Trimetrexate Inhibits Progression of the Murine 32Dp210 Model of Chronic Myeloid Leukemia in Animals Expressing Drug-resistant Dihydrofolate Reductase

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ABSTRACT

Expression of drug-resistant forms of dihydrofolate reductase (DHFR) in hematopoietic cells confers substantial resistance of animals to antifolate administration. In this study, we tested whether the chemoprotection conferred by expression of the tyrosine-22 variant DHFR could be used for more effective therapy of the 32Dp210 murine model of chronic myeloid leukemia (CML). Administration of the maximum tolerated dose of trimetrexate (TMTX) with the nucleoside transport inhibitor prodrug nitrobenzylmercaptopurine ribose-5'-monophosphate (NBMPR-P) inhibited 32Dp210 tumor progression in mice engrafted with transgenic tyrosine-22 DHFR marrow and improved survival of tumor-bearing animals as long as drug administration was continued. NBMPR-P coadministration was necessary for maximal tumor inhibition, as administration of TMTX alone delayed but did not prevent tumor progression. The chemoprotection afforded by engraftment with transgenic tyrosine-22 DHFR marrow was necessary for effective chemotherapy, as normal mice lacking transgenic marrow could not tolerate the higher TMTX dose (60 mg/kg/day) administered to mice with transgenic marrow, and the decreased dose of TMTX with NBMPR-P tolerated by normal tumor-bearing animals did not inhibit tumor progression or improve animal survival. We conclude that TMTX with NBMPR-P inhibits tumor progression in the 32Dp210 model of CML in animals engrafted with drug-resistant tyrosine-22 DHFR transgenic marrow, and that based on this model the introduction of a drug-resistant DHFR gene into marrow combined with TMTX and NBMPR-P administration may provide an effective treatment for CML.

INTRODUCTION

CML is a disease of the hematopoietic stem cell, resulting in the expansion and premature circulation of relatively mature myelocytes. In most cases, CML is characterized by a translocation between chromosomes 22 and 9, resulting in fusion of the bcr and abl genes (1). The resulting bcr-abl fusion oncogene has been shown to be necessary and sufficient for malignant transformation of hematopoietic cells (2). Many murine models of bcr-abl CML result in syndromes resembling acute leukemia or lymphoma in a subset of tumor-bearing animals (2, 3) rather than chronic-phase myeloid leukemia. One such model is the 32Dp210 cell line (4), established by insertion of the bcr-abl cDNA in the murine myeloblast cell line 32D (5). Mice infused with 32Dp210 cells exhibited rapid infiltration of myeloblastic 32Dp210 cells into a variety of tissues, in a syndrome resembling blast-phase or acute myeloid leukemia (6).

Antifolates such as MTX and TMTX inhibit the enzyme DHFR (EC 1.5.1.3), resulting in depletion of reduced folates necessary for thymidylate and purine nucleotide synthesis, and toxicity for actively dividing cells (7). Various forms of DHFR containing amino acid substitutions have been identified that are less susceptible to inhibition by antifolates than wild-type DHFR (8–10) and can render cells resistant to antifolates. Introduction of a variant DHFR gene into mouse bone marrow has been shown to confer increased antifolate resistance to transplanted mice (11–15). The chemoprotection provided by drug-resistant marrow potentially allows for improved antitumor chemotherapy at greater antifolate doses (16). However, we observed previously that the higher doses of MTX afforded by drug-resistant DHFR expression in marrow caused an increase rather than a decrease in progression of the 32Dp210 murine model CML, suggesting that use of a different DHFR inhibitor would be necessary for improved antitumor chemotherapy (6).

The antifolate TMTX is a more specific inhibitor of DHFR than MTX, as MTX polyglutamates also directly inhibit thymidylate synthase as well as glycaminamide ribonucleoetide and aminomimidazole carboxamido ribonucleotide transformylases in de novo purine biosynthesis (17). Unlike MTX, TMTX does not rely on the reduced folate carrier for transport into cells and does not require polyglutamylamation for inhibition of DHFR (18). Both MTX and TMTX have been shown to be effective alone or in combination with other chemotherapeutic agents against breast cancer and a variety of other tumors, and TMTX is also effective for the treatment of Pneumocystis pneumonia (7, 19–21). Because reduced folates contribute to the formation of purine and thymidine nucleotides, salvage of exogenous purine nucleosides and thymidine can rescue cells from antifolate toxicity (22, 23). However, nucleoside transport inhibitors can restore MTX toxicity to cells expressing wild-type DHFR while maintaining differential toxicity relative to cells expressing drug-resistant DHFR (24).

This paper describes the effect of TMTX in combination with the nucleoside transport inhibitor prodrug NBMPR-P on progression of the 32Dp210 tumor model in normal mice and in mice protected from drug toxicity by engraftment with tyrosine-22 DHFR transgenic marrow. Co-administration of TMTX + NBMPR-P in mice engrafted with transgenic marrow inhibited progression of 32Dp210 tumor and improved survival of tumor-bearing mice engrafted with DHFR transgenic marrow as long as drug administration was continued. Engraftment with drug-resistant transgenic marrow was found to be necessary for chemotherapy, as the maximal drug dose tolerated by animals lacking drug-resistant DHFR was insufficient to inhibit tumor progression or improve animal survival. These results suggest that expression of drug-resistant tyrosine-22 DHFR in the marrow with subsequent administration of an appropriate antifolate may provide effective treatment for CML.

MATERIALS AND METHODS

32Dp210 Tumor Cell Line and Culture. The C3H mouse-derived, bcr-abl enhanced-GFP myeloblast cell line 32Dp210+GFP was derived by retroviral transduction of the eGFP gene into 32Dp210 cells (6) and was maintained in RPMI 1640 (Life Technologies, Inc., Invitrogen, Carlsbad, CA) supplemented with 10% newborn calf serum (Summit Biotechnology, Fort...
Collins, CO), 2 mM glutamine (Sigma-Aldrich, St. Louis, MO), 50 units/ml penicillin, 50 μg/ml streptomycin, and 0.125 μg/ml fungizone (Life Technologies, Inc.). These cells were maintained in a humidified atmosphere at 37°C and 5% CO₂.

Animals and Bone Marrow Transplant. C3H-He/J and FVB/N mice were obtained at 6–8 weeks of age from the NIH facility at Frederick, MD. The tyrosine-22 DHFR transgenic FVB/N mice (line 11) used for these experiments were described previously (15). F1 offspring of C3H × FVB/N matings were designated C3F-F1 mice; line 11 DHFR transgenic FVB/N mice were used in mating pairs with C3H-He/J mice to generate tyrosine-22 DHFR transgenic C3F-F1 mice, as described previously (6). Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility according to institutional guidelines.

For bone marrow transplant, marrow was flushed from femurs and tibiae of euthanized transgenic C3F-F1 mice. Transgenic marrow cells (5 × 10⁶) were injected i.v. into lethally irradiated (800 rads cesium) nontransgenic C3F-F1 mice. Animals were allowed to engraft for 2 months before subsequent tumor administration and/or chemotherapy.

Tumor Administration and Therapy. 32Dp210+GFP tumor cells were injected in 0.5 ml of PBS (Celox Laboratories, Inc., St. Paul, MN) through the tail vein into C3F-F1 mice. TMTX (Medimmune Oncology, Inc., Gaithersburg, MD) and NBMPR-P (Alberta Nucleoside Therapeutics, Alberta, Ontario, Canada) were solubilized in water and stored at −20°C as stock solutions after filter sterilization. Starting 1 day after tumor injection, PBS, TMTX, and 20 mg/kg NBMPR-P were administered daily by independent i.p. injection as indicated in “Materials and Methods.” Animal weight and health were monitored daily, and moribund animals were euthanized by CO₂ asphyxiation. The spleen was collected from moribund animals and assessed for leukemia-associated splenomegaly (expressed as percentage of spleen weight; spleen weight/total animal weight at death × 100). Animals with >0.5% spleen weight were scored as leukemic, based on measurements from tumor-free control animals. At regular intervals, peripheral blood was collected from the peripheral vein into heparinized microhematocrit capillary tubes (Fisher Scientific, Pittsburgh, PA) for hematocrit determination or flow cytometric analysis. Statistical comparison of survival between different groups was conducted by using the Kaplan-Meier product limit method (25) and calculating the log-rank statistic (26).

Flow Cytometric Analysis. Flow cytometry was used to follow the in vivo growth of 32Dp210+GFP tumor. Blood samples were transferred into tubes containing an equal volume of 1,000 units/ml heparin sodium salt solution (ICN Biomedicals Inc., Aurora, OH). The RBCs were lysed, and nucleated cells were fixed either using an ammonium chloride solution (8.99 g of NH₄Cl, 1 g of KHCO₃, and 37 mg tetrasodium EDTA/liter; Sigma-Aldrich) and 0.5% paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA), respectively, or by using Optifyle B solution (Immunotech, Marseille, France). Approximately 10,000 cells/sample were analyzed for GFP⁺ tumor cells using the FITC/GFP channel on a Becton Dickinson FACScalibur (BD Immunocytometry Systems, San Jose, CA) within 96 h of preparation. The background level of fluorescence intensity was determined using a sample prepared from a control animal that had not received tumor, whereas a sample of 32Dp210+GFP cells was used as a positive control. A GFP⁺ cell percentage of ≥1% was deemed substantial, based on samples from negative control animals. Flow cytometric data were analyzed using FlowJo 3.2 software (Tree Star, Inc., San Carlos, CA) to determine the percentage of cells in each sample that exhibited GFP fluorescence greater than background.

RESULTS

TMTX with NBMPR-P Improves Survival and Inhibits Tumor Progression in 32Dp210 Tumor-bearing Mice Engrafted with DHFR Transgenic Marrow. To test the effect of TMTX and NBMPR-P on 32Dp210+GFP tumor progression, marrow from tyrosine-22 DHFR transgenic C3F-F1 mice was transplanted into lethally irradiated normal C3F-F1 recipients (see “Materials and Methods”). After engraftment, animals were infused with 10⁶ 32Dp210+GFP tumor cells and subsequently administered either PBS or 80 mg/kg/day TMTX and 20 mg/kg/day NBMPR-P (based on parallel dose-response experiments in C57BL/6 × FVB/N F1 animals). This dose was toxic for both tumor-bearing and tumor-free control animals after 8 days as evidenced by animal mortality in both groups (Fig. 1A). Therefore, drug administration was withdrawn for 4 days and then resumed at a lower TMTX dose of 60 mg/kg/day, maintaining the NBMPR-P dose at 20 mg/kg/day.

We found that whereas PBS-administered control animals succumbed to tumor ~20 days after infusion of tumor cells, there was no tumor-related mortality observed in drug-treated animals out to 45 days during drug administration (Fig. 1, A and B). At this point, it appeared as if TMTX + NBMPR-P had cured the animals of the 32Dp210 tumor. However, after drug administration was stopped (on day 49) the majority of surviving animals succumbed to tumor-related mortality within 1 month (Fig. 1B). Thus, TMTX + NBMPR-P delayed 32Dp210 tumor onset but did not completely eliminate the tumor. The difference in survival between tumor-bearing animals administered TMTX + NBMPR-P and animals administered PBS was statistically significant (P < 0.011), with an extension in median survival time from 18 days for the PBS group out to 64 days for the TMTX + NBMPR-P group. Thus, the maximum tolerated dose of 60

![Image](https://example.com/image.png)
mg/kg/day TMTX with NBMPR-P inhibited tumor progression and improved survival of tumor-bearing animals as long as drug administration was continued.

**NBMPR-P Coadministration Improves Survival and Enhances Tumor Inhibition by TMTX in 32Dp210+GFP Tumor-bearing Mice Engrafted with DHFR Transgenic Marrow.**

To determine whether nucleoside transport inhibition was necessary for TMTX to inhibit 32Dp210+GFP tumor progression, C3F-F1 mice engrafted with DHFR transgenic drug-resistant marrow (as in the previous experiment) were infused with 10^6 32Dp210+GFP tumor cells and subsequently administered PBS, 60 mg/kg TMTX, or 60 mg/kg TMTX with NBMPR-P daily. Drug toxicity again required a brief period of withdrawal (from days 20 to 23 after tumor administration), after which drug administration was resumed at the same dose. The difference in animal survival between tumor-bearing animals administered PBS and those administered drug was not statistically significant, but the median survival time was extended from 29 days for animals administered PBS to 61 days for animals administered TMTX, and to 83 days for animals administered TMTX with NBMPR-P (Fig. 2A). Animals administered TMTX or TMTX + NBMPR-P also exhibited a delay in tumor-related mortality compared with animals administered PBS (Fig. 2B). During the course of drug administration, no tumor-related deaths were observed in animals administered TMTX with NBMPR-P. In striking contrast, 71% tumor-related deaths were observed in animals administered TMTX without NBMPR-P (Fig. 2B), indicating the effectiveness of the drug combination in comparison with TMTX alone. To determine whether prolonged treatment could completely eliminate 32Dp210+GFP tumor, drug administration was continued out to 78 days, after which time both drug-related and tumor-related deaths were observed in animals treated with TMTX + NBMPR-P (as determined by the absence and presence of splenomegaly, respectively). Thus, 60 mg/kg/day of TMTX alone delayed tumor-related mortality compared with animals administered PBS, but coadministration of NBMPR-P along with TMTX provided an even greater delay in tumor-related mortality.

Tumor load in each animal was measured by flow cytometry to detect fluorescent GFP^+ tumor cells in peripheral blood samples collected during the course of the experiment. As observed previously (6), PBS-administered animals exhibited only background levels of GFP^+ cells in their peripheral blood (Fig. 3A). Many of the animals administered 60 mg/kg/day of TMTX exhibited detectable (>1%) GFP^+ tumor in the peripheral blood (Fig. 3B). This result is reminis-
cent of the exacerbation of 32Dp210 tumor progression caused by MTX that we reported recently (6). In contrast, GFP+ cells were not detectable in animals administered 60 mg/kg/day of TMTX with NBMPR-P (Fig. 3C). Taken together with the survival and tumor-related morbidity data (Fig. 2), these results show that administration of 60 mg/kg/day of TMTX alone improved survival and delayed tumor-related mortality but did not prevent tumor progression during the course of drug administration, whereas TMTX + NBMPR-P additionally prevented tumor expansion and emergence into peripheral blood for as long as drug administration was continued.

Engraftment with DHFR Transgenic Marrow Is Necessary for TMTX and NBMPR-P to Improve Survival and Inhibit Tumor Progression in 32Dp210+GFP Tumor-bearing Mice. To determine whether the chemoprotection afforded by drug-resistant marrow is necessary for TMTX and NBMPR-P to inhibit tumor progression, we also tested normal C3F-F1 animals lacking drug-resistant marrow. Normal mice received 107 32Dp210+GFP tumor cells followed by daily administration of PBS, 40 mg/kg of TMTX with NBMPR-P, or 60 mg/kg of TMTX with NBMPR-P. Tumor-bearing animals engrafted with drug-resistant marrow were administered 60 mg/kg/day of TMTX with NBMPR-P, the maximum dose tolerated as determined in the experiments described above. The combination of 60 mg/kg/day of TMTX with NBMPR-P was lethal for mice lacking drug-resistant marrow by day 42 (Fig. 4A), with the average hematocrit falling to 15 at day 24 (Fig. 4C). Whereas normal mice were able to tolerate a lower dose of 40 mg/kg/day of TMTX with NBMPR-P for an extended period of time, this regimen did not significantly improve animal survival compared with tumor-bearing animals administered PBS (Fig. 4A). In contrast, survival of tumor-bearing animals transplanted with drug-resistant marrow was significantly improved by administration of 60 mg/kg/day of TMTX with NBMPR-P (P < 0.0002 at day 42 compared with normal mice at the maximum tolerated drug dose), and the median survival time was extended to 54 days from 18 days for animals receiving PBS. Tumor-related deaths were observed in normal animals administered either PBS or 40 mg/kg/day of TMTX with NBMPR-P (Fig. 4B; as determined by % spleen weight, Fig. 4D), whereas tumor-related deaths were not observed in mice transplanted with drug-resistant marrow and administered 60 mg/kg/day TMTX with NBMPR-P (Fig. 4B) until after drug administration was stopped, consistent with the previous experiments (Figs. 1 and 2). The increased number of tumor-related deaths in animals transplanted with drug-resistant marrow in this experiment compared with the experiments shown in Fig. 2 was most likely the result of the higher tumor dose used (107 cells) and a shorter duration of drug administration. PBS-administered animals exhibited only background levels of GFP+ cells in the peripheral blood during the first month (Fig. 5A), whereas many of the normal mice administered 40 mg/kg/day of TMTX with NBMPR-P exhibited detectable (>1%) GFP+ tumor in peripheral blood (Fig. 5B). In contrast, GFP+ cells were not observed in either normal animals (Fig. 5C) or in animals transplanted with drug-resistant marrow (Fig. 5D) when administered 60 mg/kg/day of TMTX with NBMPR-P (except for 1 animal at the last time point tested; Fig. 5D). Taken together with the tumor-related mortality data, these results show that 60 mg/kg/day of TMTX with NBMPR-P, the dose required to inhibit tumor progression, can only be achieved in animals that have been protected from antifolate toxicity by engraftment with drug-resistant DHFR transgenic marrow, as these drug doses were rapidly toxic for animals lacking the drug-resistance gene.

Fig. 4. Effect of TMTX and NBMPR-P on tumor-bearing normal C3F-F1 mice and mice transplanted with tyrosine-22 DHFR transgenic marrow. A, Kaplan-Meier plot of animal survival. Mice received 107 32Dp210+GFP tumor cells by i.v. injection on day 0 (n = 8 for each group) and were subsequently administered PBS, 40 mg/kg/day of TMTX with NBMPR-P, or 60 mg/kg/day of TMTX with NBMPR-P by i.p. injection until day 42. B, tumor-related mortality. Presence of tumor at death was determined as described in “Materials and Methods.” C, hematocrits were determined from peripheral blood samples collected at regular intervals during the first month of drug administration. Each point is the mean hematocrit for surviving mice from the group at that time point. SDs were <10 at all points. A hematocrit level of ≥35 was deemed normal (indicated by a vertical line (“’)), based on samples from untreated control animals. D, percentage of spleen weights of moribund animals. The mean percentage of spleen weight for each group is also shown (indicated by a vertical line (“’)). A spleen weight >0.5% of the total weight of the animal (indicated by “)” was deemed evidence of tumor-associated splenomegaly.
TRIMETREXATE IN A MURINE MODEL OF CML

Fig. 5. Effect of TMTX and NBMPR-P on tumor progression in normal C3F-F1 mice and mice transplanted with tyrosine-22 DHFR transgenic marrow. Mice were administered TMTX and NBMPR-P as described in the legend to Fig. 4. Peripheral blood samples were collected from (A) normal mice administered PBS, (B) normal mice administered 40 mg/kg/day of TMTX with NBMPR-P, (C) normal mice administered 60 mg/kg/day of TMTX with NBMPR-P, or (D) mice transplanted with tyrosine-22 DHFR transgenic marrow and administered 60 mg/kg/day of TMTX with NBMPR-P. GFP+ tumor cells in peripheral blood samples were quantified by flow cytometry as described in "Materials and Methods.” Values shown are the GFP+ cells expressed as a percentage of the total number of cells analyzed from individual mice. A GFP+ cell percentage of ≥1% (indicated by “•”) was deemed substantial.

DISCUSSION

The effectiveness of TMTX and the nucleoside transport inhibitor prodigynine (NBMPR-P) as antitumor agents was assessed in the 32Dp210 murine model of CML to evaluate a drug-resistance gene therapy approach for treatment of CML. The combination of TMTX and NBMPR-P was found to improve animal survival and inhibit progression of the 32Dp210 tumor in mice engrafted with drug-resistant tyrosine-22 DHFR transgenic marrow. Co-administration of NBMPR-P was necessary for maximal inhibition of tumor, as administration of TMTX alone delayed but did not prevent tumor progression. The chemoprotection afforded by engraftment with DHFR transgenic marrow was necessary for effective chemotherapy, as mice lacking transgenic marrow could not tolerate the higher drug doses (60 mg/kg/day of TMTX + NBMPR-P) required to confer an antitumor effect.

Chemotherapy for treatment of CML has been only partially successful, consisting primarily of IFN-α, which can induce hematological remission and prolong survival but is not curative (27, 28). Recently, the selective Abl tyrosine kinase inhibitor STI571 has proved effective in inhibiting growth of bcr-abl” cells (29) and has shown great promise in clinical trials for treatment of chronic-phase CML (30), although significant numbers of patients with blast-phase CML and bcr-abl” acute lymphoid leukemia are refractory to STI571 treatment or relapse after treatment with STI571 (31). Resistance to STI571 in these patients has been attributed to bcr-abl gene amplification or point mutations resulting in amino acid substitutions in the tyrosine kinase domain of bcr-abl (32, 33).

Allogeneic marrow transplant has been established as a curative treatment for CML (27). However, the availability of suitable donor material is a concern, and graft-versus-host disease has been observed in up to 68% of CML patients transplanted with HLA-matched sibling donor marrow (34). Autologous marrow transplants for treatment of CML typically result in relapse either because of incomplete ablation of leukemia in the host during the preparative regimen for bone marrow transplant or contamination of the graft with bcr-abl” tumor cells (35). Introduction of a drug-resistance gene into autologous marrow by retroviral transduction could allow for drug administration after transplant to selectively eliminate leukemic cells that lack the drug-resistance gene. One problem with this approach is the potential for introducing the drug-resistance gene into tumor cells contaminating the donor marrow. We have investigated one means of addressing this problem for CML by introduction of antisense sequences directed against the fusion region of the bcr-abl message, thus decreasing bcr-abl expression and restoring a more normal phenotype to transduced bcr-abl” cells in the graft. This antisense strategy was examined previously in the 32Dp210 model of CML, resulting in a 3 log-fold reduction in tumorigenicity of 32Dp210 cells transduced with a retroviral vector containing the drug-resistant tyrosine-22 DHFR gene as well as antisense sequences directed against the bcr-abl breakpoint region (36).

Numerous studies have reported that expression of drug-resistant DHFR in hematopoietic cells can protect mice from toxic doses of MTX or TMTX (11–15). Additionally, TMTX in combination with nucleoside transport inhibition has been used effectively for in vivo selection of drug-resistant murine hematopoietic stem cells (38, 39). Administration of higher doses of antifolates in protected animals could allow for more effective treatment of tumors known to be sensitive to antifolates (16) or against tumors that are not usually treated with antifolates, such as CML. However, we observed previously in the 32Dp210 model of CML that administration of MTX in tumor-bearing mice engrafted with tyrosine-22 DHFR transgenic marrow did not inhibit tumor progression or improve survival of tumor-bearing animals, but surprisingly exacerbated 32Dp210 tumor progression, suggesting that other drugs or drug-resistance genes would be necessary for an antitumor effect (6).

Protection of the hematopoietic system from drug toxicity has been examined for other drug-resistance genes, in particular the MGMT gene (40) and the MDR1 gene (41). Expression of variant MGMT genes has been shown to confer resistance to the combination of O6-benzylguanine and DNA-alkylating agents such as 1,3-bis(2-chloroethyl)-1-nitrosourea and temozolomide, and to allow selective expansion of transduced hematopoietic cells in drug-administered animals without prior myeloablation (42, 43). MDR1 gene expression has been shown to confer cellular resistance to a number of chemotherapeutic agents including Taxol, and to allow selective expansion of transduced murine bone marrow cells (44). Despite this progress in demonstrating the expression of drug resistance genes for protection from toxicity in experimental animals, application of such a chemoprotective effect for improved antitumor chemotherapy has not been studied extensively. Koc et al. (45) reported a significant delay in progression of SW480 tumor xenografts in nude mice administered multiple cycles of O6-benzylguanine and 1,3-bis(2-chloroethyl)-1-nitrosourea after transplantation with marrow transduced using a retroviral vector encoding the G156A mutant MGMT. Hanania and Deisseroth (46) reported improved antitumor effect of Taxol at doses tolerated only in animals transplanted with MDR1-transduced marrow. Human clinical trials have been conducted involving hematopoietic transduction with MDR1 retrovirus (47–51), although the effectiveness of the approach as an antitumor strategy has yet to be determined. Using the DHFR system, Zhao et al. (16) reported im-
proved survivability of EO11 breast tumors in mice administered MTX at doses tolerated only after transplantation with marrow which had been transduced by a retroviral vector encoding the F31S mutant DHFR. Here we demonstrate the prevention of 32Dp210 tumor outgrowth and improved animal survival by administration of TMTX with NBMPR-P at doses that required transplantation with marrow expressing drug-resistant DHFR (L22Y DHFR in this case). Drug administration at lower doses maximally tolerated by normal, untransplanted animals did not significantly affect tumor progression, demonstrating a necessity for drug-resistance gene expression. These results additionally substantiate the concept of drug-resistance gene transfer and expression for improved antitumor chemotherapy, particularly for the use of antifolates in combination with nucleoside transport inhibition.

In light of the incidence of STI571-resistant tumor in blast-phase leukemia patients, an antisense/drug-resistance gene therapy approach may prove beneficial for treatment of STI571-resistant bcr-abl+ leukemia. Marrow transduction with antisense sequences directed against the bcr-abl fusion region may be effective in decreasing expression of bcr-abl in STI571-resistant cells, both in the case of gene amplification and in the case of mutations within the tyrosine kinase domain, potentially restoring sensitivity of the tumor cells to STI571 and decreasing cell tumorigenicity. Additionally, expression of a drug-resistant DHFR gene may allow for increased antifolate administration after engraftment of transduced marrow for effective chemotherapy against STI571-resistant leukemia.

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