Angiogenesis Inhibitor TNP-470 during Bone Marrow Transplant: Safety in a Preclinical Model

Julie W. Stern, Junjie Fang, Suzanne Shusterman, Giuliana Pierson, Rosalind Barr, Bruce Pawel, Lisa Diller, and Stephan A. Grupp

Division of Oncology [J. W. S., J. F., S. S., G. P., R. B., S. A. G.] and Division of Pathology [B. P.], Department of Pediatrics, Children’s Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, and Pediatric Oncology, Dana Farber Cancer Institute, Boston, Massachusetts 02135 [L. D.]

ABSTRACT

High-dose therapy with stem cell rescue is a treatment option for patients with advanced solid tumors. Although this approach has promise for some pediatric cancers, especially neuroblastoma, it is limited by the risk of relapse posttransplant as well as concern about possible reinfused tumor cells in autologous stem cell products. Antiangiogenic agents given during and after recovery from high-dose therapy with stem cell rescue may decrease the risk of relapse. TNP-470 is an antiangiogenic agent now in clinical trials. Although it inhibits the growth of bone marrow (BM) colony-forming cells in vitro, no significant hematological toxicity has been seen in Phase I trials. To assess the feasibility of using antiangiogenic agents during the period of posttransplant hematopoietic engraftment, we have developed a model of stem cell transplant in mice. Mice were lethally irradiated and then rescued with stem cells containing a transgene expressed in the hematopoietic lineage. Mice were then treated with TNP-470 or placebo, and assessed for survival, successful engraftment, and kinetics of engraftment. Both treated and control mice demonstrated reliable multilineage engraftment as well as normal lymphoid maturation with no excess mortality in the treated group. WBCs were lower but still within the normal range at d+28 in mice treated with bolus TNP-470, but not in those treated with continuous infusion TNP-470, compared with controls. These data indicate that inhibitors of angiogenesis do not adversely impact engraftment after stem cell transplantation.

INTRODUCTION

Intensified chemotherapy has improved survival rates for some patients with high-risk solid tumors. This has included patients with relapsed Hodgkin’s disease as well as pediatric patients with chemotherapy-responsive malignancies such as Ewing’s sarcoma and neuroblastoma (1, 2). Increases in dose-intensification have been facilitated by improved supportive care, the use of hematopoietic growth factors, the use of BM as stem cell support and, more recently, the use of peripheral blood progenitor cells to allow rapid return of marrow function after myeloablative chemotherapy. This approach has been termed “megatherapy” or high-dose chemotherapy with stem cell rescue. However, even the most dose-intensified approaches are still limited by the risk of relapse after the procedure.

One major risk factor for relapse after high-dose chemotherapy with stem cell rescue is the presence of bulk disease before the stem cell procedure. Even for patients who are in complete remission, however, relapse is still a concern. For these patients, relapse may arise from minimal residual disease within the patient or tumor inadvertently reinfused with the stem cell product. There is indirect evidence suggesting that reinfused tumor may sow the seeds for later relapse. In neuroblastoma, gene-marked tumor cells infused with BM used to support high-dose chemotherapy can be detected at sites of subsequent relapse (3, 4). In patients with lymphoma who undergo stem cell transplantation, molecular detection of tumor in the stem cell product is a predictor for relapse (5). However, no trial has shown an advantage for patients who receive stem cell products processed in an attempt to remove or decrease infused tumor.

To translate increased rates of complete remission provided by modern chemotherapy into additional improvements in survival, other approaches are needed. The antiangiogenic agents may represent one such approach. Antiangiogenic therapy has shown promising results in animal studies (6–10) and has been relatively nontoxic in early human clinical trials. Although Phase I development has inevitably focused on the treatment of relapsed patients with bulk disease, the angiogenesis inhibitors may prove to have the greatest efficacy when given in the state of minimal residual disease after achieving the best result possible with chemotherapy. Because these drugs have their effect at the level of normal (nontransformed) endothelium (11), clonal evolution or induced chemotherapy resistance within the tumor should not affect the response to angiogenic agents (10). Given post-stem cell infusion in the setting of high-dose chemi-

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2 To whom requests for reprints should be addressed, at ARC 902, Children’s Hospital of Philadelphia, 3516 Civic Center Boulevard, Philadelphia, PA 19104. Phone: (215) 590-2821; Fax: (215) 590-3770; E-mail: grupp@email.chop.edu.

3 The abbreviations used are: BM, bone marrow; BMT, bone marrow transplant; TBI, total body irradiation; Tg, transgene; CFU, colony-forming unit.
otherapy with stem cell rescue, antiangiogenic agents have potential to lessen the risk of relapse from minimal residual disease, whether within the patient or infused with the stem cell support. Here, we investigate the feasibility of antiangiogenic treatment initiated immediately after stem cell infusion in a mouse transplant model. The angiogenesis inhibitor used in these studies, TNP-470, is currently in clinical trials. TNP-470 is active in mouse xenograft models in bulk disease, with even greater efficacy apparent in the setting of minimal residual disease (12–14).

MATERIALS AND METHODS

Donors, Recipients, and Preparative Regimen. Transgenic mice expressing a human IgM Tg in the FVB background were used as the donor source of BM stem cells for transplantation. Marrow was collected from Tg+ mice from femurs flushed with sterile PBS. Recipients were FVB mice (Jax, Bar Harbor, ME) or Tg− littermates of the Tg+ donors, treated in groups of five to eight animals/intervention. Recipients received TBI in an M38–1 Irradiator (Isomedix) at a dose rate of 2.7 Gy/min in a mixed/split fashion with a 3 h interfraction interval to allow a higher dose of radiation without significant gastrointestinal toxicity. After completion of TBI, mice received stem cells via tail vein injection. The mice were maintained in a humidity and temperature controlled area in autoclaved microisolator cages and fed ad libitum and provided acidified water. Mice were assessed three times weekly after stem cell infusion. These studies were approved by the Animal Care and Use Committee of the Children’s Hospital of Philadelphia.

Drug. TNP-470 was provided by TAP Pharmaceuticals (Deerfield, IL). Before use, TNP-470 was reconstituted in sterile saline, aliquotted, and stored at −80°C. TNP-470 was used at a dose of 20–100 mg/kg given s.c. three times per week beginning at day 0 or at a dose of 10–20 mg/kg/week given by continuous i.p. infusion using an Alzet infusion pump (Alza Co., Palo Alto, CA). The continuous TNP-470 infusion began on the day before transplant to allow for pump implantation (see below).

Alzet Infusion Pump Placement. Using sterile technique after anesthesia, a 1-cm midline abdominal incision was made and 14-day Alzet micro-osmotic pumps (0.25 μl/h; Model 1002) containing either TNP-470 or saline were placed i.p. The peritoneum and skin were then secured separately using 4.0 Vicryl suture. The animals were allowed to recover overnight and then subjected to TBI and stem cell infusion on the following day.

BM Culture. Light density cells separated by density gradient centrifugation (Lymphocyte Separation Medium; ICN Pharmaceutical, Costa Mesa, CA) from normal human BM donors were plated in methylcellulose medium with recombinant cytokines. This medium, MethoCult GF (Stem Cell Technologies, Vancouver, Canada), contains stem cell factor (50 ng/ml), granulocyte macrophage colony-stimulating factor (10 ng/ml), interleukin-3 (10 ng/ml), and erythropoietin (3 units/ml). BM cells (5 × 10^5)/dish were cultured with TNP-470 at concentrations ranging from 1 μg/ml to 1 mg/ml, with duplicate cultures at each dose. The plates were scored after 14 days of culture, enumerating CFU-granulocyte/macrophage, CFU-mix, CFU-erythrocyte, and burst-forming unit erythrocyte.

Analysis of Engraftment. Engraftment of donor stem cells was demonstrated by both flow cytometry and by PCR. After cervical dislocation, BM and splenocytes were collected from recipient mice. Analysis was performed after red cell lysis by NH4Cl. For flow cytometric analysis of lymphoid engraftment, Tg IgM expressed only in B cells derived from the donor was detected by antibodies recognizing human IgM (RAHM; Jackson ImmunoResearch, West Grove, PA) and mouse CD45R (B220; PharMingen, Torreyana, CA) in a two-color protocol on a FACS Caliber cytometer (Becton Dickinson, Franklin Lakes, NJ). Splenocytes from untransplanted Tg− and Tg+ mice provided negative and positive controls, respectively. Lymphoid engraftment was defined as the percentage of lymphoid cells in spleen and BM that were B220/RAHM-positive. In addition to flow cytometry, genomic DNA from tail snips, blood, BM, and splenocytes was analyzed by PCR for the presence of the Tg, using a procedure and primers previously reported (15). The Tg is detectable by PCR in all donor-derived cells in the recipient, whereas the tail snips of Tg− mice provided a negative control. Genomic DNA was also isolated from splenic and peripheral blood T cells. Splenic T cells were isolated using the Cellect T isolation column (Biotex, Edmonton, Canada) according to the manufacturer’s protocol. Peripheral blood T cells were sorted on the FACS Vantage (Becton-Dickson) after staining with antibodies recognizing mouse CD3 (PharMingen). Recovery of peripheral blood counts was also analyzed. Blood was collected from cardiac puncture and placed in EDTA tubes. Analysis was then performed using a HemeVet instrument using mouse-specific parameters. Hemoglobin was measured and WBCs and platelets were enumerated.

RESULTS

Effect of TNP-470 on BM Colony-forming Cells. Although hematological toxicity has not been described in the TNP-470 Phase I trials (16, 17), there is one report of in vitro evidence of BM toxicity (18). To confirm this finding, we investigated the effect of relatively high concentrations of TNP-470 (0–100 μg/ml) on the growth of human hematopoietic progenitors in standard methylcellulose culture. As shown in Fig. 1, inhibition of colony formation in the presence of TNP-470 was observed for both myeloid and erythroid colonies. Four μg/ml of TNP-470 caused >80% inhibition of colony formation, and higher doses caused complete inhibition of colony-forming cells. Although this assay is not necessarily predictive of in vivo BM toxicity, the result emphasizes the need to develop a preclinical model of stem cell transplantation to assess the effect of antiangiogenic agents on engraftment.

Validating the Stem Cell Transplant Model. Our goal in developing a model of stem cell transplant in which to test the effects of angiogenesis inhibitors on engraftment was to define a dose of TBI that was lethal without stem cell rescue and then to define a threshold stem cell dose that reliably provided engraftment. The lethal dose of radiation was determined by tail vein injection of 0 or 5 × 10^7 BM cells after varying doses of TBI. As shown in Table 1, mortality with and without marrow support was investigated at TBI dose levels of 500 cGy (300 cGy followed by 200 cGy), 700 cGy (400/300) and 900 cGy (500/400). Mice given 900 cGy had 80–100% mortality when
Safety of TNP-470 Post-BMT

These experiments, engraftment was defined as demonstrating a small effect of TNP-470 on engraftment. For a threshold stem cell dose would increase the likelihood of reliably providing engraftment in most recipients. Choosing such studies, we then sought a minimum stem cell dose that would support hematologic recovery. Eight to 10 recipient mice/group with two (500 cGy) or three (700 and 900 cGy) groups evaluated at each dose.

Lethally irradiated mice cells in the spleen or BM as detected by flow cytometry on day 28 after stem cell infusion (d28). No stem cell support was given and only 0–12.5% mortality when 5 × 10^6 BM cells were infused.

Having chosen the TBI dose of 900 cGy for additional studies, we then sought a minimum stem cell dose that would reliably provide engraftment in most recipients. Choosing such a threshold stem cell dose would increase the likelihood of demonstrating a small effect of TNP-470 on engraftment. For these experiments, engraftment was defined as >5% Tg + B cells in the spleen or BM as detected by flow cytometry on day 28 after stem cell infusion (d+28). Lethally irradiated mice were injected with BM doses ranging from 0 to 5 × 10^6 cells in 1 × 10^6 dose intervals. Mortality was determined by observation, and engraftment of B cells was determined at each dose level by flow cytometry (Table 2). A cell dose of 2–3 × 10^6 stem cells/mouse was found to have an 8–40% rate of mortality with a 75–100% rate of BM engraftment thus assuring consistent engraftment at a minimum cell dose. The radiation and cell doses established a baseline that was used in TNP-470 experiments.

**Effect of TNP-470 on Engraftment.** Several dose levels of TNP-470 were explored to assess any effect on BM engraftment and engraftment kinetics. These dosing regimens are summarized in Table 3. Immediately after lethal irradiation and tail vein injection of Tg + BM cells, recipient mice were given either TNP-470 or saline starting on day 0. Dose schedules were also varied from a single dose at the time of stem cell infusion to an initial dose on day 0 with subsequent administration of TNP-470 or saline three times a week (Table 3). Mice were initially sacrificed on day 28–32 at all dose levels. Comparative kinetics were then analyzed further by sacrificing groups of mice at days 21, 24, and 28 at dose level 3.

Overall survival at all dose levels was 73% for mice treated with placebo and 66% for TNP-470 treated animals (Fig. 2). When analysis was completed at dose level 1 (20 mg/kg on day 0 and then thrice weekly), survival among treated mice was 57%; survival was 64% for controls. Furthermore, for dose level 2, (100 mg/kg on day 0 only), 71% of treated mice survived to experiment completion, whereas 76% of control mice were alive at day 28–32, which was statistically not significant. At the doses tested, these data provide no indication of a dose-dependent effect of TNP-470 on post-BMT survival. Toxicities overall were minimal, although the treated mice at dose level 4 (100 mg/kg, three times/week) experienced greater weight loss than the control animals and showed evidence of skin irritation at the injection sites.

Lymphocyte engraftment was not affected by treatment with TNP-470. When analyzed by flow cytometry to determine the percentage of B lymphocytes expressing the donor-origin transgenic IgM (B220+/IgM+), TNP-470-treated and control-transplanted mice expressed similar percentages of Tg IgM+ cells in spleen and BM. (Table 4). Engraftment kinetics were then explored at dose level 3 (100 mg/kg on day 0, and then 20 mg/kg three times/wk). Splenic reconstitution in treated animals at days 21, 24 and 28 was comparable with control. BM engraftment for TNP-470-exposed mice was significantly better than controls at day 21, although this difference disappeared at days 24 and 28 (Table 4).

We also performed PCR on variable cell populations to either TNP-470 or saline starting on day 0. Dose schedules were also varied from a single dose at the time of stem cell infusion to an initial dose on day 0 with subsequent administration of TNP-470 or saline three times a week (Table 3). Mice were initially sacrificed on day 28–32 at all dose levels. Comparative kinetics were then analyzed further by sacrificing groups of mice at days 21, 24, and 28 at dose level 3.

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### Table 1: TBI dose with and without stem cell support

Data indicate ranges of percentage of mortality in experimental groups given the indicated TBI dose with or without 5 × 10^6 BM cells to support hematologic recovery. Eight to 10 recipient mice/group with two (500 cGy) or three (700 and 900 cGy) groups evaluated at each dose.

<table>
<thead>
<tr>
<th>TBI dose in cGy (fraction sizes)</th>
<th>Mortality—no support</th>
<th>Mortality—marrow support</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 (300/200)</td>
<td>10–25%</td>
<td>0</td>
</tr>
<tr>
<td>700 (400/300)</td>
<td>50–60%</td>
<td>10%</td>
</tr>
<tr>
<td>900 (500/400)</td>
<td>100%</td>
<td>0–12.5%</td>
</tr>
</tbody>
</table>

### Table 2: Relationship of BM cell dose to engraftment and mortality

Data indicate ranges of percentage of mortality in experimental groups given 900 cGy TBI and then the indicated dose of BM cells. Five to eight recipient mice/group with two to three groups evaluated at each dose.

<table>
<thead>
<tr>
<th>BM dose (10^6 cells)</th>
<th>Mortality</th>
<th>Engraftment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100%</td>
<td>NA^a</td>
</tr>
<tr>
<td>0.5</td>
<td>60–80%</td>
<td>0–20%</td>
</tr>
<tr>
<td>1</td>
<td>40%</td>
<td>100%</td>
</tr>
<tr>
<td>2</td>
<td>8–37.5%</td>
<td>75–100%</td>
</tr>
<tr>
<td>3</td>
<td>0–17%</td>
<td>100%</td>
</tr>
<tr>
<td>5</td>
<td>0–20%</td>
<td>80–100%</td>
</tr>
</tbody>
</table>

^a NA, not applicable.

### Table 3: TNP-470 Dose Levels

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Dose on day 0</th>
<th>Subsequent dose and schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 mg/kg</td>
<td>20 mg/kg t.i.w. ^a</td>
</tr>
<tr>
<td>2</td>
<td>100 mg/kg</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>100 mg/kg</td>
<td>20 mg/kg t.i.w.</td>
</tr>
<tr>
<td>4</td>
<td>100 mg/kg</td>
<td>100 mg/kg t.i.w.</td>
</tr>
</tbody>
</table>

Continuous infusion 10 mg/kg/week starting on day 1

^a TNP-470 was given s.c. t.i.w. (three times/week).
detect cells that carry the Tg. The Tg is detectable in any cell derived from the graft, regardless of lineage (B cells or non-B cells). In Fig. 3A, DNA from BM and splenocytes from transplanted mice treated with TNP-470 or saline were analyzed for the presence of the Tg by PCR. The Tg was detected in all samples analyzed, regardless of treatment with TNP-470. Tail DNA from a Tg− animal provided a negative control, and BM and spleen from a Tg+ animal were used as a positive control. As seen in Fig. 3B, T cells were isolated from spleen and peripheral blood of transplanted animals 1 month after transplant and subjected to PCR detection of the Tg. For peripheral blood, T cells were isolated after Ficoll separation of the mononuclear cell fraction using the FACS Vantage cell sorter to sort CD3+ cells. For splenocytes, T cells were isolated using a mouse T cell isolation column. Cytometric analysis of the splenic T cells showed that the T cells were 92–95% CD3+ after column isolation (data not shown). Post-sort analysis of peripheral blood T cells was not possible because of the extremely small number of cells isolated. Again, repopulation of the T cell lineage with donor-derived cells was seen in both TNP-470-treated and control animals, with the Tg detected in all splenic T cell samples, three of three TNP-470-treated peripheral blood T cell samples, and two of three control peripheral blood T cell samples.

As summarized in Table 5, peripheral blood parameters were also assessed for hematological recovery. No statistical differences were found in hemoglobin or platelet count between control and treated mice (data not shown). However, control

### Table 4 Lymphoid engraftment after stem cell rescue

<table>
<thead>
<tr>
<th></th>
<th>Spleen</th>
<th>Bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B220+/IgM+</td>
<td>P</td>
</tr>
<tr>
<td><strong>Day 28 post-BMT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>54</td>
<td>11% ± 1.2b</td>
</tr>
<tr>
<td>All TNP-470 dose levels</td>
<td>54</td>
<td>14% ± 1.4</td>
</tr>
<tr>
<td><strong>Engraftment kinetics, dose level 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 21 control</td>
<td>8</td>
<td>12% ± 4.2</td>
</tr>
<tr>
<td>Day 21 TNP-470</td>
<td>5</td>
<td>13% ± 2.8</td>
</tr>
<tr>
<td>Day 24 control</td>
<td>6</td>
<td>22% ± 5.3</td>
</tr>
<tr>
<td>Day 24 TNP-470</td>
<td>6</td>
<td>31% ± 5.4</td>
</tr>
<tr>
<td>Day 28 control</td>
<td>10</td>
<td>9% ± 1.1</td>
</tr>
<tr>
<td>Day 28 TNP-470</td>
<td>14</td>
<td>11% ± 2.0</td>
</tr>
</tbody>
</table>

* Lymphoid engraftment at all TNP-470 dose levels as determined by flow cytometry is indicated in the upper half of the table. Engraftment kinetics at dose level 3 are indicated in the lower half of the table. BM and splenic reconstitution with donor-derived cells were measured at days 21, 24, and 28 post-BMT.

b Mean ± SE. Only the value indicated in bold is significantly different from control. Recipient mice were assayed for engraftment by detection of the IgM Tg derived from donor stem cells. Percentage of B220+/Tg IgM+ cells in the lymphoid size/granularity gate are indicated.

c NS, not significant.
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In other studies, we have shown that significantly lower total doses provide similar antitumor effects and are tolerated in the continuous infusion setting (data not shown and Ref. 14). Thus, we chose a dose of 10 mg/kg/week given continuously as the highest dose that did not cause cachexia in transplanted and xenografted animals. In these experiments, pumps were implanted on day 1 before BMT. TBI and stem cell infusion occurred on day 0, and the animals were sacrificed on day 28.

Lymphoid engraftment was assessed in these animals as above, and no differences were observed between TNP-470-treated animals, saline-treated animals (both by continuous infusion), or transplanted animals with no pump implanted (data not shown). As with the bolus dosing, we saw no significant differences in hemoglobin or platelet recovery between TNP-470-treated and control animals. However, in distinction to the bolus-dosed animals, we observed no differences in WBC recovery in the animals given TNP-470 by continuous infusion (Table 5) compared with animals that had been transplanted but had no pump implanted (data not shown). Among these animals, we did see lower WBCs in the saline controls; WBC counts at day 28 post-BMT in control animals and animals treated with TNP-470, continuous PBS, as well as untransplanted untreated mice as a normal reference (FVB).

Table 5 Recovery of peripheral WBCs after stem cell rescue

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>WBC(^a)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>6.3 ± 0.7b</td>
<td></td>
</tr>
<tr>
<td>TNP-470 intermittent dosing(^c)</td>
<td>10</td>
<td>3.6 ± 0.7</td>
<td>.04</td>
</tr>
<tr>
<td>TNP-470 continuous infusion(^d)</td>
<td>10</td>
<td>6.3 ± 0.4</td>
<td>NS(^e)</td>
</tr>
<tr>
<td>PBS continuous infusion</td>
<td>10</td>
<td>4.6 ± 0.3</td>
<td>.03</td>
</tr>
<tr>
<td>Untreated FVB mice</td>
<td>10</td>
<td>7.1 ± 0.7</td>
<td>NS(^f)</td>
</tr>
</tbody>
</table>

\(^a\) White blood cell count in 10\(^3\)/\(\mu\)l. WBC counts at day 28 post-BMT in control animals and animals treated with TNP-470, continuous PBS, as well as untransplanted untreated mice as a normal reference (FVB).

\(^b\) Mean ± SE. Only the values indicated in bold are significantly different from control.

\(^c\) These animals received TNP-470 at dose level 3, s.c. three times/wk, day 0 through day 28.

\(^d\) These animals received TNP-470 IP via Alzet pump at 10 mg/kg/wk.

\(^e\) NS, not significant.

DISCUSSION

Relapse after maximal dose-intensity therapy may result in part from contamination of the stem cell product with tumor cells (3, 4). Whether relapse results from reinfused tumor cells or from cells remaining in the patient, most patients are in a state of minimal residual disease after transplant. This provides a clinical situation in which the use of angiogenesis inhibitors may be most effective. It is of importance, therefore, to establish that angiogenesis inhibitors such as TNP-470 do not inhibit engraftment of normal BM cells, and that full immune and hematological reconstitution proceeds uninterrupted. We show here that a reliable system for stem cell transplant can be developed in a mouse model using stem cells from donor mice that express a Tg, detectable by flow cytometry and PCR, that is transplanted into lethally irradiated recipients. TNP-470 can be

\(^4\) D. Milkowski, personal communication.
administered in this setting, starting on day 0 of transplantation, with minimal toxicity and with no excess mortality in the TNP-470-treated group whether the drug was administered as a bolus dose or as continuous infusion. Both treated and control mice demonstrated reliable multilineage engraftment as well as normal B cell maturation. Furthermore, engraftment kinetics were not slowed by treatment with TNP-470 immediately after the infusion of donor stem cells. There was evidence of decreased WBCs in the bolus TNP-470 group compared with controls at day 28, but the opposite effect was seen in animals given continuously administered TNP-470, where saline-treated mice showed slightly lower WBCs than TNP-470-treated mice.

Metastatic solid tumors of childhood have been historically difficult to treat, especially high-risk neuroblastoma. Surgery plus conventional chemoradiotherapy has provided only 20% survival at best (19). The addition of autologous BMT and biotherapy with 13-cis-retinoic acid improved 3-year event-free survival to ~40% in a Children’s Cancer Group Phase III randomized trial (2). Additional dose-intensification with tandem transplantation and the use of peripheral blood progenitor cells as stem cell support has provided evidence of additional improvement in event-free survival (20, 21); but this approach has not yet been validated in a Phase III study. Despite these relative improvements in outcome, the majority of children with high-risk neuroblastoma still experience relapse. Chemotherapy dose-intensification has reached its limit; novel agents and approaches are needed.

Folkman first reported that analogues of fumagillin are potent inhibitors of endothelial cell proliferation, leading to the discovery of TNP-470 (22). Cohn and associates reported that increased vascularity in neuroblastoma is associated with aggressive disease and poor outcome (23) suggesting that there may be a role for angiogenesis inhibitors in the treatment of advanced disease. Since that time, several studies have explored the use of TNP-470 in animal models of malignant tumors. We and others have shown that TNP-470 seems to be most effective when used in the setting of minimal disease burden (12), especially when used before objective evidence of disease establishment (14). Other studies have found that TNP-470 first administered 10 days after inoculation of mice with two different neuroblastoma cell lines decreased both the primary tumor volume and the size and number of lymph node and liver metastases (24). Similar results have been seen with other xenograft models using malignant human cell lines such as choriocarcinoma, ovarian cancer, and endometrial cancer (25).

The data presented here show that TNP-470 does not adversely impact engraftment after stem cell transplant and may provide a complimentary approach to the treatment of advanced pediatric solid tumors. Taken together with the xenograft model experience (see Shusterman et al., in this issue) and the sense that angiogenesis inhibitors may work best when disease burden is at its least, our data point to a potential study design where antiangiogenic agents are given in the posttransplant period in an attempt to consolidate a remission and possibly increase the likelihood of long-term disease control.

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