potentially induce premature exhaustion, leading to reduced proliferative potential. Alternatively, the quality of transplanted stem cells may be impaired as a consequence of a reduced ability

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of chemotherapy-treated stem cells to migrate through the bone marrow endothelium and home successfully to the bone marrow microenvironment of the conditioned recipient. Various adhesion molecules belonging to the families of integrins (very late antigen [VLA]-4 and -5), selectins (L-selectin), and cadherins play important roles in migration, homing, and engraftment of hematopoietic stem cells [18–21]. It has long been recognized that efficient homing is confined to cells in G_0-phase [19,22,23], which might be associated with differences in adhesion molecule expression [24]. Actively dividing cells have fewer cells in G_0-phase and subsequently may have a lower homing efficiency. Therefore, multiple cycles of chemotherapy may not only lead to distortion in migration characteristics of stem cells, but may also cause them to cycle more actively, resulting in a homing deficiency.

In the present study we assessed the engraftment potential of bone marrow cells that were repeatedly exposed to 5-FU. Quantitative and qualitative stem cell characteristics, such as stem cell number, number of cells in S-phase, adhesion molecule expression, migration capacity, and in vivo homing of cells of the stem cell graft were determined. Our results demonstrate that cells repeatedly exposed to chemotherapy have a diminished repopulating capacity posttransplantation.

Materials and methods

Mice and treatment

Female C57BL/6 mice (C57BL/6JOlaHsd Pep3^a (CD45.2))(Harlan Nederland, Horst, The Netherlands), were used as control and chemotherapy-treated donors, and as bone marrow transplantation recipients. In competitive repopulation assays [25], 2 to 3×10^6 cells from untreated B6.SJL (C57BL/6.SJL.Ptprc^a Pep3^b/BoyJ) congenic mice (kindly provided by Prof. Dr. Willem E. Fibbe, Leiden University Medical Center, Leiden, The Netherlands) were mixed with 1.5 to 3×10^6 cells of 5-FU–treated or untreated C57BL/6 cells, and transplanted into lethally irradiated recipients. The animals were housed in conventional clean facilities certified to be free of pathogens and fed commercial rodent chow and acidified water ad libitum. All experimental protocols were approved by the institutional ethical committee on animal experiments.

C57BL/6 mice between 8 and 12 weeks of age were treated with three consecutive cycles of chemotherapy, each consisting of a single intraperitoneal (i.p.) injection of 5-FU, (fluracedyl; Pharmachemie B.V., Haarlem, The Netherlands), at a dose of 150 mg/kg. Mice were monitored regularly by white blood cell counting and occasional bone marrow sampling under isoflurane anesthesia. The second and third treatments were administered after full recovery of peripheral white blood cell counts (i.e., 16 days after the first and 17 days after the second injection). Following full recovery from the third 5-FU dose, bone marrow cells were collected for analysis of progenitor and stem cell numbers, progenitor cell cycling activity, and competitive repopulation. Recipient C57BL/6 mice received a single dose of 9 Gy lethal total-body irradiation (TBI) at a dose rate of 0.772 Gy/minute, using a ^{137}Cs γ-irradiation unit (IBL 637, CIS Biointernational, Gif-sur-Yvette Cedex, France).

Bone marrow transplantation and determination of peripheral blood cell chimerism

To determine the engraftment potential of control bone marrow cells and of 5-FU–treated cells, a competitive repopulation assay was performed. To this end, 1.5 to 3×10^6 unfractionated bone marrow cells obtained from either CD45.2 control mice or CD45.2 5-FU–treated mice were mixed with 2 to 3×10^6 unfractionated reference bone marrow cells of CD45.1 donor mice 24 hours. Cells were mixed immediately prior to intravenous injection into the retro-orbital plexus of 900-cGy irradiated female CD45.2 recipient mice. Peripheral blood cell chimerism (CD45 isotype expression) in recipients was determined by flow cytometry (anti-CD45.1 and anti-CD45.2 mAbs from Pharmingen, San Diego, CA, USA), at first every 2 weeks (up to week 12), and thereafter every 4 weeks until week 26. The competitive repopulation index (CRI) was calculated as follows: CRI = Ratio of CD45.2 to CD45.1 leukocytes in recipient/Ratio CD45.2 to CD45.1 marrow cells transplanted. The ratio of competitive repopulation indexes for transplantation of normal control marrow and marrow from mice treated with 5-FU was calculated as a measure for the relative quality of the two different marrow grafts.

Stem cell and progenitor cell assays

To quantify the number of progenitors and stem cells transplanted in each group, the in vitro limiting dilution cobblestone area–forming cell (CAFC) assay [26,27] was performed as described previously [28]. In addition, the number of colony-forming units–granulocyte/macrophage (CFU-GM) was determined using standard methylcellulose cultures (0.8% methylcellulose, 30% fetal calf serum [FCS] in α-medium) supplemented with 10 ng/mL recombinant mouse granulocyte-macrophage colony-stimulating factor (GM-CSF) (Behringwerke, Marburg, Germany) and 100 ng/mL recombinant rat pegylated stem cell factor (peg-SCF) (Amgen, Thousand Oaks, CA, USA). Cells were cultured at 37°C and 5% CO_2 and counted on day 6 or 7 using an inverted microscope.

The proliferative activity of progenitor and stem cells was assessed by a 1-hour in vitro incubation at 37°C with hydroxyurea [HU] (Sigma, St. Louis, MO, USA, 200 μg/mL) prior to plating cells in the CAFC assay as previously reported [28]. The fraction of cells killed by HU was calculated and was considered to reflect the percentage of cells in S-phase.

Migration assays

The motility of stem cells in response to a chemotactic gradient was investigated with an in vitro actin polymerization assay [29]. Cells were diluted in α-medium to a concentration of 2.5×10^6 cells/mL. SDF-1 (100 ng/mL; R&D Systems Europe Ltd, Abingdon, UK) was added to the cell suspension and incubated for 0, 10, 30, and 120 seconds at 37°C. The reaction was stopped by adding 200 μL cold fixation solution (6% paraformaldehyde and 0.2 mg/mL sonificated L-α-lysophosphatidylcholine [Sigma] in phosphate-buffered saline [PBS]), followed by 10 minutes incubation at room temperature. Thereafter, 4 μL FITC-phalloidin (Oregon Green 514-Phalloidin; Molecular Probes, Europe BU, Leiden, The Netherlands) was added to visualize F-actin polymerization, incubated for 30 minutes at room temperature in the dark, and analyzed by flow cytometry.

A functional migration assay was performed using a transwell system in which cells migrate towards an SDF-1 gradient [30]. 7.5×10^6 bone marrow cells of control or chemotherapy-treated mice were suspended in IMDM medium +5% FCS and seeded in

triplicate in the upper chambers of 6-well transwell plates (pore size: 3 μm; Corning Incorporated, Corning, NY, USA). The lower chambers contained IMDM medium +5% FCS, with or without 100 ng/mL murine SDF-1. Cells were collected after incubation for 16 hours at 37°C, 5% CO_2 from both the upper and lower chamber. Triplicate wells were pooled, nucleated cells were counted, and CAFC assays were initiated. The number of cells migrating specifically toward SDF-1 was calculated.

Adhesion molecule and CXCR4 expression

Bone marrow cells were isolated from 5-FU–treated and control mice for stem cell purification. Unfractionated cells were incubated with normal rat serum for 15 hours after erythrocyte lysis. Subsequently, cells were stained with a panel of biotinylated lineage-specific antibodies (lineage panel: anti-CD3e, anti-CD45R, anti-CD11b, anti-Ly76, anti-Gr-1, Pharmingen), FITC-anti-Sca-1, APC-anti-c-kit (Pharmingen), and one of the following PE-labeled antibodies directed against different adhesion molecules: anti-CD49d, anti-CD49e, anti-CD11A, anti CD62L, anti-CD43, or anti-CD44 (Pharmingen). Cells were stained with streptavidin-PerCP (Pharmingen). Primitive stem cells (Lin^- $Sca\text{-}1^+c\text{-}kit^+$) were defined as the 5% least intense PerCP-fluorescent bone marrow cells (lineage-negative) and Sca-1- and c-kit-positive cells in this fraction.

CXCR4 expression was determined using a rabbit-anti-mouse anti-CXCR4 antibody (a generous gift from Dr. J.C. Guttierez-Ramos, Millennium Pharmaceuticals, Cambridge, MA, USA). Staining was visualized with a secondary PE-conjugated goat-anti-rabbit antibody. CXCR4 staining was combined with PerCP-labeled lineage markers, FITC-anti-Sca-1, APC-anti-c-kit. Adhesion molecule and CXCR4 expression was measured in total cell population as well as in the Lin^- and $Lin^-Sca\text{-}1^+c\text{-}kit^+$ fraction by flow cytometry.

In vivo homing of bone marrow cells

To determine in vivo homing potential of chemotherapy-treated or untreated bone marrow cells, approximately 40×10^6 unfractionated bone marrow cells were intravenously injected into lethally irradiated recipients. Twenty-four hours later, recipient bone marrow cells were isolated and seeded in a CAFC assay. To quantify the number of endogenous stem cells surviving radiation, bone marrow cells of lethally irradiated, nontransplanted recipients were assayed in a separate CAFC assay. CAFC-day 7 and CAFC-day 35 were monitored and percent recovery in bone marrow (% of input cells) was calculated.

Statistics

Unpaired Student's *t*-tests were performed to determine significant differences between engraftment levels of 5-FU–treated mice and untreated controls. Ninety-five percent confidence limits (CL) were calculated in each CAFC subset. Nonoverlapping 95% CL were interpreted as $p < 0.05$.

Results

Peripheral blood cell values and bone marrow cellularity after chemotherapy

Mice were treated with three consecutive courses of 5-FU and white blood cell counts (WBC) were determined regularly after each injection to ascertain full recovery of peripheral blood cell values before the next dose of chemotherapy was administered. Figure 1 demonstrates that WBC values had normalized between 16 and 18 days after each 5-FU treatment. To verify whether recovery of leukocyte values reflected normalization of bone marrow cellularity, nucleated cells in bone marrow were monitored at distinct time points (Fig. 1). The number of nucleated cells in the bone marrow was also normalized when WBC values had recovered. We did not observe any signs of accumulating toxicity of repeated administration of the cytotoxic drug.

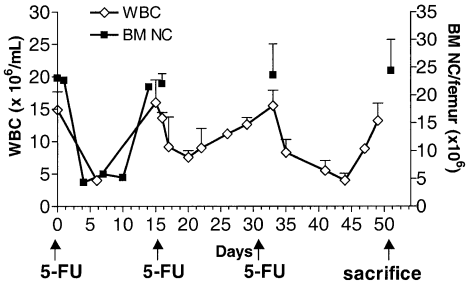

Figure 1. Recovery of hematopoiesis after three doses of 5-FU. The fluctuation in white blood cell (WBC) counts (left axis) and bone marrow cellularity (right axis) after three injections of 5-FU (150 mg/kg). 5-FU was administered at day 0, day 16, and day 33, after full recovery of WBC values had been achieved.

Donor cell engraftment after transplantation

To detect potential engraftment defects of stem cells harvested from 5-FU–treated mice, we performed a competitive repopulation assay. Engraftment of CD45.2 cells and CD45.1 cells was determined by fluorescein-activated cell sorting (FACS) analysis (Fig. 2, top panel). 5-FU–treated stem cells

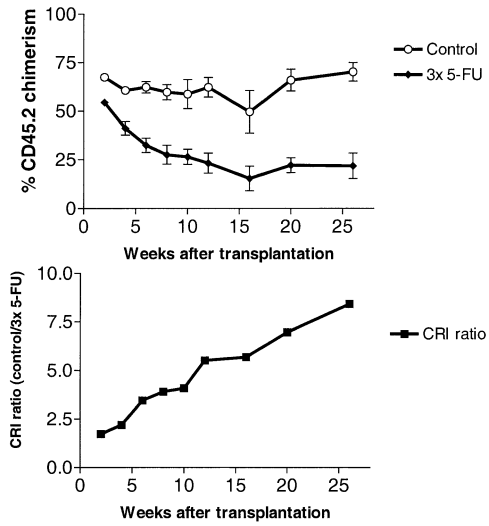

Figure 2. Long-term repopulating ability of stem cells from 5-FU-pretreated donors. Percentage donor-derived (CD45.2) leukocytes in peripheral blood of lethally irradiated recipient mice after transplantation of 1.8×10^6 control cells or 5-FU–treated cells, mixed with 2.4×10^6 congenic (CD45.1) bone marrow cells. Data are mean ± SEM for four mice (top panel). Competitive repopulation indexes (CRI) of two separate experiments (a total of 6 mice per group) were calculated as explained in materials and methods and the ratio of CRI for control vs 5-FU treated cells is plotted in bottom panel.

showed a lower percentage of chimerism than untreated cells as early as 2 weeks after transplantation ($p < 0.05$). In addition, the difference between engraftment levels of control stem cells compared to 5-FU–treated stem cells increased slowly over time ($p < 0.0001$). When competitive repopulation indexes were calculated, the same pattern was observed. Twenty-six weeks posttransplantation an eightfold difference in the competitive repopulation index between control stem cells compared to 5-FU–treated stem cells was found (Fig. 2, bottom panel). These data clearly indicate a severe engraftment defect of chemotherapy-treated stem cells.

Number and cell cycle status of transplanted cells

To test whether equal numbers of stem cells in both the control group and the chemotherapy-treated group had been transplanted, we performed CAFC assays on both bone marrow sources. Figure 3 demonstrates that bone marrow of mice sacrificed 18 days after third 5-FU injection contained equal numbers of progenitor (CAFC-day 7, Fig. 3A) and stem cells (CAFC-day 35, Fig. 3B) as bone marrow of untreated mice ($p > 0.05$). Untreated CD45.2 bone marrow cells contained 71 CAFC-day 35 per million cells (95% confidence interval (c.i.): 52–98), whereas 5-FU–treated bone marrow cells contained 58 CAFC-day 35 (c.i.: 42–80), which was not significantly different and could not explain the difference in engraftment.

To investigate whether an increased cell cycle activity of 5-FU–treated stem cells could be responsible for decreased engraftment posttransplantation, we compared the fraction of cells in S-phase in control and treated progenitor and stem cell populations prior to transplantation. We did not find any evidence for enhanced cell cycle activity of either progenitors or stem cells in chemotherapy-treated mice (Fig. 3). In fact, the fraction of cells in S-phase in these mice appeared to be reduced from 49% to 12% for CAFC-7 and from 7.5% to less than 2% for CAFC-35. Thus, the engraftment defect posttransplantation could not be explained by changes in cell cycle activity.

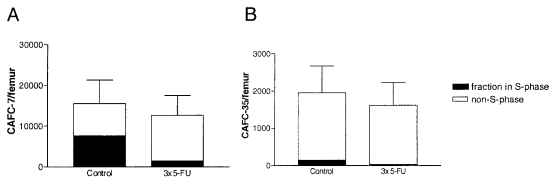

Figure 3. Effect of 5-FU treatment on the number and proliferative status of progenitors and stem cells. **Panel A** shows the absolute number of CAFC-day 7 (+95% confidence limits) per femur in mice treated with 3 doses of 5-FU compared to control values. Assays were performed 18 days after the third course of 5-FU or saline (control). The black bar depicts the percentage of CAFC-day 7 in S-phase. **Panel B** shows the absolute number of CAFC-day 35 (+95% confidence limits) per femur and percentage in S-phase (black bars) in pooled bone marrow of 5-FU–treated mice or control mice.

Migration and homing

To investigate possible effects of 5-FU treatment on the homing potential of stem cells, we performed an actin polymerization assay to determine whether motility of 5-FU–treated cells as response to a chemotactic stimulus was affected. Figure 4A demonstrates lower but not significantly different actin polymerization of chemotherapy-treated vs untreated cells, indicating a normal coordinated formation of lamellipodia and actomyosin-based contractility necessary for cell movement [31,32]. A functional in vitro migration assay was performed to obtain insight into the migration capacity of 5-FU–treated and control cells towards an SDF-1 gradient. Figure 4B demonstrates that the total number of 5-FU–treated nucleated cells which migrated in the transwell assay was reduced to ~50% compared to untreated control cells ($p = 0.05$). The fraction of migrating 5-FU–treated progenitors (CAFC-day 7) was significantly reduced to 10% of control progenitors (Fig. 4C, $p = 0.01$). Migration of CAFC-day 35 could not be detected due to the low frequency of this stem cell subset.

It was further investigated whether reduced expression of CXCR-4 could explain this reduced migration (Fig. 4D).

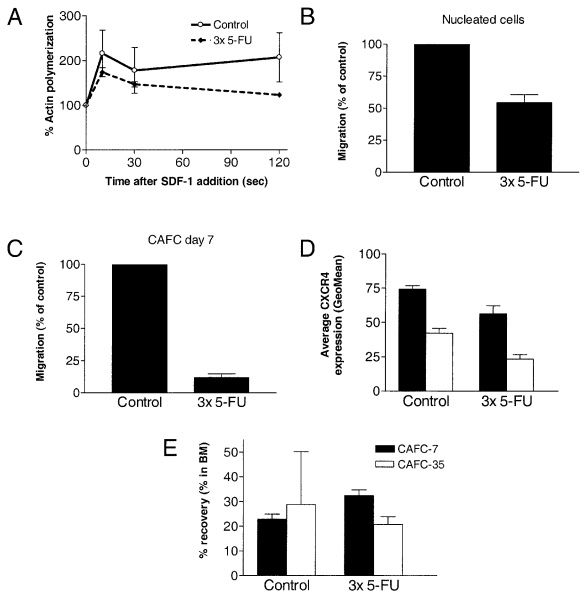

Figure 4. Effect of 5-FU treatment on in vitro and in vivo migratory capacity. SDF-1-induced actin polymerization ± SEM in bone marrow of 5-FU-exposed mice is compared with control values **(panel A)**. SDF-1-induced migration in a transwell assay is shown in **panel B** for all bone marrow nucleated cells and in **panel C** for CAFC-day 7. 5-FU-exposed mice and control mice are compared. Data are mean ± SEM. **Panel D** depicts the CXCR-4 expression (expressed as mean fluorescence (GeoMean)) in bone marrow nucleated cells (black bars) and Lin^- cells (white bars) of 5-FU-exposed mice and control mice. **Panel E**: in vivo homing of 5-FU–treated and control bone marrow cells. Percentage of recovered CAFCd7 (black bars) and CAFCd35 (white bars) in total bone marrow 24 hours after transplantation of bone marrow cells into lethally irradiated recipients assuming that one femur represents $1/17^{th}$ of the total bone marrow. Data are mean ± SEM.

CXCR4 expression was reduced in unfractionated ($p = 0.02$), the Lin^- fraction ($p = 0.003$) and the most primitive $Lin^-Sca\text{-}1^+c\text{-}kit^+$ fraction ($p = 0.16$) of 5-FU–treated bone marrow cells.

Various adhesion molecules such as β-integrins (VLA-4/5) and selectins (L-selectin) are known to be involved in the migration, homing, and engraftment potential of hematopoietic stem cells. Therefore, we compared the expression of 6 adhesion molecules, CD49d, CD49e, CD11a, CD62L, CD43, and CD44 in the $Lin^-Sca\text{-}1^+c\text{-}kit^+$ cells of 5-FU–treated and untreated mice. Table 1 demonstrates a significantly lower expression of CD11a (LFA-1) and CD62L (L-selectin) in the 5-FU–treated cells whereas the expression of other adhesion molecules was not affected. Finally, to test whether in vivo homing of stem cells was affected, we investigated the homing potential of treated or untreated cells. Thirty-five to 40×10^6 cells were transplanted into lethally irradiated recipients. After 24 hours, bone marrow cells were isolated and stem cells were quantified in a CAFC assay. Figure 4E demonstrates a significantly higher retrieval of transplanted progenitors (CAFC-day 7) from 5-FU–treated animals compared with untreated progenitors (32% vs 23%, n = 3, $p < 0.05$), but homing of stem cells (CAFC-day 35) was not significantly different between both groups.

Discussion

In the present study we have subjected mouse bone marrow cells to repeated administration of 5-FU. We failed to document any deleterious effect of this treatment on the hematopoietic system in the treated animals. Complete recovery of white blood cell counts, cellularity of bone marrow, and number of in vitro–cultured progenitors and stem cells was achieved already at 18 days after the last of three consecutive administrations of 5-FU. Our failure to detect stem cell damage is very likely to be highly dependent on the timing of the administration of 5-FU. When consecutive doses of 5-FU are administered within short time intervals, stem cells are stimulated to proliferate rapidly in order to restore the hematopoietic system and will be more susceptible for a subsequent dose of chemotherapy [13]. In the current study we deliberately postponed subsequent 5-FU treatment until complete hematopoietic recovery had occurred on the basis of blood and bone marrow cellularity. In fact, our data suggested that the hematopoietic system of 5-FU–treated mice had completely recovered because not only peripheral blood and marrow counts but also bone marrow progenitor and stem cell numbers were normal. However, upon transplantation of these bone marrow cells in a competitive repopulation assay, a dramatic and permanent engraftment defect was observed. Whereas engraftment levels in recipients reconstituted with control bone marrow cells remained stable at ~60% during the entire study period, engraftment gradually decreased in recipients transplanted with 5-FU–treated bone marrow, finally resulting in a threefold reduction in levels of chimerism. Comparison of the competitive repopulation index between 5-FU–treated and control bone marrow cells indicated an eightfold difference in stem cell functionality. Clearly, the deleterious effect of repeated chemotherapy administration on stem cells only became apparent upon transplantation in a competitive repopulation assay. These observations point to a significant reduction in stem cell quality due to repeated exposure to 5-FU. It should be noted that in autologous bone marrow transplantation after induction chemotherapy in cancer patients the chemotherapy-treated cells are the only source of stem cells. Clinically, a functional defect can only be detected when the number of transplanted stem cells becomes limited [2,4,6]. In stem cell transplantation, engraftment of stem cells depends on the ability of transplanted stem cells to migrate to the recipient bone marrow environment and lodge to appropriate niches, and on the extent that newly seeded donor stem cells contribute to short- and long-term reconstitution of the host hematopoietic system. Although these two parameters are mechanistically distinct, together they define the "quality" of a stem cell graft. While the exact experimental design was somewhat different, several studies have shown that competitive repopulation of stem cells obtained from mice treated with specific cytotoxic drugs was impaired [16,23, 33,34]. Exhaustion of stem cells can be demonstrated by serial transplantation [35] or prolonged growth factor administration in vivo [16,36], which only becomes apparent when stem cells are transplanted in a competitive repopulation assay.

Table 1. Adhesion molecule expression in $Lin^-Sca\text{-}1^+c\text{-}kit^+$ cells

Adhesion molecule: Common name:	CD49d VLA-4	CD49e VLA-5	CD11a LFA-1	CD62L L-selectin	CD43 Leukosialin	CD44 HCAM
Untreated cells	99.8 ± 0.2	96.9 ± 1.0	93.6 ± 1.3 *	70.7 ± 5.5 †	99.4 ± 0.3	99.9 ± 0.2
5-FU-treated cells	99.9 ± 0.1	97.0 ± 2.4	90.1 ± 2.8	53.3 ± 8.0	99.5 ± 0.6	99.9 ± 0.2

Individual expression of adhesion molecules was determined by flow cytometry as described.

Data represent the mean percentage of positive $Lin^-Sca\text{-}1^+c\text{-}kit^+$ cells ± SD (for each adhesion molecule: n = 4 untreated group and n = 6 in 5-FU-treated group).

Unpaired two-tailed *t*-test shows a significant difference in expression between treated and untreated cells of $^*p < 0.05$ and $^\dagger p < 0.01$ in LFA-1 and L-selectin expression, respectively.

To investigate whether 5-FU treatment alters qualitative stem cell characteristics, we studied parameters such as percentage of cells in S-phase, adhesion molecule expression, in vitro migration, and in vivo homing capacity. From previous studies it is known that cells in S-phase have a low seeding and homing efficiency upon transplantation [19,22,23]. We show that after repeated 5-FU administration the number of cells in S-phase had not increased. Hence, this cannot explain reduced engraftment posttransplantation.

The transendothelial migration of hematopoietic progenitor cells induced by the chemoattractant SDF-1 involves motility-associated processes such as shape changes in hematopoietic cells through actin polymerization and changes in adhesion molecule expression [18,30,37–39]. Our data demonstrate that, although actin polymerization is normal in 5-FU–treated cells, migration capacity in vitro assayed in a transwell assay is severely impaired. The total numbers of nucleated cells as well as progenitors show a significantly reduced migration towards SDF-1. In addition, the level of CXCR4 expression was lower on 5-FU–treated cells. However, this did not lead to a reduction in the homing capacity of these cells. This observation does not correspond with previous data [40] where human stem cell engraftment in NOD/SCID mice was shown to be completely dependent on the expression of the receptor for the chemokine SDF-1, CXCR4. These data constitute the first report showing no correlation in murine bone marrow transplantation between CXCR4 expression or in vitro SDF-1-induced migration with in vivo homing. However, rapid oscillations in CXCR4 expression and upregulation upon transplantation have been reported and could explain discrepancies between in vitro and in vivo CXCR4 function [41]. Furthermore, we assessed that expression of CD62L (L-selectin) and CD11a (LFA-1) in the chemotherapy-treated cells was significantly reduced compared to expression in untreated cells. In our model a reduction in expression of these adhesion molecules after repeated cycles of chemotherapy does not seem to affect homing. Therefore, changes in adhesion molecule expression cannot explain the engraftment defect following transplantation of chemotherapy-treated cells.

The data demonstrate that stem cell numbers and their homing capacity were not affected by prior chemotherapy. Thus, residual damage from 5-FU exposure or indirectly through replicative stress, induced by repeated cycles of chemotherapy, causes premature exhaustion and leads to a severe reduction in stem cell quality rather than quantity.

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