Transient in vivo selection of transduced peripheral blood cells using antifolate drug selection in rhesus macaques that received transplants with hematopoietic stem cells expressing dihydrofolate reductase vectors

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One of the main obstacles for effective human gene therapy for hematopoietic disorders remains the achievement of an adequate number of genetically corrected blood cells. One approach to this goal is to incorporate drug resistance genes into vectors to enable in vivo selection of hematopoietic stem cells (HSCs). Although a number of drug resistance vectors enable HSC selection in murine systems, little is known about these systems in large animal models. To address this issue, we transplanted cells transduced with dihydrofolate resistance vectors into 6 rhesus macaques and studied whether selection of vector-expressing cells occurred following drug treatment with trimetrexate and nitrobenzylmercapturin-eriboside-phosphate. In some of the 10 administered drug treatment courses, substantial increases in the levels of transduced peripheral blood cells were noted; however, numbers returned to baseline levels within 17 days. Attempts to induce stem cell cycling with stem cell factor and granulocyte-colony stimulating factor prior to drug treatment did not lead to sustained enrichment for transduced cells. These data highlight an important species-specific difference between murine and nonhuman primate models for assessing in vivo HSC selection strategies and emphasize the importance of using drugs capable of inducing selective pressure at the level of HSCs.

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Introduction

One of the main barriers for effective gene therapy of the hematopoietic system is the inability to obtain adequate numbers of genetically corrected hematopoietic cells in many disorders. In some disorders, such as severe combined immunodeficiency, a naturally occurring selective advantage for corrected cells exists, allowing therapeutic levels of normally functioning lymphocytes to develop following gene therapy. However, for most other disorders, particularly those of the myeloid system, such an advantage does not exist. For instance, in clinical trials for chronic granulomatous disease, detectable levels of biochemically corrected cells were obtained by using a gene therapy approach, but numbers were insufficient to confer a significant therapeutic effect. The same limitation is predicted for hemoglobin disorders such as beta thalassemia and sickle cell anemia, liposomal storage diseases, and other neutrophil disorders. This problem is compounded by the avoidance of myeloablative conditioning therapy prior to the administration of transduced cells, thereby leading to even lower levels of engrafted, genetically modified cells.

One strategy to increase the number of corrected blood cells has been referred to as “in vivo selection.” This general approach uses a dominant selectable marker within the vector to allow for in vivo enrichment of genetically modified cells following transplantation and engraftment. Selectable markers have included 2 broad categories, drug resistance genes for use in conjunction with cytotoxic drugs and genes that directly induce proliferation such as modified cytokine receptors or growth-promoting transcription factors. Many of these vectors allowed in vivo selection in mouse transplantation models, including those containing the multidrug resistance 1 (MDR1) gene, the dihydrofolate reductase (DHFR) gene, methylguanine methyltransferase (MGMT) resistance genes, and the HOXB4 gene.

In contrast, there are few, if any, examples of successful in vivo HSC selection in clinical trials or large animal studies. Early clinical trials using MDR1 vectors yielded equivocal results, with a later study demonstrating possible selection in bone marrow progenitors. A recent study has shown MDR1-mediated in vivo selection in a canine transplantation model, although it was unclear if this occurred at the HSC level. There are no examples of in vivo selection in nonhuman primate models, which is a highly relevant model system for recapitulating gene transfer into human hematopoietic cells. Important differences between nonhuman primates and mouse gene transfer models include much lower HSC transduction efficiency in the primate system, the fact that nonhuman primates are genetically outbred, and the fact that primates are more appropriate clinical models for assessing drug dose and toxicity of selective drug regimens.

In this study, we evaluated the use of a DHFR-based selection system in a rhesus macaque (Macaca mulatta) transplantation model.
model. Our prior experience in murine models has shown that vectors expressing antifolate resistant DHFR genes can be selected at the HSC level in mice treated with trimetrexate (TMTX) and nitrobenzylmercaptopuriniriboside-phosphate (NBMPR-P). The use of NBMPR-P inhibits a thymidine rescue mechanism used by murine HSCs, thereby sensitizing unmodified HSCs to antifolate drugs. An advantage of this system is relative lack of mutagenic effects, because antifolate agents have relatively little DNA-damaging activity. On the basis of these considerations, we tested the DHFR/TMTX/NBMPR-P selection system in 6 monkeys that both received transplants and had received a total of 10 courses of drug selection.

### Materials and methods

#### Thymidine transport assays

Assays for measurement of erythrocyte thymidine transport were performed essentially as previously described. Erythrocytes from 0.1 mL peripheral blood were incubated with 1 mM 3H-thymidine (DuPont, Boston, MA) for 5 minutes at 37°C. NBMPR (20 μM) was also added to some tubes to prevent thymidine uptake and thereby serve as a control to define baseline activities. After incubation, the cells were pelleted, washed, and then lysed with ammonium chloride. The lysates were then analyzed on a gamma-scintillation counter (Becton Dickinson, San Jose, CA).

#### Stem cell collection, transduction, and autologous transplantation

Mobilization of peripheral blood stem cells and CD34 immunoselection in rhesus macaques was performed by using an apheresis procedure that has been previously described. Transduction with the retroviral vectors in rhesus macaques was performed by using an apheresis procedure that Mobilization of peripheral blood stem cells and CD34 immunoselection in rhesus macaques was performed by using an apheresis procedure that has been previously described. Transduction with the retroviral vectors in rhesus macaques was performed by using an apheresis procedure that

#### Retroviral vectors

The MGirL22Y/RD114 vector has been previously described. The MSCV-YFP-ir-DHFR vector was made by inserting a YFP reporter gene (Clontech, Palo Alto, CA) under control of the murine stem cell virus (MSCV) promoter; and a human DHFR L22Y, F31R cDNA was placed downstream under control of the encephalomyocarditis internal ribosomal entry sequence (IRE). This vector was packaged in a vescular stomatitis virus (VSV-G) envelope-expressing packaging cell line kindly provided by Dr Richard Mulligan. The MDHFR/wc vector was made by inserting the DHFR L22Y, F31R cDNA under control of the MSCV promoter and inserting downstream a 591-bp mRNA-processing element from the woodchuck hepatitis virus. Transient VSV-G virus was used to transduce PG13 packaging cells as previously described, and a high-titer clone was selected. The G1Na vector has been previously described.

#### Drug treatments

Trimetrexate glucuronate was purchased from US Bioscience (W Conselho, PA), diluted to a 2-mg/mL solution in 5% dextrose. NBMPR-P was a generous gift from Dr Alan Paterson (University of Alberta) and was diluted to a 0.6-mg/mL solution in 5% dextrose. On any given day of treatment, half of the dose of NBMPR-P was first given over 60 minutes, the line was then flushed, and the total TMTX was given as a bolus dose over 5 to 10 minutes. The line was again flushed, and the second half of the NBMPR-P dose was given over an additional 60 minutes. Recombinant human granulocyte-colony-stimulating factor (G-CSF) and pegylated human SCF were provided by Amgen and given as subcutaneous injections at doses of 10 μg/kg/d and 200 μg/kg/d, respectively.

### Results

#### In vivo inhibition of thymidine transport with NBMPR-P

Our previous studies in mice have shown that coadministration of NBMPR-P with TMTX was necessary to obtain selection of DHFR-transduced HSCs. NBMPR-P blocks the thymidine salvage mechanism in HSCs and thereby sensitizes unmodified HSCs to TMTX. Therefore, we designed a set of experiments to determine whether thymidine transport could be blocked in vivo by NBMPR-P in rhesus macaques. NBMPR-P was infused intravenously over 1 hour at doses of either 1 mg/kg or 3 mg/kg. At various time points after the infusion, peripheral blood was sampled to study thymidine transport in circulating erythrocytes. These erythrocytes were incubated with 3H-thymidine, and intracellular accumulation of radiolabeled thymidine was then assessed at various time points after drug infusion. These studies showed that at both doses of NBMPR-P, thymidine transport was inhibited to less than 5% of baseline activity for a full 6 hours (Figure 1). The next time point analyzed 24 hours later showed thymidine transport...
had returned to baseline levels. Toxicities included only mild vomiting. These data show that significant in vivo inhibition of thymidine transport can be achieved using NBMPR-P, over a time period that would coincide with active drug levels following a TMTX infusion.

Drug treatment and in vivo selection in the first 2 monkeys that received transplants

The first monkey (RC701) received a transplant with G-CSF/SCF–mobilized PB CD34⁺ stem cells transduced with an MSCV vector containing a yellow fluorescent protein (YFP) marker in the upstream position and an IRES-driven DHFR resistance gene containing the L22Y and F31R substitutions in the downstream position (Table 1). This vector was produced in stable packaging cells expressing the VSV-G envelope protein. Initial marking levels in PB leukocytes were quite low, averaging approximately 0.3% of total cells. The first drug treatment course was administered 137 days after transplantation and consisted of TMTX 6 mg/kg per day for 5 days and NBMPR-P at 3 mg/kg per day for 5 days. At this dose, myelosuppression was mild and was associated with a modest but significant increase in YFP-expressing PB granulocytes, increasing from 0.3% on the day before treatment to 1% 9 days after the start of treatment. This enrichment disappeared by day 15. A second drug treatment course was administered by increasing the dose of TMTX to 6 mg/kg for 6 days followed by a 7th day at 8 mg/kg. The dose of NBMPR-P was 2.5 mg/kg daily for 7 days. Myelosuppression was increased, but flow cytometry data were available for only 2 posttreatment days, disallowing any conclusions regarding selection. A third treatment course was administered 210 days after transplantation, consisting of TMTX at 6 mg/kg for the first 2 days, then followed by an additional 5 days at 8 mg/kg, with coadministration of NBMPR-P at 3 mg/kg for 7 days. This treatment resulted in the greatest degree of myelosuppression of the 3 courses, with the absolute neutrophil count (ANC) falling to below 0.5 × 10⁹/L (500 cells/µL) for 3 days, and platelets falling beneath 100 × 10⁹/L (100 000 cells/µL) for 1 day. YFP marking in granulocytes again rose from 0.3% to 1.3% 12 days after the beginning of treatment, with decay back down to baseline levels 24 days after initiation of the third course.

Table 1. Summary of rhesus monkey transplants

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Vector</th>
<th>Drug treatment</th>
<th>Days ANC was below 500</th>
<th>Pre-Rx GFP marking PB granulocytes (%/mononuclear fractions)</th>
<th>Peak GFP marking PB granulocytes (%/mononuclear fractions)</th>
<th>Days until decay to baseline</th>
<th>Toxicity</th>
</tr>
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<tr>
<td>RC701</td>
<td>VSV-G, MYFP-ir-DHFR²</td>
<td>Rx 1, d 137</td>
<td>T6 × 5, N3 × 5</td>
<td>No</td>
<td>0.3/ND</td>
<td>1.0/ND</td>
<td>15</td>
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<tr>
<td></td>
<td></td>
<td>Rx 2, d 174</td>
<td>T6 × 6, T8 × 1, N2.5 × 7</td>
<td>No</td>
<td>ND/ND</td>
<td>ND/ND</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rx 3, d 210</td>
<td>T6 × 2, T8 × 5, N3 × 7</td>
<td>No</td>
<td>0.3/ND</td>
<td>1.3/ND</td>
<td>24</td>
</tr>
<tr>
<td>RQ2278</td>
<td>RD114, MgiL22Y</td>
<td>Rx 1, d 80</td>
<td>T6 × 2, T8 × 5, N3 × 7</td>
<td>No</td>
<td>At least 3</td>
<td>1/3</td>
<td>12/19</td>
</tr>
<tr>
<td>RQ2258</td>
<td>RD114, MgiL22Y</td>
<td>Rx 1, d 109</td>
<td>T6 × 5, N3 × 5</td>
<td>No</td>
<td>2</td>
<td>1/2</td>
<td>10/13</td>
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<tr>
<td>96E113</td>
<td>RD114, MgiL22Y</td>
<td>Rx 2, d 193</td>
<td>T6 × 5, N3 × 5</td>
<td>Yes</td>
<td>At least 2</td>
<td>0.8/1.4</td>
<td>9/5</td>
</tr>
<tr>
<td>RQ2282</td>
<td>PG13, MDHFR/wc and ampho G1Na</td>
<td>Rx 1, d 424</td>
<td>T6 × 5, N3 × 5</td>
<td>Yes</td>
<td>1</td>
<td>0.8/0.9</td>
<td>No change</td>
</tr>
<tr>
<td>RQ2788</td>
<td>PG13, MDHFR/wc and ampho G1Na</td>
<td>Rx 2, d 480</td>
<td>T8 × 5, N2 × 5</td>
<td>Yes</td>
<td>4</td>
<td>0.8/1.0</td>
<td>Increased DNA copy no.</td>
</tr>
</tbody>
</table>

Rx indicates drug treatment; T6, TMTX 6 mg/kg per day; N3, NBMPR-P 3 mg/kg per day; ND, not determined; T8, TMTX 8 mg/kg; N2.5, NBMPR-P 2.5 mg/kg; NA, not applicable; plts, platelets; N2, NBMPR-R 2 mg/kg; ampho, amphotrophic.
Considering the possibility that in vivo selection may have been limited by low levels of baseline marking with the VSV-G vector, a second animal (RQ2278) received a transplant with cytokine-mobilized PB CD34+ cells that had been transduced with the previously described MGIRL22Y vector,32 packaged in HT1080 cells expressing the feline endogenous retrovirus (RD114) envelope protein.25 Eighty days after transplantation, RQ2278 was treated with TMTX 6 mg/kg for the first 2 days followed by 8 mg/kg for the next 5 days. NBMPR-P was coadministered at 3 mg/kg for 7 days. This drug dose caused severe myelosuppression with the ANC dropping below 0.5×10^9/L (500 cells/μL) on days 6 to 8 following the initiation of the cycle. This animal died on day 9 after treatment, with an ANC of 0 cells/μL and a platelet count of 24×10^9/L (24,000 cells/μL), and clinical manifestations of gastrointestinal (GI) bleeding. During this short time interval, significant increases in the percentage of GFP+ PB cells were noted (Figure 2A). On day 8, marking in granulocytes had increased from less than 1% to approximately 12%, gated monocytes from 3% to 19% on day 8, and a modest increase of lymphocyte marking from about 3% baseline to 5% on day 8 (Figure 2B).

**Significant levels of transient in vivo selection in animals RQ2258 and 96E113**

Monkey RQ2258 received a transplant with cells transduced with the RD114 MGIRL22Y vector (Table 1). Given the toxicity noted in RQ2278, this animal was treated at a reduced TMTX dose 109 days after transplantation (TMTX 6 mg/kg for 5 days and NBMPR-P 3 mg/kg for 5 days). This drug dose caused 2 days of neutropenia and 1 day of platelets below 100×10^9/L (100,000 cells/μL) (Figure 3A). Transient increases in the proportion of GFP+ PB cells were noted in granulocytes and monocytes after drug treatment (Figure 3B). With baseline marking levels less than 2%, peak granulocyte marking was noted on day 6 with an increase to almost 10%. The elevation above baseline was sustained through day 8. Marking returned to baseline levels by 17 days after the beginning of treatment. Toxicity associated with this course included dehydration and positive testing for fecal blood, both successfully managed with supportive care.

We reasoned that the transient nature of in vivo selection may have been due to the lack of HSCs cycling during the drug treatment course, considering that TMTX is an S-phase specific drug that requires active cell cycling for killing. To induce HSCs to cycle, we administered a second treatment course in RQ2258 that included treatment with peg-SCF and G-CSF, a combination previously shown to induce HSC amplification in mice.33 This treatment specifically was done by administering pegylated human SCF at 200 μg/kg and G-CSF at 10 μg/kg together for 8 days, starting 3 days before the first dose of TMTX and NBMPR-P and continuing through 5 days of cytotoxic drug...
treatment. The addition of cytokine stimulation prior to and during the 2nd course was associated with increased myelosuppression relative to the first treatment course, with ANC near 0 cells/μL on days 6 and 7, and a decrease in the platelet count to $24 \times 10^9/L$ (24,000 cells/μL) on day 7. This animal died late on day 7 because of staphylococcal sepsis and pneumonia. An autopsy showed this condition was due to a previously unrecognized catheter track infection. Between days 5 and 7 of the second treatment course, substantial in vivo selection of GFP-marked granulocytes was again noted (Figure 3B).

The fourth monkey, 96E113, has been previously reported for having achieved high levels of marking with the MGirL22Y vector prior to drug selection. These high levels of GFP-expressing cells subsequently decayed and were between 1% and 2% 690 days after transplantation. DNA analysis at this late time point showed correspondingly low levels of provirally marked cells, demonstrating that the decrease seen in GFP+ cells was not due to silencing of gene expression (Figure 4A). At this relatively late time point, the animal was treated with peg-SCF/G-CSF stimulation as before, along with TMTX 6 mg/kg for 5 days and NBMPR-P 3 mg/kg for 5 days. This drug treatment course caused significant myelosuppression between days 8 and 10 after treatment, with ANC below $0.5 \times 10^9/L$ (500 cells/μL) and platelet counts below $150 \times 10^9/L$ (150,000 cells/μL) during this 3-day interval (Figure 4B).

This treatment course was associated with the greatest degree of in vivo selection seen in our series. Baseline granulocyte marking went from approximately 1% to 19% on days 6 and 7 and did not decay to baseline until day 21 (Figure 4C-D). Similarly, monocyte marking went from less than 5% to 60% on day 7, with increased levels above baseline noted through day 16. There was no evidence for significance enrichment for GFP-marked lymphocytes in this case. This drug treatment course was additionally associated with dehydration, hypernatremia, and hyperglycemia that were successfully treated with appropriate fluid management. All together, these data show that transient in vivo selection can be obtained using the DHFR system. However, despite cytokine prestimulation, myelosuppressive nadirs were relatively brief, indicating the lack of significant HSC toxicity and selective pressure.

Selection experiments using a DHFR vector lacking the GFP or YFP marker

We next examined whether the lack of sustained enrichment for GFP-expressing PB cells could be due to loss of GFP expression over time or due to potential immune rejection of GFP-expressing cells. For these purposes, we developed a DNA-based detection system for proviral sequences contained within a DHFR
The present study used a nonhuman primate gene therapy model to test a cytotoxic drug selection system previously shown to be effective for selecting transduced HSCs in a murine transplantation model. In contrast to the results obtained in the mouse model, selective increases in marked cells were not sustained over time, and decay back to baseline levels occurred within approximately 2 weeks after drug therapy. Furthermore, substantially increased toxicity was evident in the primate model with complications including death, GI bleeding, infection, and metabolic abnormalities, although in a number of cases no toxicity was seen at all. These results illustrate the importance of testing HSC selection strategies in appropriate large animal model systems, which in this case gave different efficacy and safety results than in mouse models. Other selection systems have recently been described that also resulted in transient enrichment for genetically modified blood cells in large outbred animals. 39,40

Significant short-term selection was obtained in PB granulocytes and monocytes in a number of cases in our study. DNA analyses showed that these increases were not due to a simple increase in gene expression from drug treatment but rather due to a selection of MDHFR-wc-transduced cells.

RQ2282 was treated 424 days after transplantation with SCF/G-CSF for 8 days as described earlier, with coadministration of TMTX 6 mg/kg for 5 days and NBMPR-P at 2 mg/kg for 5 days. This first cycle of chemotherapy resulted in modest levels of myelosuppression, with 1 day with the ANC below 0.5 x 10^9/L (500 cells/μL) (Figure 5A). PCR analysis of DNA samples obtained from Ficoll-separated PB granulocyte and mononuclear fractions showed no change in the average DNA copy number of the DHFR vector, remaining at approximately 1% before and after treatment. No significant toxicity was associated with this first treatment course. A second treatment course was administered 56 days later, again using SCF and G-CSF priming for 8 days, with the TMTX dose increased to 8 mg/kg per day for 5 days and given concurrently with NBMPR-P at 2 mg/kg for 5 days. This dose escalation resulted in significantly increased myelosuppression (Figure 5A) with ANCs sustained below 0.5 x 10^9/L (500 cells/μL) for 4 days and 1 day of a platelet count below 100 x 10^9/L (100 000 cells/μL). With appropriate supportive care, no significant toxicity was noted with this second treatment course. PCR DNA analysis showed transient increases in vector copy numbers in both Ficoll-purified granulocyte and mononuclear fractions (Figure 5B). Granulocyte marking increased from 0.4% prior to treatment to 1.5% on day 9. Although the mononuclear sample from day 9 was inadequate for analysis, modest increases were noted in samples from days 7 and 17. For both cycles, there was no significant change in the PCR copy number for the G1Na vector.

Similarly, animal RQ2788 was treated with SCF/G-CSF stimulation concordant with TMTX 6 mg/kg per 5 days and NBMPR-P 2.5 mg/kg for 5 days starting 207 days after transplantation. This was associated with 2 days of neutropenia and moderate GI toxicity. Despite the modest degree of myelosuppression, an increase in the vector copy number was seen both in Ficoll-purified granulocytes and mononuclear cells from peripheral blood (Figure 6). Although marked granulocytes were undetectable prior to treatment, marking was easily detectable on days 7 to 24 and decayed to baseline by day 37. Marking in mononuclear cells increased from 0.1% copies to 0.8% copies on day 17.
transient increase in marked cells. The greatest increases were seen in animal 96E113, in which granulocytes were enriched more than 10-fold at their peak levels and monocyte levels by approximately 26-fold. The magnitude of this selection demonstrates in vivo expression of the MSCV-based DHFR vector in proliferating hematopoietic cells, presumably within a myeloid progenitor compartment. It is notable that in the case of 96E113, in vivo drug selection was obtained 690 days after transplantation with transduced cells, providing clear evidence of long-term retroviral expression in vivo in primates using the MSCV promoter system. It is also interesting to note that the degree of selection obtained may be somewhat a function of the initial engraftment efficiency of transduced HSCs. The lowest levels of selection were seen in animal RC701, in which the VSV-G vector resulted in extremely low levels of marked PB cells. Intermediate levels of transient selection were noticed in the next 2 cases, RQ2278 and RQ2258, and were associated with higher levels of transduction with the RD114-based vector. The highest degree of selection occurred in monkey 96E113, which initially had a very high proportion of vector-transduced PB cells, indicating that initial engraftment was efficient. Even though this level of engraftment decayed by the time selective therapy was administered, it was still higher than in any of the other animals at baseline levels. These results indicate that strategies that increase stem cell transduction efficiency and engraftment may improve the results obtained with in vivo selection strategies. Another point is that increases in GFP-expressing granulocytes were greater than seen in lymphocytes. This increase was likely due to the relatively long half-life of lymphocytes coupled with the acute and transient nature of the selection process. It also may reflect increased sensitivity of myeloid versus lymphoid progenitors to the antifolate drug regimen.

The transient nature of selection differs significantly from the stable stem cell selection that was seen in the murine model. One likely explanation for this difference is known differences in the proportion of HSCs actively in cycle in primates versus mice and the known fact that the DHFR/antifolate systems requires cell cycling for untransduced HSCs to be eliminated. Bromodeoxyuridine (Brdu)–labeling experiments have shown that in mice most of the total body stem cells have cycled at least once within 30 days. In contrast, Brdu-labeling experiments in baboons have shown significantly lower proportions of HSCs in cycle. This decrease in cycling is consistent with the relatively short but sometimes severe myelosuppression noted in the animals. It appears that pretreatment with G-CSF and peg-SCF was not sufficient to induce significant HSC cycling. The increase in total body stem cells seen in mice treated with these cytokines indicates that HSCs may respond differently in the murine model versus the rhesus macaque model or alternatively a different dosing schedule may be required. Other drug resistance systems such as MGMT that are less likely to be cycle dependent may be useful to overcome this primate-specific limitation. Indeed, the use of MGMT-related drugs such as temozolomide and nitrosourea are frequently associated with protracted and progressive myelosuppression in patients with cancer, probably indicating that these drugs are toxic to human HSCs.

Other potential mechanisms can be considered to explain the lack of sustained HSC selection in our study. It is possible that the MSCV promoter was not expressed in primitive HSCs; therefore transduced HSCs were not protected. Although we cannot definitively rule out this possibility, we do not favor this interpretation because the MSCV promoter is highly expressed in murine HSCs and has mediated HSC selection in mice. A second possibility would be that thymidine salvage pathways in primate HSCs were not adequately blocked with NBMPR-P. Although we were able to show high-grade inhibition of thymidine uptake in erythrocytes, it is possible that HSCs express higher levels of the eN nucleoside transporter than in erythrocytes or that HSCs could express an NMBPR-P insensitive transporter such as the ei nucleoside transporter.

These studies also illustrate that drug effects vary to a greater degree in outbred large animals in comparison to inbred mouse strains. Although toxicity patterns were relatively reproducible in our prior murine experiments, we noted quite a bit of dose response variability in the rhesus macaque model. For instance, the third treatment course of RC701 used the same drug doses as the first treatment course in animal RQ2278. RC701 experienced no toxicity, whereas RQ2278 died of myelosuppressive complications in the treatment. Similarly, significant toxicity was seen with 5-day treatment courses (RQ2258) but not with repeated 7-day treatment courses in RC701. Therefore, an in vivo selection system would ideally possess the advantage of relatively low levels of pharmacokinetic variability, both in terms of avoiding toxicity and in obtaining significant HSC selective pressure.

On the basis of this experience, the use of the DHFR selection system for stem cell enrichment does not appear feasible for clinical application. It may be possible to use this system for attenuating antifolate-induced myelosuppression if a sufficient number of engrafted cells can initially be established. In fact, the selective advantage for transduced cells, although transient, clearly reflects an in vivo protective effect conferred by DHFR gene transfer. Alternative selection systems will need to be explored in nonhuman primates, particularly those like MGMT-based systems in which the drugs used for selection are known to confer stem cell toxicity in humans.

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References


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