Phase I Clinical Study of the Recombinant Oncotoxin TP40 in Superficial Bladder Cancer

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ABSTRACT

Transforming growth factor α-Pseudomonas exotoxin-40 (TP40) is a hybrid fusion protein that selectively binds to cancer cells that express the epidermal growth factor receptor. TP40 is then internalized and kills these cells by virtue of its Pseudomonas exotoxin-derived domains. We studied the safety and short-term antitumor activity of intravesical TP40 in 43 patients with refractory superficial bladder cancer. These patients had resected T₁/T₂ disease (n = 19), visible T₃ or T₄ lesions (n = 11), or carcinoma in situ (n = 13). Patients were treated with increasing dose levels of TP40 at 0.15, 0.3, 0.6, 1.2, 4.8, or 9.6 mg/week for 6 weeks and evaluated by comparing pretreatment and posttreatment cystoscopic examinations, cytology, and histopathology. All TP40 doses were well tolerated. No evidence of antitumor activity was seen in any of the patients with T₃ or T₄ lesions. However, 8 of 9 patients with evaluable carcinoma in situ were judged by histopathology of multiple biopsy specimens to exhibit clinical improvement following TP40 therapy. In most of these responsive patients, cystoscopic examination supported the histopathological findings, although cytology of urine and bladder washings persistently demonstrated malignant cells. Therefore, TP40 appears to be a well-tolerated biological agent that may prove to have utility in treating carcinoma in situ of the bladder.

INTRODUCTION

TP40 is a hybrid fusion protein composed of the mature form of TGF-α fused to a modified 40-kDa segment of the bacterial exotoxin PE40. TP40 possesses the receptor-binding properties of TGF-α, which enables it to bind to mammalian cells that express EGFRs. PE40 contains the protein translocating and ADP-ribosylating enzymatic domains of Pseudomonas exotoxin. After receptor binding and internalization, TP40 utilizes these PE40-derived domains to enter the cytoplasm of mammalian cells and to kill those cells by inhibiting protein synthesis. Thus, TP40 combines the selective cell-targeting properties of TGF-α combined with the potent cell killing properties of Pseudomonas exotoxin. Cell culture experiments have shown TP40 to be an exceptionally effective biological agent which is capable of killing a wide variety of human tumor cell lines that express EGFR, while showing remarkably little toxicity toward cells that lack EGFR. Similarly, in animal experiments, TP40 has demonstrated antitumor activity against tumors that express EGFR including glioblastoma, prostate carcinoma, and epithelial carcinoma cells (2-5). Thus, TP40 appears to be well suited to selectively target and destroy human cancers that express EGFR.

Many human cancers, including squamous cell and transitional cell carcinomas, express large numbers of EGFR on their plasma membranes (6-8). However, some normal tissues (e.g., hepatocytes and keratinoctes) also express significant numbers of EGFR (7) and might be expected to be adversely affected by TP40. A further complication to the clinical evaluation of recombinant fusion proteins such as TP40 is the formation of neutralizing antibodies that may occur following the systemic administration of these agents to animals (9, 10). Therefore, in selecting a human cancer for testing TP40’s utility we sought a malignancy that (a) currently lacks effective therapy; (b) over-expresses EGFR; and (c) is localized to an immunologically privileged site, avoiding formation of antibodies which could interfere with TP40’s biological activity.

Superficial bladder cancer is a malignancy which appears to meet these criteria (11). Traditional therapy for this cancer involves frequent local resections and a variety of intravesical chemotherapies (e.g., thiotepa or doxorubicin) or immunotherapy (e.g., BCG). However, such therapies are generally not curative and are often accompanied by local and systemic toxicities (11-14). Therefore, a more effective and less toxic therapy for recurrent bladder cancer is highly desired. Most superficial bladder cancers express EGFR and those that do have been...
Table I  Patient enrollment into phase I study of TP4O in superficial bladder cancer

<table>
<thead>
<tr>
<th>Dose (mg/wk)</th>
<th>No.</th>
<th>T2/T1 resecteda</th>
<th>T2/T1 evaluablea</th>
<th>CISa</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.3</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.6</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>1.2</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2.4</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.8</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>9.6</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>19</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>

a No visible lesions or all visible lesions resected at pretreatment cystoscopy.

b One or more visible lesions remain at pretreatment cystoscopy.

c Biopsy at pretreatment cystoscopy showed CIS, or patient presented with history of CIS.

found to exhibit a more aggressive clinical course than EGFR-negative bladder cancers (8, 15, 16). In vitro testing of human bladder cancer cell lines and explants of human bladder cancer tissues have demonstrated that TP40 is a potent cytotoxic agent against these cancer cells. Furthermore, transurethral instillation of TP40 directly into the bladder should prevent TP40 from being exposed to the immune system. Using this method of treatment, it was anticipated that TP40 would not engender a systemic antibody response. In this report, we describe the findings of a Phase I trial of intravesical antitumor therapy with TP40 in patients with superficial bladder cancer.

MATERIALS AND METHODS

Protocol. The primary objective of the Phase I study of TP40 (L-691,912) in patients with superficial bladder cancer was to evaluate the safety and tolerability of the fusion protein when administered into the urinary bladder once weekly for 6 weeks. To meet this objective, patients with superficial bladder cancer that had failed to respond to BCG and/or other local therapies (e.g., thiotepa, mitomycin) were recruited into an open, rising-dose, uncontrolled study. TP40 was administered under an investigational new drug exemption following review by the Food and Drug Administration. The protocol was approved by institutional review boards at the investigators’ institutions, and all patients provided written informed consent for the study. Recombinant TP40 was produced by expression of the protein in an Escherichia coli expression system as described previously (2). Large-scale production of TP40 was performed under the BL2-LS level of physical containment in accordance with NIH Guidelines for Research Involving Recombinant DNA Molecules.

Seven dose-levels were studied. Each was sequentially administered to 3–8 patients (Table 1, 43 patients total), with the demonstration of clinical tolerability used to justify advancement to the next higher dose level in subsequent patients. Dose levels studied were 0.15, 0.3, 0.6, 1.2, 2.4, 4.8, and 9.6 mg, administered transurethrally in 60 ml phosphate buffer (pH 7.8, approximately 0.06–4.0 μM TP40 at the time of instillation) once per week for 6 weeks. The solution was retained in the bladder for 2 h prior to voiding. Based on preclinical data, concentrations administered (particularly at higher dose levels) were comparable to or greater than those shown to be active in vitro and in nude mice bearing tumor xenographs (2). The 2 h exposure time was consistent with the in vitro cell-killing kinetics of TP40. Tolerance to TP40 was evaluated clinically.

Anti-Tumor Activity. To evaluate antitumor activity, patients were divided into 3 groups (Table I). Patients with papillary (i.e., T1 disease, n = 25) or superficially invasive (i.e., T2 disease, n = 5) disease were merged into two groups based on whether or not all visible lesions had been resected prior to treatment. Patients with evident or historical CIS were analyzed as a single group, although some had CIS alone and others had CIS with T1 or T2 disease. Assessment of activity was based on comparison of pretreatment and posttreatment cystoscopic examinations, pretreatment and posttreatment cytological examinations (voided urine and/or bladder washings), and pretreatment and posttreatment histopathological evaluation, as reported by the investigating urologist and his institutional pathologist. Activity assessment was supplemented by documentation that urine voided at the end of the dwell time retained in vitro cell-killing activity, indicating that TP40 was not inactivated when held in the bladder for 2 h.

EGFR Measurements. To guide interpretation of the results, bladder biopsy specimens collected pretreatment and posttreatment were examined by immunohistochemical assay for the presence of EGFR. Immunohistochemical studies were performed following standard avidin-biotin-peroxidase techniques (16–18). Five-μm-thick frozen sections were fixed for 10 min in acetone, washed in PBS, and incubated with 0.1% hydrogen peroxide. Endogenous biotin was blocked with avidin-biotin complexes. Tissue was incubated in 10% normal horse serum for 30 min. Sections were then incubated with anti-EGFR monoclonal antibody 528 (Oncone Science, Manhasset, NY) at a concentration of 5 μg/ml for 1 h. After washing with PBS, sections were incubated for 30 min with biotinylated horse anti-mouse antibodies (Vector Laboratories, Burlingame, CA) at a dilution of 1:100, followed by incubation with avidin-biotin peroxidase complexes for 30 min (Vector Laboratories) at a concentration of 1:25. Diaminobenzidine was used as the final counterstain. MlgS-Kp-1 antibody (Pharmingen, San Diego, CA) was used as a negative control.

Anti-TP40 Antibody Determinations. An assay was developed to quantify anti-TP40 antibodies that might arise during TP40 therapy. A dual antibody format was utilized by coating the plastic surface of a 96-well plate with recombinant TP40. The plate was then incubated for 1 h with the patient serum samples diluted 1:100 in saline to reduce nonspecific binding and then washed extensively with saline. A radiolabeled goat anti-human IgM + IgG antibody was then used to detect bound anti-TP40 antibodies. Results from each post-TP40 therapy sample were then compared to pretreatment samples from the same patient, and a 2-fold or greater increase in bound counts was deemed significant.

RESULTS

Forty-three patients were enrolled into the Phase I study. They ranged in age from 47 to 82 years. Of these patients, 35
(81%) had received BCG and 23 (53%) had received cytotoxic agents (thiotepa, mitomycin C) as prior intravesical therapy. In 27 patients, the treatment prior to TP4O was BCG, with 21 of these patients receiving their treatment within the year prior to TP4O. All patients had undergone multiple resections in addition to the intravesical treatments over the 1–2 years prior to enrollment into this study. Clinically, all patients were considered suitable for enrollment in a Phase I study. Table 1 summarizes the distribution of these patients among the dose levels of TP4O and indicates their disease classification.

Assessments of safety included clinical laboratory tests, electrocardiograms, cataloging of adverse events including reports of bladder irritation, measurement of serum anti-TP4O antibodies as an indicator of systemic exposure to TP4O, and posttreatment cystoscopic evaluation for bladder irritation. Several patients reported mild symptoms or signs of urinary tract irritation (e.g., dysuria, cystitis, urgency, or spasm). However, no evidence of dose-related local toxicity was found. In fact, patients treated with the highest dose levels (4.8 and 9.6 mg) of TP4O reported no local or systemic adverse events. There was no evidence of systemic exposure to TP4O, as evidenced by the absence of either systemic toxicity or anti-TP4O antibodies.

Thirty patients were classified as having papillary (\( T_2, n = 25 \)) or superficially invasive (\( T_1, n = 5 \)) disease. Nineteen of these patients were considered to be tumor free prior to treatment with TP4O, having undergone local resections of tumor prior to therapy (Table 1, \( T_{\text{res}}/T_1, \text{Resected} \)), Following TP4O therapy, 9 of these patients showed no evidence of recurrence. In the 10 other resected patients, visible recurrent disease was apparent 4 weeks after the last TP4O treatment at the posttreatment cystoscopy examination. Of the 11 patients with evaluable \( T_2 \) or \( T_1 \) lesions at the start of TP4O therapy (Table 1, \( T_{\text{res}}/T_1, \text{Evaluable} \)), visible tumors remained present at the posttreatment cystoscopy in all patients. No evidence of reduction in the size of these lesions was noted.

Thirteen patients with evident (\( n = 9 \)) or historical (\( n = 4 \)) CIS were treated with TP4O. Evaluation of patients with CIS focused on three components of response: visual improvement at posttreatment cystoscopy, evidence of CIS in biopsies, and cytological examination of voided urine or bladder washings. Six of the patients classified with CIS also had \( T_2 \) or \( T_1 \) disease. The effect of TP4O therapy on the \( T_2 \) or \( T_1 \) lesions in patients with concomitant CIS lesions was similar to those described above for patients without CIS. No evidence of reduction in the size of the \( T_2 \) or \( T_1 \) lesions was noted. The effect of TP4O therapy on CIS lesions was more promising (Table 2). Eight of the nine patients with evaluable CIS exhibited a response based on comparison of pretreatment and posttreatment histopathological analysis of multi-site bladder biopsies. In two patients this favorable clinical impression was supported by the absence of malignant cells in posttreatment urine samples and bladder washings, while the cytology remained positive in four patients (three of the eight responders were not evaluable for cytology, based on residual prostatic urethral or \( T_2/T_1 \) disease). Cytoscopic evaluation was generally consistent with the histopathological findings, although the reports were too subjective to be rigorously evaluated in this multi-site Phase I trial.

EGFR status was determined pretreatment and posttreatment with TP4O in 18 patients. As expected, all biopsies stained positive for EGFR on both determinations, confirming that transitional cell epithelium expresses EGFR.

### DISCUSSION

TP4O is a potent cytotoxic agent in vitro (1–5) and in vivo (2, 4, 5) for EGFR-positive tumor cells. Immunohistochemical examination of bladder cancer biopsies has documented the presence of EGFR in these tumors (8, 15, 16). Additionally, Sarosdy et al. have shown that six out of six explants of human bladder cancers were sensitive to TP4O in a tumor cell clonogenic assay, regardless of the tumor stage or histological grade of the tumor (5). Bladder cancers that exhibit more aggressive clinical courses are also frequently EGFR positive (8, 16). Superficial bladder cancer was therefore considered a reasonable clinical target to evaluate TP4O’s safety and tolerability because it permitted direct administration of TP4O to the tumor site via intravesical instillation. In this report, we describe the findings of a Phase I study in patients with superficial bladder cancer treated with TP4O.

Normal bladder epithelium possesses EGFR, which might have rendered these normal cells susceptible to the cytotoxic activity of TP4O. However, TP4O was extremely well tolerated in this study when administered into the bladder at concentrations up to three orders of magnitude above those required to kill EGFR-positive tumor cells in vitro. The only adverse effects associated with TP4O therapy were occasional reports of dysuria, urgency, and bladder spasm and these few adverse experiences were not dose dependent.

TP4O administered via intravesical instillations did not engender a humoral immune response. This result is in marked contradistinction to systemically administered TP4O in animals where intraperitoneal instillation of as little as 10 μg oncostxin elicited anti-TP4O antibodies with titers of 1:10,000.² The absence of systemic toxicity and the failure of TP4O to elicit antibody formation suggests that TP4O was confined within the bladder during each treatment cycle. It is less clear why TP4O

### Table 2. Analysis of CIS responsiveness to TP4O therapy

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Sex, Age</th>
<th>Pre-Tx Histopathology</th>
<th>Post-Tx Histopathology</th>
<th>Cytology malignant cells</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15</td>
<td>M, 49</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Residual T1</td>
</tr>
<tr>
<td>0.6</td>
<td>M, 72</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>0.6</td>
<td>M, 64</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.6</td>
<td>M, 63</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.2</td>
<td>M, 69</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.2</td>
<td>M, 71</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>4.8</td>
<td>M, 70</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.6</td>
<td>M, 53</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.6</td>
<td>M, 65</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.6</td>
<td>F, 77</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10</td>
<td>9</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

*Pre-Tx, pretreatment; Post-Tx, posttreatment.  
+ ≥, CIS present; -, no CIS reported.  
+ ≥, malignant cells present; +/-, suspicious or atypical cells present; - -, no malignant, suspicious, or atypical cells reported.
showed no apparent toxicity to the normal bladder epithelium. However, a partial explanation may be due to the fact that EGFRs are only found on the basal surface of normal transitional cell epithelium while bladder tumor cells exhibit EGFRs about their entire cell surface (19). In any event, the current study clearly establishes TP4O as a very well-tolerated cytotoxic agent even when used at high doses as an intravesical anticancer agent.

Eight of the nine patients initially presenting with histopathological CIS disease exhibited no CIS in multiple bladder biopsies following TP4O therapy. One of these patients demonstrated a 6-month disease-free interval following TP4O therapy, consistent with a complete response. Several other apparently responsive patients exhibited CIS on histopathological examination of the entire bladder following posttreatment cystectomy, however. In addition, one patient exhibited CIS in the posttreatment biopsy, where none was reported before therapy. Although cystoscopic examination of the bladder was generally consistent with the biopsy data, these encouraging results are tempered by the observation that four of the apparently responding patients exhibited malignant or suspicious cells in bladder washings, despite the absence of obvious malignancy in the urinary tract. Whether this observation reflects the existence of cryptic disease outside the bladder or inadequate sampling during posttreatment biopsy is uncertain.

Antitumor activity of TP4O was not evident in patients with visible papillary (T₂) or locally invasive (T₁) bladder cancer in this study. The reason for this poor result in T₂ and T₁ disease is not fully understood, although it is important to recall that most of these patients also failed standard chemotherapeutic regimens before enrollment in the TP4O trial. The treatment regimens used in this study were similar to those shown to be generally effective with other intravesical therapies (11–13). However, these agents were either small organic molecules (e.g., mitomycin and thiotepa) which readily penetrate into tissues and cells, or biological agents which adhere to the bladder wall and initiate an intense inflammatory reaction (i.e., BCG). By contrast, TP4O is a Mr 45,000 protein and is expected to have greater difficulty penetrating into solid tumor masses than low molecular weight cytotoxic agents. In support of this hypothesis, we recently examined the ability of radiolabeled 125I-epidermal growth factor (Mr 5000) to penetrate into the urothelium of normal dog bladders. The epidermal growth factor in this study only penetrated approximately one to two cell layers into the bladder epithelium. Tumor penetration by TP4O was not evaluated in this study. Alternative explanations for TP4O’s poor activity against T₂ and T₁ lesions seem less plausible, since several measures were taken to ensure that TP4O was properly dosed and retained its full biological activity in the urine of patients. Examination of the urine of patients immediately following therapy showed that TP4O retained substantial cell-killing activity throughout the 2-h dwell time in the bladder.

It is unlikely that higher doses of TP4O than those used in this study would have elicited a better response. The concentrations of TP4O achieved in bladder fluid were up to 1000 times higher than those shown to be effective in previous in vitro studies and were up to 2-fold higher than those used successfully in in vivo studies in nude mice (1–4). Patients whose CIS responded to TP4O therapy were scattered throughout the different doses utilized, with no discernible pattern (Table 2). Thus, using higher concentrations of TP4O did not seem to be a promising approach to significantly improve the therapeutic response to this agent. It is possible that the antitumor activity of TP4O could be improved by modifying the treatment protocol either to extend the duration of each treatment or to increase the frequency of treatments. Alternatively, the use of penetration enhancing agents such as DMSO might improve TP4O’s ability to enter solid tumor structures. However, these methods for potentially improving TP4O’s bladder cancer activity profile may also enhance toxicity and will require future clinical testing. At present, it is evident that TP4O is a relatively well-tolerated antitumor agent for the treatment of superficial bladder cancer when administered via the intravesical route. Moreover, in the course of this Phase I trial, TP4O appeared to show antitumor activity against CIS of the bladder in several patients who had failed multiple prior therapies.

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