

In Vivo Effects of Myeloablative Alkylator Therapy on Survival and Differentiation of MGMT^{P140K}-Transduced Human G-CSF-Mobilized Peripheral Blood Cells

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High-intensity alkylator-based chemotherapy is required to eradicate tumors expressing high levels of O⁶-methylguanine DNA methyltransferase (MGMT). This treatment, however, can lead to life-threatening myelosuppression. We investigated a gene therapy strategy to protect human granulocyte colony-stimulating factor-mobilized peripheral blood CD34⁺ cells (MPB) from a high-intensity alkylator-based regimen. We transduced MPB with an oncoretroviral vector that coexpresses MGMT^{P140K} and the enhanced green fluorescent protein (EGFP) (*n* = 5 donors). At 4 weeks posttransplantation into nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice, cohorts were not treated or were treated with low- or high-intensity alkylating chemotherapy. In the high-intensity-treated cohort, it was necessary to infuse NOD/SCID bone marrow (BM) to alleviate hematopoietic toxicity. At 8 weeks posttreatment, human CD45⁺ cells in the BM of mice treated with either regimen were EGFP⁺ and contained MGMT-specific DNA repair activity. In cohorts receiving low-intensity therapy, both primitive and mature hematopoietic cells were present in the BM. Although B-lymphoid and myeloid cells were resistant to *in vivo* drug treatment in cohorts that received high-intensity therapy, no human CD34⁺ cells or B-cell precursors were detected. These data suggest that improved strategies to optimize repair of DNA damage in primitive human hematopoietic cells are needed when using high-intensity anti-cancer therapy.

Key Words: gene therapy, hematopoietic stem cell, NOD/SCID mice, SCID-repopulating cell, O⁶-methylguanine DNA methyltransferase, O⁶-benzylguanine, BCNU, G-CSF-mobilized peripheral blood

INTRODUCTION

Maintenance of genome stability in hematopoietic stem and progenitor cells (HSC) is essential for normal blood cell development. Survival of HSC and their progeny can be severely compromised during exposure to DNA-damaging drugs used in anti-cancer therapy due to low levels of endogenous DNA repair activity [1–3]. In terms of anti-cancer therapy, generation of HSC that efficiently repair DNA damage due to chemotherapy may protect patients from life-threatening cytopenias commonly observed following dose-intensified therapy. In a recent phase II clinical trial, patients with nitrosourea-resistant gliomas were simultaneously treated with O⁶-benzylguanine (6BG) to deplete the DNA repair protein O⁶-

methylguanine DNA methyltransferase (MGMT) and the DNA damaging agent 1, 3-bis (2-chloroethyl)-1-nitrosourea (BCNU) [4]. Although lack of tumor progression was transiently observed in some patients, effective dose-escalation therapy could not be achieved due to severe hematopoietic toxicity. These studies provide clinical proof that strategies protecting HSC during dose-intensified therapy are indeed clearly needed in relapsed patients requiring high-dose alkylator therapy.

Numerous transplant studies have convincingly proven that murine stem cells could be selected *in vivo* with 6BG/BCNU, 6BG/temozolomide, or 6BG/CCNU [5–21]. In addition, studies performed by Neff *et al.* [11,22] used dose-escalation regimens in a canine transplant model

and demonstrated selection of MGMT^{P140K}-expressing cells over time with no signs of hematotoxicity or overt multiorgan toxicity reported. We previously used the severe combined immunodeficient (SCID)-repopulating cell (SRC) assay to investigate the extent to which human SRCs and their progeny were selected *in vivo* from submyeloablative doses of 6BG and BCNU [7]. Human HSC derived from umbilical cord blood (UCB) or granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood (MPB) that expressed MGMT^{P140K} could be selected *in vivo* by nonmyeloablative doses of 6BG and BCNU. Gerson and colleagues also reported similar results using MGMT^{P140K}-transduced UCB in the non-obese diabetic (NOD)/SCID xenograft model [9].

Treatment of nitrosourea-resistant cancers will likely require high doses of chemotherapy in the myeloablative range necessitating a stem-cell transplant. In addition, efficient and prolonged depletion of wild-type MGMT (wtMGMT) by agents such as 6BG will be necessary so that tumor DNA damage as a consequence of treatment with alkylating agents can be optimally accomplished [4,23,24]. We and others previously demonstrated simultaneous protection of murine stem cells with mutant MGMT protein and significant lack of disease progression of nitrosourea-resistant tumors in NOD/SCID mice treated with 6BG/BCNU [13,25]. An aggressive myeloablative 6BG/BCNU regimen was used in our study. Kreklau *et al.* [26,27] demonstrated that wtMGMT begins to regenerate within hours after delivery of 6BG; therefore, one dose may not allow time for sufficient DNA crosslinks to be generated prior to *de novo* synthesis of wtMGMT. They also found that administration of two boluses of 6BG 8 h apart led to a prolonged depletion of wtMGMT in a human glioma (SF767) that expresses high levels of wtMGMT [26,27]. Our group tested a myeloablative dosing schema that consisted of two boluses of 6BG delivered 8 h apart combined with one dose of BCNU delivered 1 h after the first bolus of 6BG. NOD/SCID mice were engrafted with a human glioma that expresses high levels of wtMGMT (SF767). Following transplant with murine bone marrow (BM) expressing MGMT^{P140K}, the mice received two cycles of the 6BG double-bolus regimen over a 2-week period. A significant reduction in the growth of the engrafted glioma was observed [13].

The extent to which human HSC can be protected *in vivo* by MGMT^{P140K} during delivery of high doses of alkylator therapy that kills cancer cells has not been investigated. In this report, we compare the outcome of administering a low-dose 6BG/BCNU regimen versus a high-dose regimen to NOD/SCID mice transplanted with MGMT^{P140K}-transduced MPB. The *in vivo* model used here provides the most stringent test of human HSC protection and self-renewal capacity tested to date *in vivo*. We found that low numbers of human MPB cells were protected following delivery of the myeloablative regimen and that these cells were limited to mature

lymphoid and myeloid cells. This model system can now be used to optimize protection of human HSC during high-intensity alkylator therapy.

RESULTS AND DISCUSSION

Administration of high-dose alkylator-based therapy that is myeloablative at initial diagnosis or immediately following cancer relapse may be the most effective means of eradicating drug-resistant tumors that express high levels of wtMGMT. In the current study we used the NOD/SCID xenograft model to evaluate the impact of high-dose alkylator therapy on human hematopoiesis. We used MPB CD34⁺ cells since this is the HSC source utilized in human gene therapy trials focused on preventing myelosuppression in cancer patients undergoing high-dose therapy [28,29].

Transduction Efficiency of Human CD34⁺ Cells Prior to Transplantation

We stimulated human CD34⁺ cells isolated from MPB for 48 h with a cytokine cocktail consisting of G-CSF, stem cell factor (SCF), and thrombopoietin (TPO) and transduced them with a gibbon ape leukemia virus (GALV)-pseudotyped oncoretroviral vector that coexpresses MGMT^{P140K} and enhanced green fluorescent protein (EGFP) (SF1-P140K). We performed five independent transplant experiments (MPB 1–5) using CD34⁺ MPB cells isolated from five normal donors. EGFP expression was used to determine the transduction efficiency of the MPB prior to transplantation. The transduction efficiency was $35.8 \pm 11\%$ (mean \pm standard deviation) for CD34⁺ cells and $45.2 \pm 8\%$ for committed progenitor cells (for details see supplemental information online).

Development of a Murine BM support Model to Evaluate the Impact of High-Dose 6BG/BCNU Chemotherapy on Human Hematopoiesis

Our primary objective was to evaluate to what extent human MPB CD34⁺ cells transduced with the SF1-P140K vector were protected from a low-intensity regimen consisting of 6BG and BCNU that is in the submyeloablative range (Fig. 1A., see low dose) versus a high-intensity regimen that is myeloablative (Fig. 1A, see high dose). The low-dose regimen served as an excellent control with which to compare the impact of the high-dose regimen on human hematopoiesis since we had shown previously that this regimen led to selection of human clonogenic cells in the NOD/SCID xenograft model [7]. In pilot studies we initially transplanted NOD/SCID mice with SF1-P140K-transduced human CD34⁺ cells and delivered two cycles of either low-dose or high-dose therapy commencing at 4 weeks posttransplant. Transplanted mice receiving the high-dose therapy died of severe pancytopenia within 7–10 days posttreatment, although transplanted mice treated with low-dose

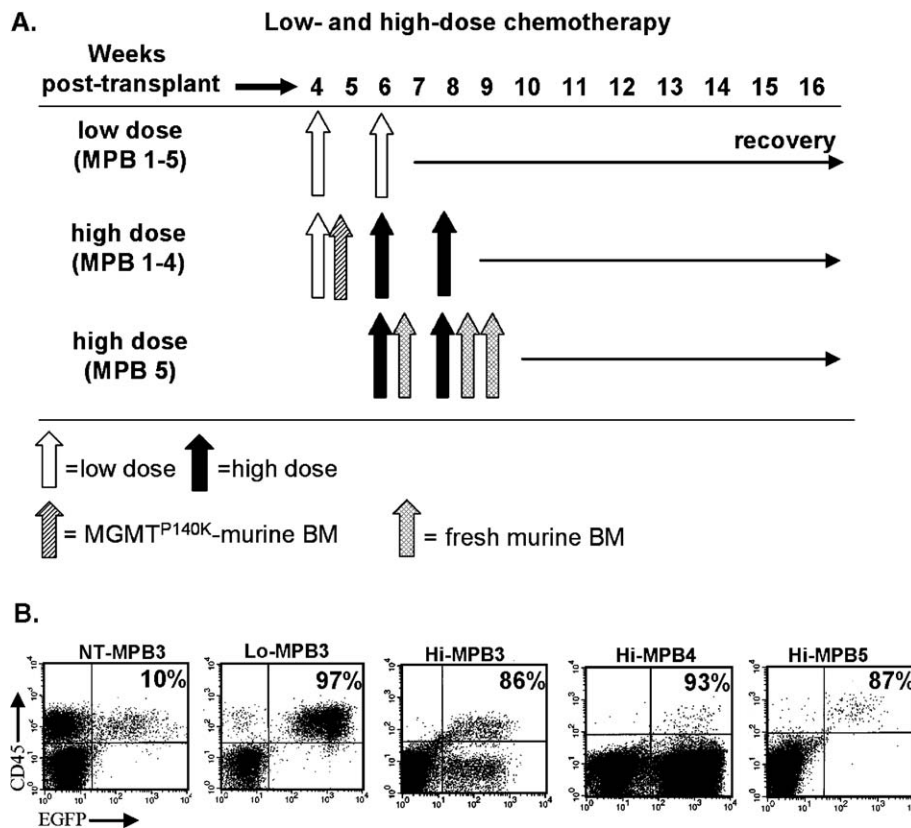


FIG. 1. *In vivo* resistance of SF1-P140K-transduced MPB cells to low- or high-dose 6BG/BCNU. (A) Human MPB CD34⁺ cells were transduced with the GALV-pseudotyped SF1-P140K oncoretroviral vector and transplanted into NOD/SCID mice. At 4 weeks posttransplant, chemotherapy was administered. In the low-dose regimen, two cycles of 20 mg/kg 6BG followed 1 h later by 5 mg/kg BCNU were administered 2 weeks apart. For MPB 1–4, the high-dose regimen consisted of one cycle of low-dose chemotherapy followed by infusion of SF1-P140K-transduced BM obtained from NOD/SCID previously treated with 6BG/BCNU. At week 6 posttransplant, the first high-dose cycle of 30 mg/kg 6BG followed 1 h later by 10 mg/kg BCNU and 7 h later by 15 mg/kg 6BG was administered. This cycle was repeated again at 8 weeks posttransplant. For MPB 5, mice received 9×10^6 fresh BM cells 2 days after the first high-dose cycle and at 2 and 7 days after the second high-dose cycle. (B) The percentage of human CD45⁺EGFP⁺ cells in the BM was determined by flow cytometry at 16 weeks posttransplantation. Representative examples of nontreated (NT), low-dose treated (Lo), and high-dose treated (Hi-MPB 3–5) are shown. The BM support used in Hi-MPB 3 and Hi-MPB 4 was SF1-P140K NOD/SCID BM (i.e., EGFP⁺) and in Hi-MPB 5 was fresh NOD/SCID BM. The percentage in the upper right quadrant denotes the percentage of the human CD45⁺EGFP⁺ cells.

chemotherapy survived (data not shown). These data suggested that although human hematopoietic cells could repopulate the BM, they did not contribute to the survival of the animal under such genotoxic conditions as delivery of high-dose chemotherapy. Therefore, to prevent life-threatening myelosuppression, we infused *in vivo* selected SF1-P140K-NOD/SCID BM into transplanted mice undergoing high-dose chemotherapy (Fig. 1, high dose). We found that by maintaining functional murine hematopoiesis, 85–100% of the transplanted mice receiving high-dose therapy survived. This strategy was used in four experiments (MPB 1–4). In another series of pilot experiments, we determined whether alkylator-induced myelotoxicity could be prevented by infusing fresh NOD/SCID BM after delivery of each high-dose cycle. We found that by injecting fairly high doses of NOD/SCID BM cells ($\sim 9 \times 10^6$) 2 days after the first high-dose cycle and on days 2 and 7 after the second high-dose cycle, 95–100% of

the mice survived (data not shown). This approach was used in MPB 5 and gave results similar to those observed in MPB 1–4.

Assessment of Transduced Hematopoietic Cells Following Delivery of Low- or High-Dose Chemotherapy

At 8 weeks postchemotherapy, we analyzed the NOD/SCID BM for the presence of human cells. In contrast to nontreated mice, the vast majority of the human CD45⁺ cells expressed EGFP in mice treated with low- or high-dose chemotherapy (Fig. 1B). Representative examples from three of the five independent experiments are shown. As expected in mice receiving high-dose therapy, human and mouse cells that expressed EGFP were present (Fig. 1B, Hi-MPB 3 and Hi-MPB 4). Flow-cytometric analyses indicated that the EGFP⁺ cells that did not express human CD45 expressed the murine

CD45 antigen (data not shown). Since nontransduced NOD/SCID BM was used to maintain murine hematopoiesis in MPB5, no murine EGFP⁺ cells were present (Fig. 1B, Hi-MPB 5).

To evaluate the effects of low- versus high-dose therapy on human hematopoiesis, we determined the percentage of human cell engraftment (Fig. 2A, %huCD45⁺) and the percentage of transduced human cells (Fig. 2B, %huCD45⁺EGFP⁺) using samples of BM from transplanted mice. The majority of the mice survived the low- and high-dose regimens (Fig. 2). In MPB 2 and MPB 4, two mice in cohorts receiving the high-dose regimen died 5–6 days after the second cycle of chemotherapy. Analysis of BM cellularity indicated that the marrow was hypocellular. In MPB 3, one mouse in the nontreated group died at 6 weeks posttransplant and in MPB 5 one mouse in the cohort receiving the low-dose regimen died after the second cycle of chemotherapy. Consistent with previous transplant studies using MPB, human cell chimerism varied among the five MPB

donors, with the drug-treated cohorts exhibiting lower levels of human cell chimerism (Fig. 2A) [7,30]. Under both low- and high-dose regimens, the majority of the remaining human cells expressed EGFP (Fig. 2B). The statistical analyses of these data are presented in Table 1. The percentage of huCD45⁺ cells in the BM was marginally decreased in the high-dose cohort compared to the low-dose cohort ($P = 0.095$), while the percentage of huCD45⁺ cells was significantly decreased in both the low- and the high-dose cohorts compared to the NT group (both $P < 0.0001$). The percentage of huCD45⁺EGFP⁺ cells was significantly increased in all comparisons (Hi vs Low, Low vs NT, and Hi vs NT) (Table 1). The increasing numerical order for the percentage of human cells that were EGFP⁺ was NT < Low < Hi. These data indicate that although human engraftment levels decreased, the remaining cells contained the SF1-P140K vector and were resistant to *in vivo* drug treatment. In addition, we found no human cells in the spleens of drug-treated mice when engraftment was $\leq 5\%$, which was the

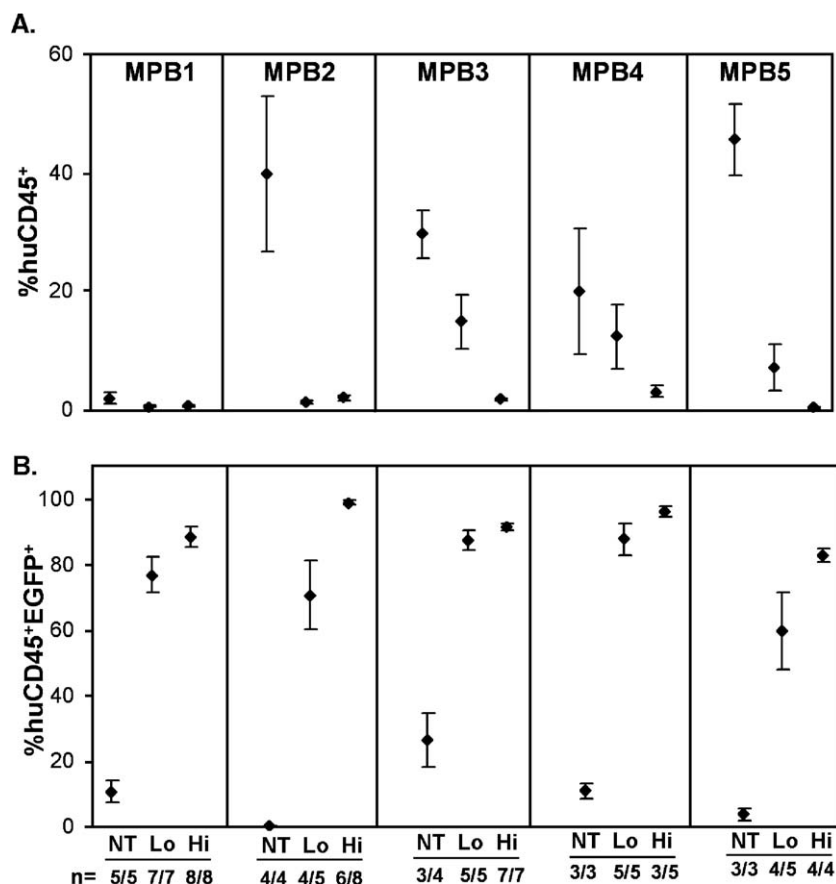


FIG. 2. Effect of low- or high-dose 6BG/BCNU on human cell engraftment and transduction efficiency. At 16 weeks posttransplantation, the BM of NOD/SCID mice was analyzed for (A) the percentage of human cell engraftment (%huCD45⁺) and (B) the percentage of human cells that are EGFP⁺ (%huCD45⁺EGFP⁺). Five independent experiments were performed (MPB 1–5). Data are presented as the means (diamonds) \pm the standard error (lines). See Table 1 for statistical significance. NT, nontreated; Lo, low-dose chemotherapy; Hi, high-dose chemotherapy; *n*, the number of mice that survived and were analyzed (numerator) versus the number of mice initially transplanted (denominator).

TABLE 1: Pair-wise treatment comparisons

Variable	Comparison	Estimate ^a	P value
% huCD45	Hi vs Low ^b	-0.052	0.095
	Low vs NT ^b	-0.187	<0.0001
	Hi vs NT ^b	-0.239	<0.0001
% huCD45 ⁺ EGFP ⁺	Hi vs Low ^b	0.143	<0.0001
	Low vs NT ^b	0.668	<0.0001
	Hi vs NT ^b	0.811	<0.0001
# EGFP ⁺ cells per femur	Hi vs Low ^b	-0.191	0.012
	Low vs NT ^b	0.130	0.120
	Hi vs NT ^b	-0.061	0.459
% huCD19	Hi vs Low ^c	0.23	0.109
	Low vs NT ^c	-1.0631	0.145
% huCD33	Hi vs NT ^c	-0.8331	0.175
	Low vs NT ^c	-0.009	0.767
	Hi vs NT ^c	0.747	0.024
% huCD34	Low vs NT ^c	0.738	0.016
	Hi vs NT ^c	-0.551	0.130
	Hi vs Low ^d	NA	<0.0001
	Hi vs NT ^d	NA	<0.0001

NT, nontreated; Low, low-dose therapy; Hi, high-dose therapy; NA, not applicable.

^a The sign of the estimate indicates the direction of the comparison between the data sets (i.e., $x > y$; positive, $x > y$, and negative, $x < y$).

^b A mixed-linear model was fit with a variance-covariance structure that incorporated correlation of multiple observations from the same donor.

^c Comparisons were analyzed as Poisson distributions of counts using the natural logarithm of total cells analyzed as the offset, which adjusts for differences between total cells analyzed in each sample. Generalized Linear Models were used to account for correlation between repeated samples from the same donor.

^d Nonparametric Wilcoxon sum rank test was utilized since all 20 values of CD34% were equal to 0 for high-dose treatment.

case for all transplanted mice that received the high-dose regimen (data not shown).

To determine if chemotherapy treatment resulted in an expansion of human transduced cells, we next determined the number of human EGFP⁺ cells per femur in the NT, Low, and Hi cohorts (Fig. 3). The increasing numerical order for the number of human EGFP⁺ cells per femur was Hi < NT < Low. However, due to variability in the number of human EGFP⁺ cells in the BM of NT cohorts, there were no significant differences between the high- and the low dose-treated mice compared to nontreated mice (both $P > 0.05$). The number of human EGFP⁺ cells was significantly decreased in the samples from the high-dose-treated compared to the low-dose-treated group (Table 1, $P = 0.012$), indicating that the SF1-P140K-transduced human cells were not protected to the same extent when exposed to high-dose alkylator therapy.

Analysis of Multilineage Engraftment of Human Cells Following Delivery of Low- or High-Dose Chemotherapy

We determined the impact of the low- or high-dose therapy on the maintenance of primitive human CD34⁺ cells and differentiation of lymphomyeloid cell lineages by flow cytometry. An enrichment of EGFP⁺CD34⁺CD38⁻ and EGFP⁺CD34⁺CD38⁺ cells was evident in mice recei-

ving low-dose chemotherapy but not high-dose chemotherapy (Fig. 4). In sharp contrast, even though we collected and analyzed via flow cytometry large numbers of events from the BM of transplanted mice that received high-dose therapy, EGFP⁺CD34⁺CD38⁻ and EGFP⁺CD34⁺CD38⁺ cells were not detected (ND) (Fig. 4). The BM of the transplanted mice (NT, low, and high) was also analyzed for clonogenic activity by the colony-forming unit (CFU) assay (Table 2). We analyzed large numbers of progenitor plates since the transduction level of the human cells in the nontreated mice and the engraftment level of the human cells in the drug-treated mice were low. The number of EGFP⁺ CFU detected in the nontreated and treated animals was consistent with our previously published study [7]. The presence of hematopoietic cells that still possessed clonogenic activity in transplanted mice receiving the high-dose regimen was extremely limited. We classified a colony as clonogenic if it contained 50 cells or greater since this is the accepted cutoff in the CFU assay. In the BM of these mice, we did occasionally observe nonclonogenic cell clusters in the methylcellulose that contained ~10–30 cells per cluster (data not shown). The two progenitor colonies that were detected in the BM of transplanted mice receiving the high-dose regimen were of myeloid origin (i.e., CFU-GM). These data suggest that although MGMT^{P140K} expression levels were sufficient to protect committed progenitors under low-dose therapy, expression levels were not adequate to maintain a pool of detectable committed progenitors following high-dose therapy.

We next determined the frequency of EGFP⁺CD34⁺CD19⁺ (B-lymphoid precursors) and EGFP⁺CD34⁻CD19⁺ (mature B-lymphoid cells) (Fig. 5A). Under low-dose therapy, both B-lymphoid progenitors and mature B-lymphoid cells were present and the majority of the cells expressed EGFP. In contrast, in the transplanted mice that received high-dose therapy, mature B-lymphoid cells but not B-lymphoid progenitors were present, again indicating that the more primitive hematopoietic cells were not protected adequately during high-dose chemotherapy. Similar to our findings showing that lymphopoiesis of transduced human cells was diminished following chemotherapy in the high-dose range, the appearance of transduced lymphocytes in a dog transplanted with allogeneic MGMT^{P140K}-transduced BM appeared to be compromised if dosing was increased above a certain threshold level [11].

Human myeloid cells (huCD45⁺CD33⁺) were protected in the BM of transplanted mice receiving either low- or high-dose therapy (Fig. 5B) and the vast majority of these cells expressed EGFP. The virtual lack of CFU-GM progenitors in transplanted mice that received the high-dose regimen (Table 2) suggests that the remaining CD33⁺ cells must be somewhat immature since they could survive for 8 weeks postchemotherapy but were still fairly differentiated overall since they had progressed

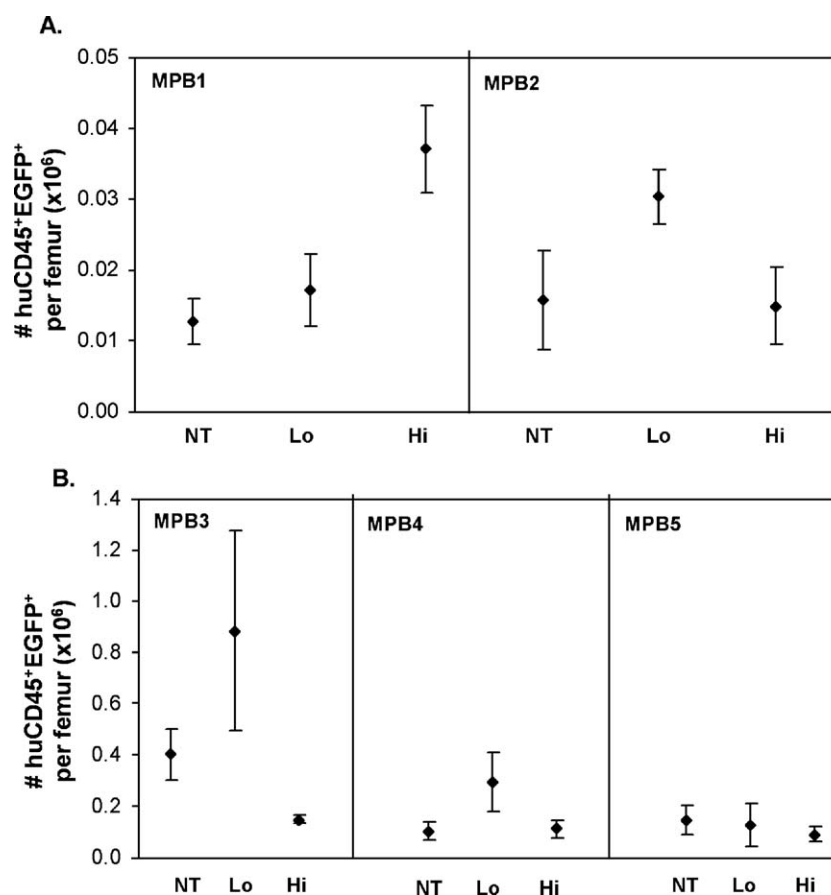


FIG. 3. Effects of low- or high-dose 6BG/BCNU on the total number of human EGFP⁺ cells. At 16 weeks posttransplantation, the BM of NOD/SCID mice was analyzed for the number of human EGFP⁺ cells per femur (# huCD45⁺EGFP⁺ × 10⁶). Different scales were used for (A) MPB 1 and 2 versus (B) MPB 3–5 due to differences in the number of human cells present. Data are presented as the means (diamonds) ± the standard error (lines). See Table 1 for statistical significance. NT, nontreated; Lo, low-dose chemotherapy; Hi, high-dose chemotherapy.

beyond the point of being clonogenic. Since engraftment was low ($\leq 5\%$ human cells) in the transplanted mice that received the high-dose regimen, this may also in part account for the lack of detectable clonogenic cells.

We also looked at the relative contribution of each hematopoietic cell phenotype (CD34, CD19, and CD33) to the human graft in the BM (Table 1 and see supplemental information online). The increasing numerical order for the percentage of CD34⁺ cells was Hi < Low < NT. There was no significant difference between NT and low-dose cohorts for the percentage of human CD34⁺ cells (Table 1, $P > 0.05$). As tested by Wilcoxon sum rank test, the percentage of human CD34⁺ cells was decreased in the high-dose cohort compared to both the low-dose and the NT cohorts (both P values < 0.0001). We observed no significant differences in the percentage of human CD19⁺ cells between the three experimental groups (Table 1, P values > 0.05). The percentage of human CD33⁺ cells was significantly increased in both the high-dose and the low-dose treatment groups compared to the NT group ($P = 0.016$ and 0.024 , respectively). The increasing

numerical order for the percentage of human CD33⁺ cells was NT < Hi < Low, although high dose and low dose were almost equal and no statistical difference was noted ($P > 0.05$). These data suggest that human myeloid cells that express MGMT^{P140K} were protected more consistently than the other hematopoietic subsets during chemotherapy administration.

Expression of MGMT^{P140K} in the BM of Transplanted Mice Following Delivery of Low- or High-Dose Chemotherapy

Using a fluorometric oligonucleotide assay, we next confirmed that MGMT activity was present in the BM of the transplanted mice following delivery of low- and high-dose therapy (Table 3) [31]. High levels of MGMT activity were present in the marrow of mice transplanted with transduced human MPB following treatment with the low- or high-dose regimens compared to NT and control BM. We observed no substantial differences in the level of MGMT activity in the BM between the low-dose and the high-dose treatments, indicating that high-dose

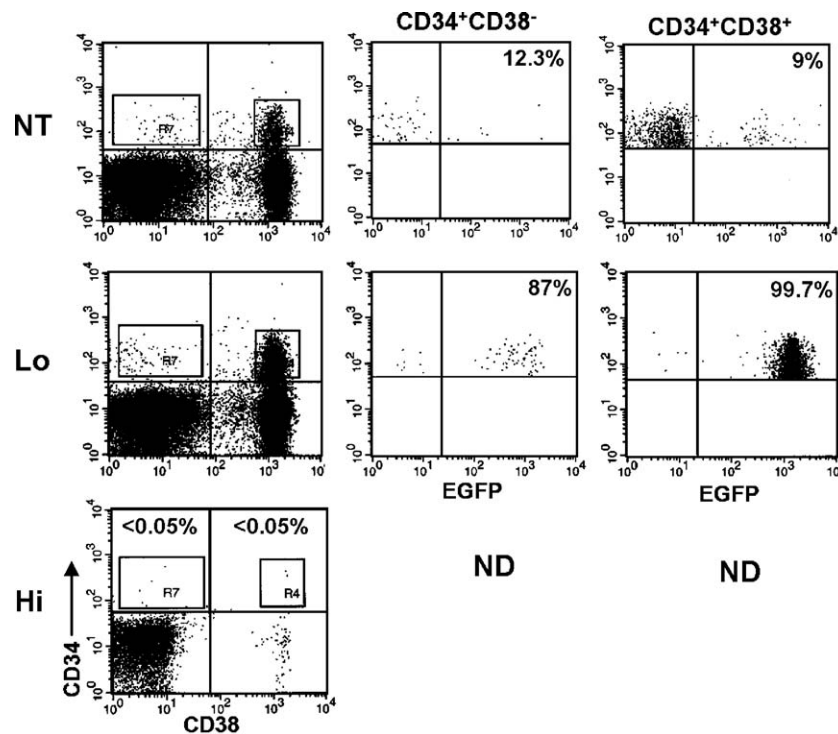


FIG. 4. Presence of human CD34⁺EGFP⁺ cells following delivery of low- or high-dose 6BG/BCNU. The percentage of human CD34⁺CD38⁻ and CD34⁺CD38⁺ cells that were EGFP⁺ following chemotherapy was assessed by flow cytometry. The percentage of EGFP⁺ cells in these populations was determined by setting gates for human CD34⁺CD38⁻ and CD34⁺CD38⁺ cells. Representative examples of nontreated (NT), low-dose-treated (Lo), and high-dose-treated (Hi) cells are shown. ND, not detected. The percentage in the upper right quadrant denotes the percentage of the human EGFP⁺ cells in the CD34 subset.

alkylator treatment did not result in selection or maintenance of hematopoietic cells expressing higher levels of MGMT^{P140K} than those mice treated with low-dose therapy. When using mice that had similar numbers of huCD45⁺EGFP⁺ cells in the BM (Table 3), we did not see a difference in MGMT DNA-repair activity in mice treated with low- versus high-dose therapy. However, data presented here suggest that protection under high-dose

therapy is suboptimal compared to the low-dose treatment in regard to the level of MGMT^{P140K}-transduced human cells following chemotherapy.

We are currently addressing whether low numbers of primitive human cells that express high levels of MGMT^{P140K} existed after the high-dose therapy and were either out-competed by the infusion of mouse BM and/or depleted due to differentiation during the recovery

TABLE 2: Analysis of clonogenic activity in the bone marrow postchemotherapy

Treatment	No. of mice ^a	%huCD45 ^{+b}	% huCD45 EGFP ^{+c}	Total No. human cells ($\times 10^6$) plated	Total No. CFU ^d	Total No. EGFP ⁺ CFU ^e	Total No. EGFP ⁺ CFU/femur	% CFU EGFP ⁺
NT ^f	4	40 \pm 13.1	0.35 \pm 0.2	2.7 (39) ^g	3599	12	1.5	0.33
Low ^h	5	1.3 \pm 0.26	71 \pm 10	0.2 (51)	928	496	49.6	53
High ⁱ	6	2.1 \pm 0.4	99 \pm 0.8	0.6 (63)	2	2	0.2	100

^a Number of mice in which the BM (i.e., femurs) was analyzed by CFU assay. Data were compiled in each treatment group and were derived from MPB 2. MPB 1 was not analyzed for CFU content. Two to four mice per treatment group were analyzed in MPB 3–5 and similar results obtained.

^b Human cell engraftment in the BM (mean \pm SE).

^c Percentage of human cells that were EGFP⁺ in the BM (mean \pm SE).

^d Total number of human CFU detected (BFU-E and CFU-GM combined).

^e Total number of EGFP⁺ human CFU detected in all femurs.

^f Not treated.

^g To maximize the number of human cells analyzed, the number of human cells plated per milliliter of complete methylcellulose depended on the level of human cell engraftment in each mouse. Mice with >30% human cell engraftment in the BM were plated at 1×10^5 total BM cells, mouse and human, per plate; mice with 10–30% human engraftment were plated at 2×10^5 total BM cells per plate and mice with <10% engraftment were plated at 4×10^5 total BM cells per plate. (n) is the number of plates analyzed.

^h Low-dose 6BG/BCNU.

ⁱ High-dose 6BG/BCNU.

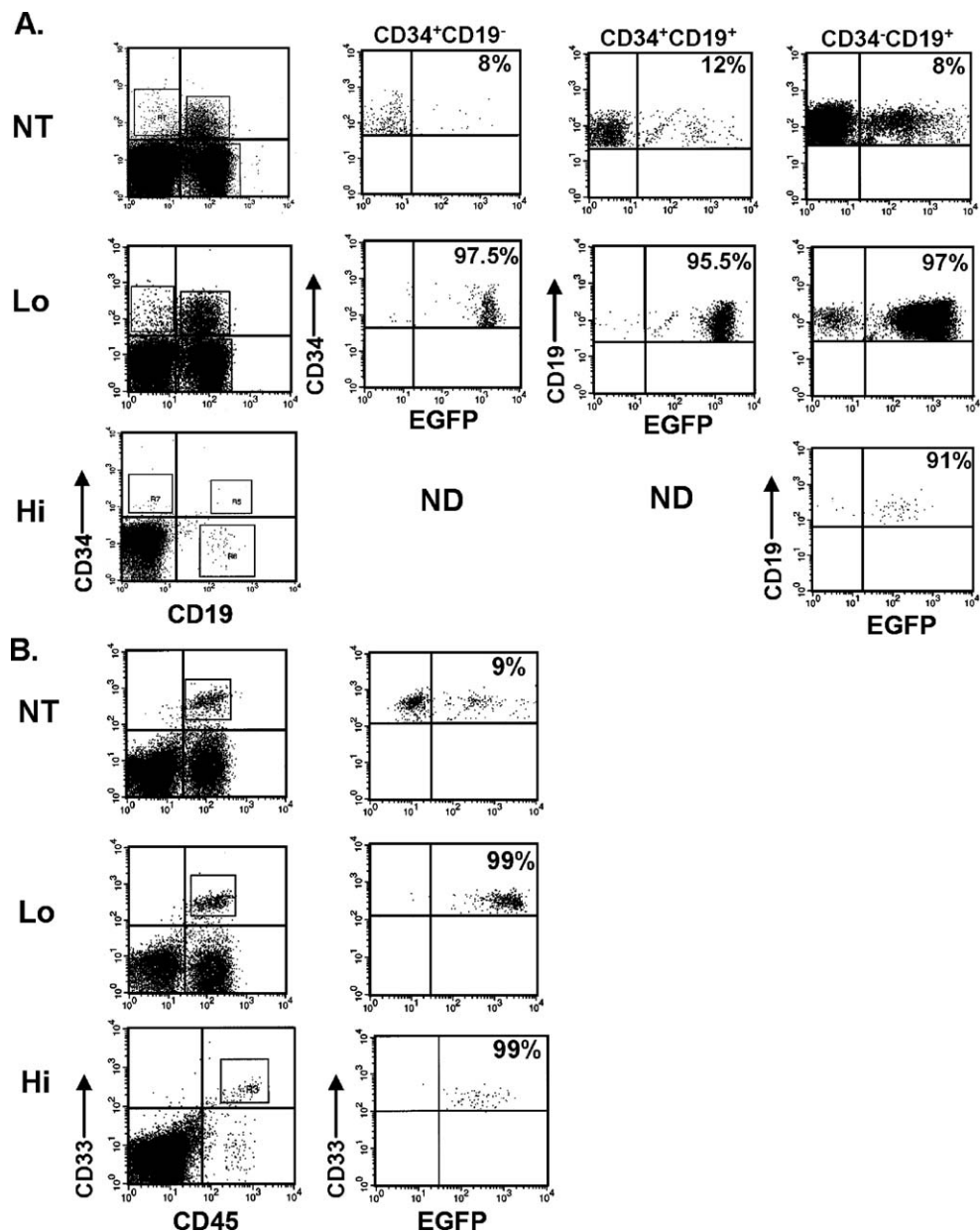


FIG. 5. Effects of low- or high-dose 6BG/BCNU on multilineage engraftment. (A) For B-lymphoid cells, the percentage of EGFP⁺ cells was determined after gating on human CD34⁺CD19⁻, CD34⁺CD19⁺, and CD34⁻CD19⁺ cells. (B) For myeloid cells, the percentage of EGFP⁺ cells was determined by setting gates for human CD45⁺CD33⁺ cells. Representative examples of nontreated (NT), low-dose treated (Lo), and high-dose treated (Hi) are shown. ND, not detected. The percentage in the upper right quadrant denotes the percentage of the human EGFP⁺ cells in the denoted subset.

period. The reason for the lack of primitive MPB cells detected in mice receiving the high-dose regimen is most likely the low number of SRC transduced by the oncoretroviral vector. The challenge for future investigations lies in delivering MGMT^{P140K} DNA sequences into sufficient numbers of human HSC derived from MPB that can maintain long-term expression. Cytokine-mediated entry into cell cycle and subsequent breakdown of the nuclear envelope are a prerequisite for integration of

oncoretroviral vectors into the host genome. This requirement for provirus integration in conjunction with our previous and current findings may preclude the use of oncoretroviral vectors for gene transfer into MPB. We demonstrated previously that transduction into MPB SRC using oncoretroviral vectors was problematic and that substantial differences in the “transducibility” of SRC derived from MPB and UCB existed [30]. In this previous study, we showed that although MPB and UCB engrafted

TABLE 3: Analysis of MGMT activity in the BM postchemotherapy

MPB 5 mouse ID	Treatment	% huCD45 ⁺	No. huCD45 per femur ($\times 10^6$)	% huCD45 ⁺ EGFP ⁺	No. EGFP cells per femur ($\times 10^6$)	MGMT activity (fmol O ⁶ -MeG removed/mg)
Control ^a	—	—	—	—	—	3072
Mo 2 ^b	NT ^c	46.0	3.8	2.2	0.10	14,600
Mo 4	Low ^d	5.0	0.1	40.4	0.04	21,800
Mo 6	Low	3.0	0.13	42.5	0.10	28,600
Mo 8	High ^e	0.4	0.14	82.5	0.12	26,100
Mo 9	High	0.7	0.17	88.6	0.15	30,500

^a Control NOD/SCID BM from a nontransplanted mouse.

^b Mouse number corresponding to mice in MPB 5.

^c Not treated.

^d Low-dose 6BG/BCNU.

^e High-dose 6BG/BCNU.

at similar levels in the NOD/SCID mice, transduction efficiency of SRC derived from MPB with oncoretroviral vectors was significantly lower compared to that obtained with SRC derived from UCB. We hypothesize that this difference is due to an overall lack of cytokine responsiveness in MPB SRC compared to UCB SRC and that once MPB SRCs enter the cell cycle, many of them differentiate. In addition, Srour and colleagues demonstrated previously that while UCB SRC in G₀ or G₁ of the cell cycle engrafted similarly in NOD/SCID mice, MPB SRC in G₁ did not engraft as efficiently as MPB SRC in G₀ [32,33]. To increase the number of SRCs transduced with the oncoretroviral vector that expresses MGMT^{P140K}, we recently transduced CD34⁺ cells isolated from UCB. The striking difference in the transducibility of UCB and MPB is further highlighted by these experiments. MGMT^{P140K}-transduced UCB SRC and progeny were protected in the transplanted mice following delivery of the high-dose regimen, suggesting that if the oncoretroviral vector can be delivered to adequate numbers of SRC, detection of protected human cells from high-dose therapy is possible (Cai and Pollok, manuscript in preparation). It is important to note, however, that clinical gene therapy trials are using oncoretrovirus vectors and autologous MPB as the target stem cell source. To what extent oncoretroviral vectors can adequately transduce and protect adequate numbers of primitive cells derived from MPB in humans is being addressed in a phase I clinical trial [34]. The *in vivo* high-dose model described in our study can now be used to test other transduction strategies and vector systems designed to enhance the transduction efficiency of primitive MPB HSC. Alternative vector systems such as lentivirus and foamy virus may help overcome the problem of low transduction efficiency into SRC derived from MPB, and studies are currently in progress.

The NOD/SCID xenograft/SRC assay provides a reliable method with which to test the impact of relevant anticancer therapies on human tumorigenesis and hematopoiesis in multiple independent experiments. In our study we compared the impact of low-dose versus high-

dose therapy in experiments using isolated CD34⁺ cells from five normal MPB donors, and we noted variability in terms of engraftment and composition of the graft following treatment. This study underscores the fact that studying sources of stem cells relevant to the future proposed therapies is necessary. How information obtained using this model coincides with that observed in humans awaits the results of clinical gene therapy trials investigating the capability of MGMT^{P140K}-transduced CD34⁺ cells to protect cancer patients during alkylator-based therapy. In summary, these data suggest that improved strategies for transfer of MGMT^{P140K} into primitive hematopoietic precursor cells to increase DNA repair in hematopoietic cells during high-dose alkylator therapy are needed, particularly when using MPB as a stem cell source. This *in vivo* model provides an innovative approach with which to determine the impact of high-dose alkylator therapy on human HSC and also to test strategies predicted to increase substantially the number of primitive human hematopoietic cells protected from cytotoxic drug therapy.

MATERIALS AND METHODS

Animals. A breeding colony of NOD.CB17-Prkdc^{scid} (NOD/SCID) mice was established at the Laboratory of Animal Research Center at the Indiana University School of Medicine (IUSM) (Indianapolis, IN, USA). All protocols were approved by the Institutional Animal Care and Use Committee. Animals were housed in positive-airflow ventilated racks, bred, and maintained in microisolators under specific-pathogen-free conditions. Following transplantation, animals were placed on static racks and housed under a Biobubble (The Colorado Clean Room Co., Ft. Collins, CO, USA). All mice received 1% neomycin sulfate supplemented with 1.64% glucose in autoclaved water with three changes weekly.

Retrovirus backbones for expression of MGMT^{P140K} in HSC. The oncoretroviral vector SF1-MGMT^{P140K}-IRES-EGFP (SF1-P140K) was utilized to coexpress MGMT^{P140K} and EGFP in human CD34⁺ cells and was described previously [7]. Retroviral vectors were pseudotyped with the GALV envelope using the PG13 packaging cell line (American Type Culture Collection, Manassas, VA, USA) [35]. Titers were initially determined on human erythroleukemia cells by limiting-dilution analysis. A high-titer clone, SF1-MGMT^{P140K}-IRES-EGFP (clone 40), produced $2-3 \times 10^5$ infectious units per milliliter and was negative for replication-competent retrovirus.

Isolation of MPB CD34⁺ cells. All protocols described were approved by the Institutional Review Board at IUSM. For isolation of MPB CD34⁺ cells, healthy adult volunteers were treated subcutaneously for 4 days with 10 $\mu\text{g}/\text{kg}/\text{day}$ of human G-CSF (Filgrastim, Neupogen; Amgen, Thousand Oaks, CA, USA). Mononuclear cells were collected by apheresis and CD34⁺ cells were isolated by immunomagnetic methods using the Isoplex 3000 cell selection device according to the manufacturer's instructions (Baxter Immunotherapy, Irvine, CA, USA). The range in purity of CD34⁺ cells was 80–90%.

Transduction of MPB CD34⁺ cells. The transductions were done as previously described by our laboratory [7,30]. The starting cell number prior to prestimulation and transduction was 4×10^6 per mouse transplanted. Isolated CD34⁺ cells were prestimulated at a cell density of 5×10^5 cells/ml in Ex Vivo-10 serum-free medium containing 1% human serum albumin. The medium was supplemented with G-CSF, SCF (a generous gift from Amgen, Inc.), and TPO (PeproTech, Rocky Hill, NJ, USA). Each cytokine was used at 100 ng/ml for prestimulation. Non-tissue-culture 10-cm plates (Falcon, Franklin Lakes, NJ, USA) were coated with 2 $\mu\text{g}/\text{cm}^2$ Retronectin (Takara Shuzo, Otsu, Japan) overnight at 4°C. Cells were plated at a concentration of 2×10^5 cells/ cm^2 for transduction. Cells were infected with a 1:1 ratio of retrovirus supernatant:complete medium with cytokines for 4 h on 2 consecutive days, with a change to complete medium containing cytokines for overnight incubation. After the second round of infection, cells were allowed to remain overnight on Retronectin-coated plates with fresh medium and cytokines. The following day, the medium was changed and the cells were allowed to adhere to Retronectin in the presence of 100 ng/ml SCF for an additional 2 days prior to transplant as previously described [36]. The transduction efficiency was determined by flow cytometry on the day of the transplant.

MGMT bioactivity assay. MGMT activity was determined as previously described by Kreklaui *et al.* [31]. Briefly, lysates prepared from BM samples were incubated with a fluorometric 5'-hexachlorofluorescein phosphoramidite-labeled oligonucleotide containing a single O⁶-methylguanine residue nested within a PvuII restriction site. Following a phenol-chloroform extraction, the oligonucleotide was digested with PvuII. Cleavage of the oligonucleotide by PvuII occurs only if the O⁶-methylguanine residue is repaired by MGMT. Samples were run on a polyacrylamide gel and the amount of cleaved product was quantitated using the Hitachi FMBio II fluorescence imaging system (Hitachi Genetic Systems, South San Francisco, CA, USA) Specific activity was equal to femtomoles of O⁶-methylguanine removed per milligram of total protein.

Transplantation of NOD/SCID mice. Prior to transplant, NOD/SCID mice were conditioned with 300 cGy total-body irradiation using a GammaCell 40 (Nordion International, Inc., On, Canada) equipped with two opposing ¹³⁷Cs sources. Cells were washed once and resuspended in IMDM containing 0.2% endotoxin-free BSA and injected into the lateral tail vein of each animal. The number of cells injected per mouse for MPB 1–5 was 28×10^6 , 25×10^6 , 38×10^6 , 45×10^6 , and 49×10^6 , respectively.

Chemotherapy administration. 6BG (Sigma, St. Louis, MO, USA) was dissolved in 40% polyethylene glycol-400 (v/v) and 60% saline (v/v). BCNU (Sigma) was dissolved in 10% ethanol (v/v) and 90% normal saline solution (v/v). BCNU was placed on ice and used immediately after reconstitution. At 4 weeks posttransplant, cohorts of mice were randomly grouped and either not treated or treated with low- or high-dose therapy according to the schema in Fig. 1. A low-dose cycle consisted of 20 mg/kg 6BG followed by 5 mg/kg BCNU 1 h later. A high-dose cycle consisted of 30 mg/kg 6BG followed by 10 mg/kg BCNU 1 h later and 15 mg/kg 6BG 7 h later. *In vivo*-selected SF1-P140K-transduced NOD/SCID BM or fresh NOD/SCID BM was infused following high-dose chemotherapy to maintain murine hematopoiesis as detailed under Results and Discussion and Fig. 1A.

Analysis of human cell engraftment. Mice were sacrificed at 8 weeks post-6BG/BCNU injection and single-cell suspensions of the BM prepared. Human cell engraftment measured by human CD45 staining and the proportion of engraftment in various lineages was determined by immunostaining and flow-cytometric analysis. Aliquots of $1\text{--}2 \times 10^5$

cells/tube were stained with various antibodies (2 μl per sample) for 25 min at 4°C in complete medium and washed one time in PBS containing 1% FBS. All antibodies were titered and used at saturating concentrations. The lack of cross-reactivity of human-specific antibodies with murine cells was confirmed in every experiment by staining BM from a nontransplanted mouse with each antibody combination. Cells were stained with peridinin chlorophyll-conjugated anti-human CD45 (anti-HLE-1; Becton–Dickinson Immunocytometry, San Jose, CA, USA) alone or in combination with phycoerythrin (PE)-conjugated anti-human CD33 (anti-Leu-M9; Becton–Dickinson). Identical aliquots were stained with allophycocyanin-conjugated anti-human CD34 (clone 581; Pharmingen, San Diego, CA, USA) in combination with anti-human CD19–PE (PharMingen) or anti-human CD38–PE (anti-Leu-17; Becton–Dickinson). The forward- and right-angle light scatter parameters were used to set the gates for analysis. In experiments in which engraftment of human cells was >5%, ~20,000–40,000 events were collected and analyzed. In experiments in which human engraftment was <5%, ~200,000 events were collected and analyzed. All samples were acquired and analyzed on a Becton–Dickinson FACSCalibur using CellQuest software (Becton–Dickinson).

Statistical analysis. Analysis was done using SAS version 9 (Cary, NC, USA). Statistical tests were conducted as two-sided at the $\alpha = 0.05$ significance and 0.10 marginal levels. Pair-wise comparisons between the three groups, nontreated and low-dose and high-dose treatment, were adjusted for multiple comparisons using the Bonferroni method ($P < 0.017$ for each comparison to maintain overall α of 0.05). The P values for overall significance, difference between groups, and pair-wise comparisons are presented.

The percentage of huCD45⁺, the percentage of huCD45⁺EGFP⁺, and the number of huCD45⁺EGFP⁺ obtained per femur were analyzed separately. Due to nonconstant variance for each measure, a violation of the assumption of normality, transformations of the data were performed for analysis. A natural logarithm transformation was used for the number of huCD45⁺EGFP⁺ and arcsine of the square root transformation was used for proportions of huCD45⁺ and huCD45⁺EGFP⁺. For each analysis, a mixed linear model was fit with a variance-covariance structure that incorporated correlation of multiple observations from the same donor. Typically, samples obtained from the same donor will be more similar than samples obtained from different donors. The percentages of CD19⁺, CD33⁺, and CD34⁺ in the BM were compared separately between the three groups. Except for the percentage of CD34⁺ cells in the mice treated with high-dose therapy, comparisons were analyzed as Poisson distributions of counts using the natural logarithm of total cells as the offset (count of CD19⁺ or CD33⁺ cells of the total cells analyzed per sample). Generalized Linear Model analysis was used to account for the correlation between repeated samples from the same donor. Sources of correlation include input percentage of EGFP⁺ cells, percentage of EGFP⁺CFU⁺, total number of cells injected, and number of EGFP⁺ cells, which are constant for each donor. Nonparametric Wilcoxon sum rank test was used to compare the percentage of CD34⁺ obtained in the mice treated with high-dose therapy to percentages in the other two treatment groups, since all 20 values of CD34 percentage were equal to 0.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ymthe.2005.11.017.

REFERENCES

- Park, Y., and Gerson, S. L. (2005). DNA repair defects in stem cell function and aging. *Annu. Rev. Med.* **56**: 495.
- Sorrentino, B. P. (2002). Gene therapy to protect hematopoietic cells from cytotoxic cancer drugs. *Nat. Rev. Cancer* **2**: 431.
- Gerson, S. L. (2002). Clinical relevance of MGMT in the treatment of cancer. *J. Clin. Oncol.* **20**: 2388.
- Quinn, J. A., et al. (2002). Phase II trial of carmustine plus O(6)-benzylguanine for patients with nitrosourea-resistant recurrent or progressive malignant glioma. *J. Clin. Oncol.* **20**: 2277.
- Sawai, N., Zhou, S., Vanin, E. F., Houghton, P., Brent, T. P., and Sorrentino, B. P. (2001). Protection and in vivo selection of hematopoietic stem cells using temozolomide, O⁶-benzylguanine, and an alkyltransferase-expressing retroviral vector. *Mol. Ther.* **3**: 78.
- Sawai, N., Persons, D. A., Zhou, S., Lu, T., and Sorrentino, B. P. (2003). Reduction in hematopoietic stem cell numbers with in vivo drug selection can be partially abrogated by HOXB4 gene expression. *Mol. Ther.* **8**: 376.
- Pollok, K. E., et al. (2003). In vivo selection of human hematopoietic cells in a xenograft model using combined pharmacologic and genetic manipulations. *Hum. Gene Ther.* **14**: 1703.
- Milsom, M. D., Woolford, L. B., Margison, G. P., Humphries, R. K., and Fairbairn, L. J. (2004). Enhanced in vivo selection of bone marrow cells by retroviral-mediated coexpression of mutant O⁶-methylguanine-DNA-methyltransferase and HOXB4. *Mol. Ther.* **10**: 862.
- Zielske, S. P., Reese, J. S., Lingas, K. T., Donze, J. R., and Gerson, S. L. (2003). In vivo selection of MGMT(P140K) lentivirus-transduced human NOD/SCID repopulating cells without pretransplant irradiation conditioning. *J. Clin. Invest.* **112**: 1561.
- Reese, J. S., Davis, B. M., Liu, L., and Gerson, S. L. (1999). Simultaneous protection of G156A methylguanine DNA methyltransferase gene-transduced hematopoietic progenitors and sensitization of tumor cells using O⁶-benzylguanine and temozolomide. *Clin. Cancer Res.* **5**: 163.
- Neff, T., et al. (2003). Methylguanine methyltransferase-mediated in vivo selection and chemoprotection of allogeneic stem cells in a large-animal model. *J. Clin. Invest.* **112**: 1581.
- Neff, T., Beard, B. C., Peterson, L. J., Anandakumar, P., Thompson, J., and Kiem, H. P. (2005). Polyclonal chemoprotection against temozolomide in a large-animal model of drug resistance gene therapy. *Blood* **105**: 997.
- Kreklaue, E. L., et al. (2003). Hematopoietic expression of O(6)-methylguanine DNA methyltransferase-P140K allows intensive treatment of human glioma xenografts with combination O(6)-benzylguanine and 1,3-bis(2-chloroethyl)-1-nitrosourea. *Mol. Cancer Ther.* **2**: 1321.
- Persons, D. A., et al. (2003). Successful treatment of murine [beta]-thalassemia using in vivo selection of genetically modified, drug-resistant hematopoietic stem cells. *Blood* **102**: 506.
- Davis, B. M., Humeau, L., and Dropulic, B. (2004). In vivo selection for human and murine hematopoietic cells transduced with a therapeutic MGMT lentiviral vector that inhibits HIV replication. *Mol. Ther.* **9**: 160.
- Davis, B. M., Koc, O. N., and Gerson, S. L. (2000). Limiting numbers of G156A O(6)-methylguanine-DNA methyltransferase-transduced marrow progenitors repopulate nonmyeloablated mice after drug selection. *Blood* **95**: 3078.
- Davis, B. M., Koc, O. N., Reese, J. S., and Gerson, S. L. (1999). O⁶-benzylguanine-resistant mutant MGMT genes improve hematopoietic cell tolerance to alkylating agents. *Prog. Exp. Tumor Res.* **36**: 65.
- Davis, B. M., Reese, J. S., Koc, O. N., Lee, K., Schupp, J. E., and Gerson, S. L. (1997). Selection for G156A O⁶-methylguanine DNA methyltransferase gene-transduced hematopoietic progenitors and protection from lethality in mice treated with O⁶-benzylguanine and 1,3-bis(2-chloroethyl)-1-nitrosourea. *Cancer Res.* **57**: 5093.
- Ragg, S., et al. (2000). Direct reversal of DNA damage by mutant methyltransferase protein protects mice against dose-intensified chemotherapy and leads to in vivo selection of hematopoietic stem cells. *Cancer Res.* **60**: 5187.
- Jansen, M., et al. (2002). Hematoprotection and enrichment of transduced cells in vivo after gene transfer of MGMT(P140K) into hematopoietic stem cells. *Cancer Gene Ther.* **9**: 737.
- Choi, U., et al. (2004). Nuclear-localizing O⁶-benzylguanine-resistant GFP-MGMT fusion protein as a novel in vivo selection marker. *Exp. Hematol.* **32**: 709.
- Neff, T., et al. (2004). Efficient gene transfer to hematopoietic repopulating cells using concentrated RD114-pseudotype vectors produced by human packaging cells. *Mol. Ther.* **9**: 157.
- Dolan, C., Pegg, A. E., Mullen, Friedman, and Moschel, R. C. (1994). Metabolism of O⁶-benzylguanine, an inactivator of O⁶-alkylguanine-DNA alkyltransferase. *Cancer Res.* **54**: 5123.
- Dolan, M. E., Moschel, R. C., and Pegg, A. E. (1990). Depletion of mammalian O⁶-alkylguanine-DNA alkyltransferase activity by O⁶-benzylguanine provides a means to evaluate the role of this protein in protection against carcinogenic and therapeutic alkylating agents. *Proc. Natl. Acad. Sci. USA* **87**: 5368.
- Koc, O. N., Reese, J. S., Davis, B. M., Liu, L., Majczenko, K. J., and Gerson, S. L. (1999). DeltaMGMT-transduced bone marrow infusion increases tolerance to O⁶-benzylguanine and 1,3-bis(2-chloroethyl)-1-nitrosourea and allows intensive therapy of 1,3-bis(2-chloroethyl)-1-nitrosourea-resistant human colon cancer xenografts. *Hum. Gene Ther.* **10**: 1021.
- Kreklaue, E. L., Kurpad, C., Williams, D. A., and Erickson, L. C. (1999). Prolonged inhibition of O(6)-methylguanine DNA methyltransferase in human tumor cells by O(6)-benzylguanine in vitro and in vivo. *J. Pharmacol. Exp. Ther.* **291**: 1269.
- Kreklaue, E. L., Liu, N., Li, Z., Cornetta, K., and Erickson, L. C. (2001). Comparison of single- versus double-bolus treatments of O(6)-benzylguanine for depletion of O(6)-methylguanine DNA methyltransferase (MGMT) activity in vivo: development of a novel fluorometric oligonucleotide assay for measurement of MGMT activity. *J. Pharmacol. Exp. Ther.* **297**: 524.
- Korbling, M., and Anderlini, P. (2001). Peripheral blood stem cell versus bone marrow allotransplantation: does the source of hematopoietic stem cells matter? *Blood* **98**: 2900.
- Couban, S., et al. (2002). A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. *Blood* **100**: 1525.
- Pollok, K. E., et al. (2001). Differential transduction efficiency of SCID-repopulating cells derived from umbilical cord blood and granulocyte colony-stimulating factor-mobilized peripheral blood. *Hum. Gene Ther.* **12**: 2095.
- Kreklaue, E. L., Limp-Foster, M., Liu, N., Xu, Y., Kelley, M. R., and Erickson, L. C. (2001). A novel fluorometric oligonucleotide assay to measure O(6)-methylguanine DNA methyltransferase, methylpurine DNA glycosylase, 8-oxoguanine DNA glycosylase and abasic endonuclease activities: DNA repair status in human breast carcinoma cells overexpressing methylpurine DNA glycosylase. *Nucleic Acids Res.* **29**: 2558.
- Wilpshaar, J., et al. (2000). Similar repopulating capacity of mitotically active and resting umbilical cord blood CD34(+) cells in NOD/SCID mice. *Blood* **96**: 2100.
- Gothot, A., van der Loo, J. C., Clapp, D. W., and Srour, E. F. (1998). Cell cycle-related changes in repopulating capacity of human mobilized peripheral blood CD34(+) cells in non-obese diabetic/severe combined immune-deficient mice. *Blood* **92**: 2641.
- Reese, J., Linguas, K., Ksenich, P., Sweeney, C., Koc, O., and Gerson, S. (2004). Preliminary results of a phase I trial using retroviral gene transfer of G156A MGMT to protect hematopoiesis during BG and BCNU therapy of advanced malignancies. *Mol. Ther.* **9**: S385.
- Miller, A. D., Garcia, J. V., von Suhr, N., Lynch, C. M., Wilson, C., and Eiden, M. V. (1991). Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus. *J. Virol.* **65**: 2220.
- Takatoku, M., et al. (2001). Avoidance of stimulation improves engraftment of cultured and retrovirally transduced hematopoietic cells in primates. *J. Clin. Invest.* **108**: 447.