Antitumor Activity of TP3(anti-p80)-Pokeweed Antiviral Protein Immunotoxin in Hamster Cheek Pouch and Severe Combined Immunodeficient Mouse Xenograft Models of Human Osteosarcoma

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ABSTRACT

TP3-pokeweed antiviral protein (PAP) immunotoxin is directed against the p80 antigen on osteosarcoma cells. Previous studies have demonstrated that TP3-PAP kills clonogenic human osteosarcoma cells in vitro and shows significant antitumor activity in a murine soft tissue sarcoma model (P. M. Anderson, et al., Cancer Res., 55: 1321–1327, 1995.) In this study, we demonstrate that TP3-PAP elicits potent in vivo antitumor activity in a hamster cheek pouch model of human osteosarcoma. Furthermore, treatment with TP3-PAP at nontoxic dose levels significantly delayed the emergence and progression of leg tumors and markedly improved tumor-free survival in severe combined immunodeficient mice challenged with OHS human osteosarcoma cells. Thus, TP3-PAP may be useful in the treatment of poor risk osteosarcoma.

INTRODUCTION

Osteosarcoma accounts for approximately 60% of primary bone neoplasms in the first two decades of life (1–5). The long-term survival of pediatric patients with osteosarcoma has dramatically improved as a result of contemporary multimodal treatment programs (1–5). However, osteosarcoma patients who present with metastatic disease or develop pulmonary metastases as well as a subgroup of patients whose tumors show an inadequate necrotic response to neoadjuvant chemotherapy continue to have a very poor prognosis (4–8). Presently, the major challenge in the treatment of pediatric osteosarcoma is to cure such poor risk patients who require more effective chemotherapy. Therefore, the development of new potent anti-osteosarcoma drugs has become one of the focal points in translational cancer research.

TP3-PAP is a potent immunotoxin directed against a Mr 80,000 surface antigen on osteosarcoma cells (9). TP3-PAP was shown to selectively kill >99.9% of human osteosarcoma cells at picomolar concentrations in vitro (10). The purpose of the present study was to further evaluate the clinical potential of this immunotoxin by examining its in vivo toxicity profile and its efficacy in preclinical animal model systems.

MATERIALS AND METHODS

TP3(anti-p80)-PAP Immunotoxin. TP3-PAP was constructed by covalent conjugation of the TP3(IgG2b) murine monoclonal antibody (Refs. 11, 12; ATCC accession number HB-12340) to the plant-derived ribosome inhibitory hemitoxin PAP. The procedures used for the production and purification of TP3-PAP immunotoxin have been described previously in detail (10). B43-PAP, an anti-CD19 immunotoxin directed against human leukemia cells (13, 14), was used as a control agent.

Cells. The OHS human osteosarcoma cell line (15) was maintained by serial passages in RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (HyClone Laboratories, Logan, UT) and 1% (vol/vol) penicillin-streptomycin (Life Technologies), as reported previously (10). Cells were cultured in tissue culture flasks at 37°C in a humidified 5% CO2 atmosphere. Before s.c. injection into the right hind leg of SCID mice, cells were washed twice in PBS. SCID mice were then inoculated s.c. with 0.2 ml of the cell suspension containing a total of 1 × 106 OHS cells.

HCP Tumor Assay. The effects of in vivo exposure to TP3-PAP on tumor development and angiogenesis were examined in the HCP assay system using 2-month-old female Golden Syrian hamsters (Harlan, Indianapolis, IN), as described previously in detail (16, 17). Hamsters with established OHS cheek tumors were treated systemically with 1 mg/kg TP3-PAP administered in daily i.p. boluses for a total of 5 treatment days.

Toxicity Studies in BALB/c Mice. All of the BALB/c mice used in the toxicity studies were obtained from the SPF breeding facilities of NIH (Bethesda, MD) at 6–8 weeks of age. The mice were housed in the American Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved and -accredited Research Animal Resources Mouse Facility of the University of Minnesota (Minne-
apoli, MN). All of the husbandry and experimental contact made with the mice maintained SPF conditions. The mice were kept in Micro-Isolator cages (Lab Products, Inc., Maywood, NY) containing autoclaved food, water, and bedding. In each of the toxicity studies, female BALB/c mice were administered an i.p. bolus injection of TP3-PAP in 0.2 ml PBS or of 0.2 ml PBS alone (control mice). In the first study, groups of four to six mice received a single treatment of one of five different dose levels of TP3-PAP ranging from 1 mg/kg to 5 mg/kg. In the second study, groups of four to six mice received five consecutive daily treatments of TP3-PAP at five different cumulative dose levels ranging from 1 mg/kg to 5 mg/kg. In each study, four control mice were administered 0.2 ml PBS i.p. in accordance with the experimental protocol. No sedation or anesthesia was used throughout the treatment period. Mice were monitored daily for mortality in order to determine the day-30 LD50 values. Multiple organs were collected within 4 h after death, grossly examined, and processed for histopathological examination, as reported previously (14). Mice who survived 30 days after the treatment were killed; the tissues were immediately collected from randomly selected mice and preserved in 10% neutral buffered formalin.

Efficacy Studies in SCID Mice. All of the SCID mice used in the efficacy study were produced by SPF CB-17 SCID/SCID breeders (originally obtained from Dr. Melvin Bosma, Fox Chase Cancer Center, Philadelphia, PA) in the AAALAC-approved and -accredited Research Animal Resources SCID Mouse Facility of the University of Minnesota (Minneapolis, MN). All of the husbandry and experimental contact made with the mice maintained SPF conditions. The mice were housed in Micro-Isolator cages containing autoclaved food, water and bedding. Trimethoprim sulfamethoxazole (Bactrim) was added to the drinking water of the mice three times a week. Female SCID mice (ages 4–6 weeks) were inoculated s.c. with 1 × 10^6 OHS cells followed by i.p. administration of the TP3-PAP immunotoxin at one of the three cumulative dose levels of 0.25 mg/kg, 0.50 mg/kg, or 1.0 mg/kg. Throughout the experimental period, they were monitored daily for overall health and survival. At the time of death, necropsies were performed, and the osteosarcoma burden of the mice was assessed as described previously (18–23). For histopathological studies, tissues were fixed in 10% neutral buffered formalin, dehydrated, and embedded in paraffin by routine methods. Glass slides with affixed 6-μm tissue sections were prepared, stained with H&E, and submitted to the veterinarian pathologist for examination. The control group, comprising 78 female SCID mice, was treated with three daily i.p. injections of 0.2 ml PBS (n = 53), TP3 monoclonal antibody (1 mg/kg cumulative dose) + unconjugated PAP (0.25 mg/kg cumulative dose; n = 5), the control immunotoxin B43-PAP (n = 5) at a total cumulative dose of 1 mg/kg, adriamycin (25 mg/m²/day for 3 days; n = 5), a single dose of methotrexate (12.5 g/m²/day for 1 day; n = 5), or cyclophosphamide (50 mg/kg/day for 2 days; n = 5). The primary end points of interest were tumor growth and tumor-free survival outcome. Estimation of life table outcome and comparisons of outcome between groups were done with the log rank test. The efficacy of TP3-PAP against established tumors was examined by treating SCID mice who had s.c. OHS xenografts of 0.5-cm or 1.0-cm diameter with a cumulative dose of 1 mg/kg TP3-PAP given in three i.p. injections (injection volume, 0.2 ml) 24 h apart from each other. Control mice were treated with 0.2 ml PBS/day for 3 days.

RESULTS

Anti-Osteosarcoma Activity of TP3-PAP Immunotoxin in the HCP Model. Within 7 days after transplantation to the cheek pouches of female Golden Syrian hamsters (cell dose, 5 ×
10^6 cells/hamster; volume of inoculated cell suspension = 0.1 ml), proliferating OHS cells formed highly vascularized \( > 10^{-6} \) mm^3 tumors in four of four hamsters (Fig. 1, A and B). Importantly, when hamsters carrying established 9–16 mm^3 cheek pouch OHS tumors received a 5-day (i.e., days 8–12) systemic therapy with i.p. injections of TP3-PAP, there was a 57 ± 19% tumor shrinkage in three hamsters, as determined by tumor measurements 48 h after completion of the treatment (i.e., on day 14) without any obvious side effects (Fig. 1, C and D). Thus, TP3-PAP showed significant in vivo antitumor activity in our initial pilot study that used the HCP model, which provided the rationale for detailed toxicity and efficacy studies in mice.

**Mouse Toxicity of TP3-PAP Immunotoxin.** In the first study, groups of four to six female BALB/c mice treated with a single i.p. bolus dose of TP3-PAP at one of five different dose levels (1, 2, 3, 4, and 5 mg/kg) developed signs of toxicity at doses of 3 mg/kg and higher (Table 1A). At these high-dose levels of immunotoxin, the mice became weak and lethargic, had decreased levels of activity, lost weight, and developed scruffy skin. All of the 12 mice treated with 4 mg/kg or 5 mg/kg TP3-PAP died within 15 days with median survival times of 11.5 days and 9.5 days, respectively (Table 1). The day-30 LD50 was approximately 3 mg/kg with 67 ± 19% of the mice remaining alive on day 30. The histopathological lesions in the TP3-PAP-treated mice were dose-dependent and were observed at ≥2 mg/kg dose levels. The lesions attributed to TP3-PAP toxicity included: (a) acute to subacute degeneration and mild to severe necrosis of cardiac muscle fibers with variable mineralization and satellitosis of necrotic muscle fibers at dose levels ≥3 mg/kg; (b) mild to moderate necrosis of skeletal muscle fibers with variable degrees of mineralization at dose levels ≥4 mg/kg; (c) mild pericardial vacuolization of hepatocytes and mild scattered pinpoint hepatocellular necrosis at dose levels ≥4 mg/kg; and (d) mild and subtle kidney damage with widely scattered necrotic tubules and/or regenerative tubules that contained variable amounts of intraluminal granular cellular debris at dose levels ≥3 mg/kg. Control mice that were given 0.2 ml PBS i.v. developed no clinical signs of toxicity and had no gross or histological lesions.

In the second study, groups of four to six female BALB/c mice were treated with a single daily i.p bolus injection of TP3-PAP for 5 consecutive days at one of five cumulative dose levels (1, 2, 3, 4, and 5 mg/kg). Similar to the mice who received single doses of TP3-PAP, these mice developed signs of toxicity at cumulative doses of 3 mg/kg and higher. All of the 18 mice treated at ≥3 mg/kg of TP3-PAP died with a median survival of 13 days (Table 1B). The histopathological lesions in the TP3-PAP-treated mice were dose-dependent and were observed at ≥3 mg/kg dose levels. The lesions attributed to TP3-PAP toxicity were virtually identical to those observed in mice treated with single-dose TP3-PAP injections and included: (a) acute to subacute degeneration and mild to severe necrosis of cardiac muscle fibers with variable mineralization and satellitosis of necrotic muscle fibers at dose levels ≥3 mg/kg; (b) mild to moderate necrosis of skeletal muscle fibers with variable degrees of mineralization at dose levels ≥4 mg/kg; (c) mild pericardial vacuolization of hepatocytes at dose levels ≥3 mg/kg; and (d) mild and subtle kidney damage with widely scattered necrotic tubules and/or regenerative tubules that contained variable amounts of intraluminal granular cellular debris at dose levels ≥3 mg/kg. Control mice that were given 0.2 ml of PBS i.v. developed no clinical signs of toxicity and had no gross or histological lesions.

**In Vivo Antitumor Activity of TP3-PAP Immunotoxin in SCID Mice Xenografted with OHS Human Osteosarcoma Cells.** All of the 25 CB-17-SCID mice that were inoculated s.c. with 1 × 10^6 OHS osteosarcoma cells in their hind legs developed tumors at a median of 12 days. These tumors rapidly progressed to reach a mean (± SE) volume of 3.3 ± 2.2 cm^3 on day 52 (n = 25) and 4.9 ± 0.6 cm^3 on day 77 (n = 10). The tumors were poorly circumscribed, fingered into the adjacent skeletal muscle, and invading the femur. The tumor masses were highly cellular and were divided into incomplete lobules by thin fibrous septa. There were large, often confluent, areas of necro-

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### Table 1: Toxicity of TP3-PAP in BALB/c Mice

<table>
<thead>
<tr>
<th>Treatment schedule</th>
<th>Total dose of TP3-PAP (mg/kg)</th>
<th>Body weight (g)</th>
<th>Survival after TP3-PAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretreatment</td>
<td>Day 30 or at death</td>
<td>Median survival (days)</td>
</tr>
<tr>
<td>A. Single dose a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (n = 4)</td>
<td>24.2 ± 1.4</td>
<td>25.8 ± 0.6</td>
<td>&gt;30</td>
</tr>
<tr>
<td>1 (n = 4)</td>
<td>25.5 ± 1.1</td>
<td>24.2 ± 0.6</td>
<td>&gt;30</td>
</tr>
<tr>
<td>2 (n = 4)</td>
<td>24.9 ± 0.2</td>
<td>24.2 ± 0.1</td>
<td>&gt;30</td>
</tr>
<tr>
<td>3 (n = 6)</td>
<td>25.7 ± 0.4</td>
<td>22.1 ± 0.9</td>
<td>&gt;30</td>
</tr>
<tr>
<td>4 (n = 6)</td>
<td>24.7 ± 0.5</td>
<td>16.9 ± 1.1</td>
<td>11.5</td>
</tr>
<tr>
<td>5 (n = 6)</td>
<td>26.1 ± 0.6</td>
<td>17.9 ± 1.2</td>
<td>9.5</td>
</tr>
<tr>
<td>B. Five daily doses b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (n = 4)</td>
<td>22.4 ± 0.5</td>
<td>25.4 ± 0.5</td>
<td>&gt;30</td>
</tr>
<tr>
<td>1 (n = 4)</td>
<td>25.7 ± 0.7</td>
<td>24.9 ± 0.3</td>
<td>&gt;30</td>
</tr>
<tr>
<td>2 (n = 4)</td>
<td>24.1 ± 0.3</td>
<td>23.1 ± 0.4</td>
<td>&gt;30</td>
</tr>
<tr>
<td>3 (n = 6)</td>
<td>25.6 ± 0.6</td>
<td>16.8 ± 0.9</td>
<td>13</td>
</tr>
<tr>
<td>4 (n = 6)</td>
<td>25.0 ± 0.3</td>
<td>18.3 ± 0.7</td>
<td>13</td>
</tr>
<tr>
<td>5 (n = 6)</td>
<td>26.1 ± 0.6</td>
<td>17.9 ± 1.2</td>
<td>13</td>
</tr>
</tbody>
</table>

a) All of the mice were electively killed on day 30. See “Materials and Methods” for experimental details.

b) Mice were treated with either a single i.p. bolus dose (A) or five daily i.p. bolus doses (B).

c) n, number of mice.
sis in the centers of many lobules. The cells in the viable regions were closely packed into sheets with little intercellular stroma. The cells were large with abundant, clearly outlined, amphophilic to slightly eosinophilic, finely vacuolated cytoplasm. The large, vesicular, round to ovoid nuclei were often eccentrically located and had one to two large eosinophilic nucleoli. Mitotic figures were numerous. Microscopic metastases were found in the lung and spleen.

We examined the in vivo antitumor activity of TP3-PAP in our SCID mouse xenograft model of human osteosarcoma described above. We first sought to determine whether TP3-PAP could delay the emergence and progression of tumors when administered according to a nontoxic 3-day treatment program at cumulative dose levels of 0.25 mg/kg, 0.50 mg/kg, or 1.00 mg/kg commencing 2 h after the s.c. tumor cell inoculation. Control mice were treated with PBS, unconjugated TP3 antibody (1 mg/kg cumulative dose) + unconjugated PAP (1 mg/kg cumulative dose), or B43-PAP (1 mg/kg cumulative dose), a control immunotoxin directed against the CD19 surface antigen on human leukemia cells. All of the 63 control mice treated with PBS (n = 53), TP3 antibody + PAP (n = 5), or B43-PAP (n = 5) developed tumors within 45 days with a median tumor-free survival of only 19 days (Fig. 2). Tumors rapidly progressed and reached a size of 4.0 cm³ by 53.7 ± 2.6 days in PBS-treated mice, by 43.6 ± 2.9 days in mice treated with TP3 + PAP, and by 46.2 ± 3.3 days in B43-PAP-treated mice. At the 0.25- and 0.50-mg/kg dose levels, TP3-PAP delayed the emergence of tumors (mean tumor-free survival = 26.5 days at 0.25 mg/kg and 32.0 days at 0.50 mg/kg). On day 30, only 15.9 ± 4.6% of the control mice were tumor-free, whereas no tumors were detected in 33.3 ± 19.2% of mice treated with 0.25 mg/kg TP3-PAP and 50 ± 20.4% of mice treated with 0.50 mg/kg TP3-PAP (Table 2). However, once developed, OHS tumors in TP3-PAP-treated mice progressed as rapidly as in control mice (Fig. 2). TP3-PAP showed remarkably potent antitumor activity at the 1.0-mg/kg cumulative dose level; none of the SCID mice treated with TP3-PAP at this dose level developed a tumor for up to 150 days after inoculation (Fig. 2), and 67 ± 19% were still alive and tumor-free at 150 days (Table 2). In contrast, all of the 15 mice that were treated with the chemotherapeutic agents adriamycin, methotrexate, or cyclophosphamide developed tumors within 45 days with a median tumor-free survival.

**Fig. 2** Anti-osteosarcoma activity of TP3-PAP in SCID mice. A, the tumor volume of s.c. OHS xenografts according to the amount of time (days) after the inoculation of OHS cells and the specific treatment program applied to prevent tumor growth. B, tumor-free survival curves of SCID mice inoculated with OHS cells and treated with TP3-PAP, TP3 antibody (Ab) plus PAP, B43-PAP, PBS, or chemotherapy. For details of the treatment programs, see the "Materials and Methods" section.
Table 2  In vivo anti-osteosarcoma activity of TP3-PAP immunotoxin

Female SCID mice were inoculated s.c. with 1 × 10^6 OHS human osteosarcoma cells. Two hours later, mice were started on treatment programs employing TP3-PAP immunotoxin (0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg in daily i.p. injections over 3 days) or chemotherapeutic agents (adriamycin, methotrexate, cyclophosphamide) as described in “Materials and Methods.” Control mice were treated with PBS, TP3 antibody + unconjugated PAP, or B43-PAP immunotoxin. Results are expressed as the cumulative proportion of mice surviving tumor-free according to the time after the inoculation of OHS cells. Life table analysis used the log-rank statistics.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of mice</th>
<th>30 days</th>
<th>60 days</th>
<th>90 days</th>
<th>120 days</th>
<th>Median tumor-free survival (days)</th>
<th>P (log rank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63</td>
<td>16 ± 5</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>TP3-PAP, 0.25mg/kg</td>
<td>6</td>
<td>33 ± 19</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>26.5</td>
<td>0.04</td>
</tr>
<tr>
<td>TP3-PAP, 0.5mg/kg</td>
<td>6</td>
<td>50 ± 20</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>32</td>
<td>0.04</td>
</tr>
<tr>
<td>TP3-PAP, 1mg/kg</td>
<td>6</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>83 ± 15</td>
<td>&gt;150</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Chemotherapeutic agents</td>
<td>15</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>15</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Fig. 3  Anti-osteosarcoma activity of TP3-PAP in SCID mice with established xenograft tumors. A, SCID mice with 0.5-cm diameter OHS tumors were treated with PBS, TP3-PAP, or chemotherapeutic agents, as described in the “Materials and Methods” section. Tumor progression over time is shown for each of the treatment groups. B, SCID mice with 1.0-cm diameter OHS tumors were treated with TP3-PAP (1 mg/kg) or PBS as described in the “Materials and Methods” section.
antigen recognized by its monoclonal antibody moiety has to fulfill at minimum the following essential requirements: (a) it has to be expressed on clonogenic cells from the majority of patients; (b) it has to be expressed on the self-renewing clonogenic osteosarcoma cell populations; (c) it has to undergo antibody-induced internalization and thus allow the toxin moiety to be transported into the targeted osteosarcoma cells; (d) it has to be absent from the peripheral blood myeloid/erythroid elements and thus allow immunotoxin to reach and effectively kill the small number of clonogenic osteosarcoma cells; (e) it should not be shed from the surface nor circulate in blood in soluble form competing with surface-bound antigen for the administered immunotoxin molecules; and (f) it has to be absent from the parenchymal cells of the life-maintaining nonhematopoietic organs.

The tumor-associated antigen p80 is expressed at very high levels on the surface membrane of human osteosarcoma cells (9). The very limited normal-tissue distribution of p80 antigen (9, 11, 12, 27, 28) makes it an attractive target for biotherapy of osteosarcomas. The murine IgG2b anti-p80 antibody TP3 reacts with mesenchymal tumors including osteosarcomas, hemangio-pericytomas, chondrosarcomas, malignant fibrous histiocytomas, and synovial cell sarcomas (11). In a previous study, we found that the PAP immunoen conjugate of the TP3 antibody (i.e., TP3-PAP immunotoxin) kills >99.99% of clonogenic human osteosarcoma cells within 18 h; suggesting that this biotherapeutic agent may be useful in the treatment of therapy-refractory osteosarcoma (10). This report provides a detailed characterization of the pharmacokinetics, toxicity, and antitumor activity of TP3-PAP immunotoxin directed against osteosarcoma cells. The in vivo toxicity profile as well as pharmacokinetics of TP3-PAP in mice were identical to the previously reported toxicity profiles and pharmacokinetic features of other PAP immunotoxins that are presently under clinical investigation (14, 26, 29). In efficacy studies, TP3-PAP elicited potent in vitro and in vivo antitumor activity in a HCP model of human osteosarcoma. Treatment with TP3-PAP at nontoxic systemic exposure levels significantly delayed the emergence and progression of leg tumors and markedly improved tumor-free survival in SCID mice challenged with OHS human osteosarcoma cells.

Several investigators have evaluated the toxicities associated with the administration of immunotoxins (24–27). There is limited information about the toxicity profiles of immunotoxins containing the plant hemotoxin PAP (14, 29). B43-PAP, an anti-CD19 immunotoxin directed against B-lineage leukemia cells, was reported previously to cause dose-limiting renal and cardiac toxicities in mice (30) and dose-limiting renal toxicity due to severe and diffuse renal tubular necrosis in cynomolgus monkeys (14). In addition, B43-PAP caused a mild hepatic injury in cynomolgus monkeys, characterized by transient elevation of transaminases and a mild multifocal hepatitis detected histopathologically and a transient episode of proteinuria and hypoalbuminemia, which could be due to a mild capillary leak. The toxicities associated with TXU-PAP immunotoxin detailed in a recent report were reminiscent of those associated with B43-PAP immunotoxin (30). In mouse toxicity studies, TP3-PAP immunotoxin caused compound-related histological lesions. TP3-PAP-related histological lesions in BALB/c mice included mild to severe myocardiocyte necrosis, characterized by the presence of occasional necrotic myofiber segments in one or both ventricles, and mild or moderate skeletal muscle necrosis, characterized by occasional to many necrotic myofiber segments. There was also mild acute multifocal hepatocellular necrosis, characterized by the presence of a small number of individual necrotic hepatocytes in the livers of some mice. This toxicity profile is identical to that seen in BALB/c mice treated with B43-PAP immunotoxin (30), which is in clinical trials under BB-IND-3864, or with TXU-PAP immunotoxin (14), which is in clinical trials under BB-IND-6982. Lesions that might be expected to cause morbidity are myocardial necrosis and skeletal muscle necrosis. The hepatocellular necrosis may have been a direct toxic effect of TP3-PAP inasmuch as it was seen only at higher dose levels, but it may also have been a consequence of impaired cardiac function.

Humoral immune responses to the monoclonal antibody as well as toxin portions of immunotoxins have contributed to their limited clinical utility (13). Immunogenicity of TP3-PAP might be reduced by replacing the mouse antibody with a chimeric or humanized version of TP3 as well as by attaching allergens, haptens, or chemical agents, such as polyethylene glycol, that suppress immune responses.

On the basis of the encouraging anti-osteosarcoma activity of TP3-PAP presented herein, we postulate that the incorporation of this immunotoxin into clinical treatment protocols may improve the prognosis for therapy-refractory osteosarcoma patients. TP3 antibody also reacts with budding capillaries of a wide variety of tumors (9, 10). Therefore, toxin conjugates of the TP3 antibody can inhibit the growth of tumors not only by selectively destroying p80 antigen positive cancer cells but also by damaging the tumor neovascularature. Such damage to the tumor neovascularature (31) may also facilitate the tumor entry of antineoplastic agents by providing “windows” in the vasculature through which the antineoplastic agents may easily enter the tumor.

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O Ek, B Waurzyniak, D E Myers, et al.

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