A Phase I Trial of TNFerade Biologic in Patients with Soft Tissue Sarcoma in the Extremities

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ABSTRACT

Purpose: TNFerade is a second-generation replication-deficient adenovector carrying a transgene encoding human tumor necrosis factor α under control of a radiation-induced promoter. The objective of this study was to assess the tolerance of combining TNFerade and radiation therapy in patients with soft tissue sarcomas of the extremity.

Experimental Design: TNFerade was administered in combination with single-daily fractionated radiation therapy in 14 patients with soft tissue sarcoma of the extremities. Three escalating dose levels of TNFerade (4 × 109–4 × 1011 particle units) were planned, given in 1 log increments by intratumoral injections, twice weekly during week 1 and once weekly during weeks 2–5 of radiation therapy.

Results: TNFerade was well tolerated with no dose-limiting toxicities noted. Grade 1–2 chills (50.0%), fever (43.0%), fatigue (36.0%), and flu-like symptoms (21.0%) were the most common side effects. Serum-tumor necrosis factor α levels were low in all of the patients (<15 pg/mL). No patients had virus-detected blood, sputum, or urine cultures. Of the 13 evaluable patients, 11 received TNFerade preoperatively, and 2 received the treatment for palliation. Eleven patients (85%) showed objective or pathological tumor responses (2 complete and 9 partial), and 1 had stable disease. Partial responses were achieved despite some of these tumors being very large (up to 675 cm2). Of the 11 patients who underwent surgery, 10 (91%) showed a pathological complete response/partial response.

Conclusion: TNFerade + radiation therapy was well tolerated in the treatment of patients with soft-tissue sarcoma of the extremity. The high number of objective responses observed warrants additional studies of this approach in a larger controlled prospective trial.

INTRODUCTION

Tumor necrosis factor α (TNF-α) is a polypeptide proinflammatory cytokine, which has a critical role in the host response to infection (1). TNF-α induces in vitro cytotoxic effects in 30–50% of tumor lines studied, although under most experimental conditions, the addition of a protein synthesis inhibitor is required to demonstrate TNF-mediated cytotoxicity (2, 3). TNF-α derives its name from the finding that hemorrhagic tumor necrosis occurs after intravenous injection of the protein into tumor-bearing mice. This tumor necrosis occurs as a result of direct cytotoxic effects on the tumor endothelium and enhanced procoagulant activity within the tumor vessels, leading to thrombosis of the vasculature and subsequent necrosis (1, 4). These preclinical investigations lead to extensive testing of recombinant TNF-α in humans as an anticancer therapy (5–11). However, relatively low concentrations of TNF-α achieved intravenously produced undesirable side effects, including shock and death. To circumvent the toxicity, perfusion of extremities with high concentrations of TNF-α has been used to enhance local and regional control of large soft tissue sarcoma and in-transit metastases for melanoma. In these investigations, TNF-α is administered intra-arterially and sometimes combined with melphalan, interferon γ, or hyperthermia (12, 13). The demonstration that limb perfusion is associated with significant antitumor activity has supported a role for TNF-α as a local and regional antitumor agent. The strategy of regional perfusion with TNF-α has also been extended to patients with liver metastases (14).

The EGR-1 gene is activated by a range of doses of ionizing radiation (15). Studies of the mechanism of gene in-
duction revealed that the CaRG sequences in the EGR-1 promoter mediates ionizing radiation-induced gene transcription. On the basis of the finding that the CaRG sequences are activated by ionizing radiation, the concept of "genetic radiotherapy" was proposed (16–18). This concept hypothesized that when injected into a tumor, radioinducible DNA sequences linked to a cDNA encoding a toxin (i.e., TNF-α) could provide ionizing radiation-induced spatial and temporal control of gene expression and synergistic antitumor effects. Preclinical studies confirmed this hypothesis. The results demonstrated that a replication-defective adenoviral vector, which contains the EGR-1 promoter upstream to a cDNA encoding human TNF-α (Ad.Egr-TNF) produced high levels of intratumoral TNF-α and synergistic antitumor effects with radiation in diverse preclinical human tumor xenograft models, including prostate, head and neck, gliomas, and esophagus cancers (16, 19–22). Histopathological examination demonstrated destruction of the tumor microvasculature with intravascular thrombosis and necrosis within the tumor (19, 21).

TNFerade is a second generation E1, partially E3 and E4 deleted adenoviral vector that contains a human TNF cDNA under the control of the CaRG elements of EGR-1 promoter. TNFerade was evaluated in three good laboratory practice toxicology studies (23). Two of these investigations were conducted in nude mice and one in immunocompetent BALB/c mice. TNFerade in combination with radiotherapy was well tolerated in these studies and demonstrated a therapeutic window. As in previous tumor studies, TNF-α levels were low, and a high intratumoral TNF-α level was achieved. Dose-limiting toxicity noted at 4 × 10¹¹ particle units (pu) in immunocompetent mice was local ulceration and necrosis at the site of injection. These data, combined with the preclinical antitumor data, demonstrated that TNFerade could be effectively combined with radiotherapy.

The present Phase I study of TNFerade was conducted to evaluate the safety of antitumor administration of TNF-α in combination with radiotherapy for patients with large soft tissue sarcoma of the extremity. These patients would have received preoperative radiotherapy or radiotherapy for palliation. Soft tissue sarcomas were selected for this investigation because they are sensitive to the antitumor effects of TNF-α in limb perfusion, accessible for repeated injections, and not expected to be locally cured with doses of 5000 cGy.

MATERIALS AND METHODS

Patient Selection. Inclusion criteria were: (1) histologically confirmed soft tissue sarcoma located in an extremity (including the limb girdle); (2) tumor size >5 cm (greatest diameter); (3) radiotherapy indicated either preoperatively or for palliation; (4) age >18 years; (5) Karnofsky Performance Status ≥60%; (6) negative pregnancy test and using effective means of contraception; (7) disease measurable by computed tomography or magnetic resonance imaging scan; and (8) life expectancy >3 months.

Exclusion criteria were: (1) liver enzymes >2 × upper limit of normality (aspartate aminotransferase, alanine aminotransferase, and bilirubin); (2) significant anemia, defined by hematocrit <33%, hemoglobin <11g/dL, or thrombocytopenia (platelet count <100,000); (3) evidence of active infection of any type, including adenovirus, hepatitis, or HIV; (4) chemotherapy or experimental medications within the last 4 weeks before day 1; and (5) any acute illness within 1 week of the start of the study or any other illness considered by the investigator to significantly interfere with study outcome.

Regulatory Approvals. All of the patients provided signed informed consent. The protocol was approved by the Institutional Review Boards as well as the Institutional Biosafety Committees in all of the participating institutions. The protocol was also reviewed by the Recombinant DNA Advisory Committee to the NIH Director in accordance with NIH guidelines for Research Involving Recombinant DNA Molecules as well as by the Food and Drug Administration as a supplement to an Investigational New Drug application.

Treatment Plan. Biosafety Level 2 physical and biological containment procedures were followed during the preparation and administration of TNFerade, in accordance with the NIH Guidelines for Research Involving Recombinant DNA Molecules. TNFerade was administered by intratumoral injection, twice weekly during week 1 and once weekly from weeks 2 to 5. Each dose of TNFerade was divided into 3–8 aliquots for injection. Total injection volume was 3–8 mL depending on tumor size. A volume of 1 cc was given per injection. Injections were given in straight lines down the center of the tumor for a corridor of administration. The penetration depth for each injection was 2–3 cm, depending on tumor volume and accessibility. Injections were spaced so as to allow the maximum area of the tumor to be infected with TNFerade but also to allow for excision of the injection site if necessary and skin flap preservation. A 21–22-gauge needle, depending on tumor size and accessibility, was used. Three to 6 patients were to be treated per dose level, starting with a dose of 4 × 10⁹ pu and escalating in 1-log increments to a dose of 4 × 10¹¹ pu or until the maximal tolerated dose was reached.

Radiation Therapy Schedule. All of the patients received concomitant external beam radiotherapy administered in 1.8–2.0 Gy single-daily fractions for 5 days a week for up to 5 weeks for a total dose of 36–50.4 Gy. The tumor volume targeted included a minimum of 5-cm margins proximally and distally around the gross tumor volume, visualized on either computed tomography or magnetic resonance imaging scan.

All of the patients were evaluated twice weekly during week 1, once weekly during weeks 2–5, and at 2 and 6 weeks after completion of treatment (or just before surgery). Patients who underwent surgery were also evaluated within 7 days after surgery. Physical examination, patient symptomatology, and laboratory safety tests were used during and after the study. Surgery was performed in patients whose tumor was resectable 3–9 weeks after completion of radiotherapy. Across all of the patients at a particular site, 1–3 pathologists performed pathological assessment of tumor response.

Definition of Dose-Limiting Toxicities and Schedule for Dose Escalation. Patients were monitored for safety and tolerability using National Cancer Institute Common Toxicity Criteria. Initially, 3 patients were to be included at each dose level. Dose-limiting toxicity was defined as: (1) any grade 3 or higher nonhematologic toxicity (except alopecia, nausea, vomiting, and diarrhea); (2) grade 4 thrombocytopenia, neutropenia, or ane-
mia; or (3) grade 3 or higher nausea, vomiting, or diarrhea
despite maximal antiemetic and/or anti diarrheal agents,
possibly, probably, or definitely related to TNFerade or the com-
bination of TNFerade and radiotherapy occurring during the com-
bined modality treatment period and up to 2 weeks after. If no
dose-limiting toxicity developed, dose-escalation would con-
tinue until the highest dose of $4 \times 10^{11}$ pu was reached. If 1 of
3 patients developed dose-limiting toxicity during the specified
treatment and observation period, another 3 would be enrolled at
that dose level. If 2 of 3 or 3 of 3 developed dose-limiting
toxicity, the previous dose level would be classified as the
maximal tolerated dose, and there would be no additional dose
escalation. If 1 of 6 patients developed dose-limiting toxicity,
dose-escalation would be allowed. If 2 or more of 6 developed
dose-limiting toxicity, the previous lower dose would be desig-
nated the maximal tolerated dose. Once determined, a total of 6
patients would be enrolled at the maximal tolerated dose. There
was no intrapatient dose escalation. Once 3 patients completed
TNFerade + radiotherapy and had been observed for at least 2
weeks for acute toxicity, additional patients could start treatment
at the next dose level.

Special Pharmacodynamic and Safety Measures. Se-
rum TNF-α levels were monitored at baseline and on study days
1, 2, 4, and 5 during the first week, on day 5 during weeks 2–5,
at 2 weeks after completion of treatment and after surgery. An
ELISA kit (Quantikine HS, R & D Systems, Minneapolis, MN)
with a level of detection of 0.18 pg/mL was used. Neutralizing
antibody titer against adenovirus was monitored at baseline, on
day 5 during week 1, at 2 weeks after completion of treatment,
and after surgery using a standard assay. Cultures of blood,
sputum, and urine taken at baseline and 2 weeks after comple-
tion of treatment were analyzed for the presence of virus.

Construction of TNFerade. TNFerade was constructed
by using GenVec’s AdFAST technology as described previ-
ously (23). Briefly, TNFerade is a human adenovirus serotype 5
deleted of early regions 1A, 1B, and 4 and partially of early
region 3 and with a transcriptionally inert spacer sequence
inserted into the E4 region (23). The TNF-α expression cassette
contains the nucleotides 1–455 of Ad2, the mouse EGR-1
promoter including the radiation inducible CArG elements, a
splice acceptor and splice donor from SV40 late genes, the
complete TNF-α cDNA, and an SV40 poly(A) site (23). TN-
ferade was propagated and expanded in 293-ORF6 cells as
described (24). 293-ORF6 cells were grown in media containing
serum, infected with TNFerade, and E4-ORF6 expression
was induced by the addition of ZnCl₂. Adenovirus particles were
purified using three successive rounds of CsCl gradient centri-
fugation. The adenovector was formulated for storage and as-
sayed for potency, purity, sterility, and absence of replication-
competent adenovirus.

Statistical Analyses. All of the data are presented as
means ± SE unless otherwise indicated.

Role of Funding Source. Representatives of the funding
source were involved in the study design, data collection,
and article preparation. However, the interpretation of the data
were the responsibility of the authors themselves. In addition, all
aspects of study design, data collection, data interpretation, and
article preparation were overseen by the principal investigator
A. J. Mundt.

RESULTS

Patient Characteristics. Fourteen patients were enrolled
from August 28, 2001 to August 13, 2002 at 5 institutions. One
patient, (patient 8, Table 3) who received a dose of $4 \times 10^{11}$ pu
withdrew due to “flu-like” symptoms (National Cancer Institute
Common Toxicity Criteria grade 2) after the first week of
dosing and was not assessable for activity. The remaining 13
patients were evaluable for both safety and antitumor activity.
Demographic details of the 14 patients are provided in Table 1.

Safety. No dose-limiting toxicities were observed (Table
2), and the maximal tolerated dose was not reached. One patient
(patient 8, Table 3) reported grade 2 flu-like symptoms after the
second injection and elected to withdraw from the protocol,
although the toxicity did not qualify as a dose-limiting toxicity.
Two grade 4 toxicities of wound infection and gangrenous
necrosis were observed at the $4 \times 10^{11}$ pu dose; however, these
events occurred 133 and 330 days after the last administration
of TNFerade respectively and were, therefore, not considered
dose-limiting toxicities. Potentially drug-related side effects
most commonly reported were fever and chills, described in
50% and 43% of the patients, respectively. Flu-like symptoms
were described in 21% of the patients. All possibly, probably, or
definitely related side effects are listed in Table 2. The adverse
events increased in frequency and severity by dose. There were
no National Cancer Institute Common Toxicity Criteria grade 2
events at the lower dose of $4 \times 10^{9}$ pu as compared with grade 5
events at the highest dose of $4 \times 10^{11}$ pu. Fever was not
reported at the lowest dose but occurred in 4 of 7 (57%) patients
at the highest dose. The two reported grade 4 events of wound
infection and gangrenous necrosis also occurred in patients
treated at the highest vector dose. Typically, fever was most
pronounced with the initial administration of TNFerade and was
blunted with subsequent administrations often due to acetamino-
phen prophylaxis. Serum TNF-α levels remained low in all of
the patients; no patients had levels in excess of 15 pg/mL, and
the majority of measurements were in the 3–10 pg/mL range
(normal range, <8.3 pg/mL). There was no significant increase
in serum TNF-α from baseline. All of the patients had relatively
low titers (<1:50) of neutralizing antibodies against adenovirus
type 5 at baseline. Almost all of the patients exhibited signif-

Table 1  Patient characteristics (n = 14)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex:</td>
<td>8 males, 6 females</td>
</tr>
<tr>
<td>Age, years: median, 62 years (range, 39–84 years)</td>
<td></td>
</tr>
<tr>
<td>Karnofsky Performance Status 100: 4</td>
<td></td>
</tr>
<tr>
<td>90: 8</td>
<td></td>
</tr>
<tr>
<td>80: 1</td>
<td></td>
</tr>
<tr>
<td>70: 1</td>
<td></td>
</tr>
<tr>
<td>60: 0</td>
<td></td>
</tr>
<tr>
<td>Race: White, 11; Black, 3</td>
<td></td>
</tr>
<tr>
<td>Tumor type Liposarcoma 6</td>
<td></td>
</tr>
<tr>
<td>Malignant fibrous histiocytoma: 4</td>
<td></td>
</tr>
<tr>
<td>Chondrosarcoma: 1</td>
<td></td>
</tr>
<tr>
<td>Leiomyosarcoma: 1</td>
<td></td>
</tr>
<tr>
<td>Spindle cell sarcoma: 2</td>
<td></td>
</tr>
</tbody>
</table>
to ≥ 1:16,384). No virus was found in blood, urine, or sputum in any samples from any patients at any time points.

**Tumor Response.** Tumor responses are listed in Table 3. Eleven of thirteen (85%) patients demonstrated an objective tumor response with 2 complete responses and 9 partial responses. Of the 11 patients who went on to surgery, 10 had either pathological complete response (2) or partial response (8). Four of the 8 partial responses demonstrated >95% tumor necrosis. All 11 of the patients were resected with negative margins. Two patients received TNFerade for palliation and did not undergo surgery. One had a partial response (patient 1, Table 3), the other stable disease (patient 6, Table 3).

**Tumor Response Assessment.** There was a substantial discrepancy in response determined by computed tomography scan and pathological assessment after resection (Table 3). In 9 of the 11 patients who underwent surgery at 3–9 weeks after completion of study drug, computed tomography scans underestimated responses as determined by histologic assessment (Table 3). For example, patient 4 showed stable disease on computed tomography scan but was classified as a pathological complete remission by histologic examination of the resected tissue. These results indicate that computed tomography scans performed shortly after completion of TNFerade + radiotherapy underestimate the magnitude of the treatment effects of TNFerade.

**Table 2 Adverse events, possibly, probably or definitely related to TNFerade**

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>TNFerade 4 × 10^9 pu (n = 3)</th>
<th>TNFerade 4 × 10^10 pu (n = 4)</th>
<th>TNFerade 4 × 10^11 pu (n = 7)</th>
<th>Overall (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1* N (%)</td>
<td>Grade 1* N (%)</td>
<td>Grade 2* N (%)</td>
<td>Grade 1* N (%)</td>
</tr>
<tr>
<td>Body as a whole</td>
<td>Fatigue 1 (33)</td>
<td>1 (25)</td>
<td>3 (43)</td>
<td>5 (36)</td>
</tr>
<tr>
<td></td>
<td>Fever 2 (50)</td>
<td>1 (25)</td>
<td>4 (57)</td>
<td>6 (43)</td>
</tr>
<tr>
<td>Flu-like symptoms</td>
<td>Hot flashes 1 (33)</td>
<td>1 (25)</td>
<td>1 (14)</td>
<td>1 (7)</td>
</tr>
<tr>
<td></td>
<td>Rigors 1 (33)</td>
<td>2 (50)</td>
<td>3 (43)</td>
<td>1 (14)</td>
</tr>
<tr>
<td></td>
<td>Weakness 1 (33)</td>
<td></td>
<td></td>
<td>1 (7)</td>
</tr>
<tr>
<td>Digestive system</td>
<td>Diarrhea 1 (33)</td>
<td>1 (25)</td>
<td>1 (14)</td>
<td>1 (7)</td>
</tr>
<tr>
<td></td>
<td>Indigestion 1 (33)</td>
<td></td>
<td></td>
<td>1 (7)</td>
</tr>
<tr>
<td></td>
<td>Stomatitis 1 (25)</td>
<td></td>
<td></td>
<td>1 (7)</td>
</tr>
<tr>
<td></td>
<td>Vomiting 1 (33)</td>
<td></td>
<td></td>
<td>1 (7)</td>
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<tr>
<td>Hemic and lymphatic system</td>
<td>Anemia 1 (14)</td>
<td>1 (7)</td>
<td>1 (7)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Metabolic and nutritional disorders</td>
<td>Anorexia 1 (25)</td>
<td></td>
<td></td>
<td>1 (7)</td>
</tr>
<tr>
<td></td>
<td>Peripheral edema 1 (14)</td>
<td></td>
<td></td>
<td>1 (7)</td>
</tr>
<tr>
<td>Musculoskeletal system</td>
<td>Myalgia 1 (14)</td>
<td>1 (25)</td>
<td>1 (14)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Skin and appendages</td>
<td>Blister 1 (25)</td>
<td>1 (25)</td>
<td>1 (7)</td>
<td>1 (7)</td>
</tr>
<tr>
<td></td>
<td>Full feeling extremity 1 (25)</td>
<td></td>
<td></td>
<td>1 (7)</td>
</tr>
<tr>
<td></td>
<td>Gangrenous necrosis 1 (14)</td>
<td>1 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection site reaction</td>
<td>Radiation dermatitis 1 (14)</td>
<td></td>
<td></td>
<td>1 (7)</td>
</tr>
<tr>
<td></td>
<td>Wound infection 1 (14)</td>
<td></td>
<td></td>
<td>1 (7)</td>
</tr>
<tr>
<td>Urogenital system</td>
<td>Genital pain 1 (33)</td>
<td></td>
<td></td>
<td>1 (7)</td>
</tr>
</tbody>
</table>

* National Cancer Institute Toxicity.

**DISCUSSION**

Systemic application of recombinant TNF-α protein in patients with cancer has been limited by substantial systemic side effects (5–11). However, the limited systemic toxicity when TNF-α is administered by isolated perfusion of the limb has resulted in durable complete tumor regressions and limb salvage in patients with sarcoma of the extremities destined for amputation (12, 13). We have proposed that a gene transfer approach could produce high intratumoral levels of TNF-α without untoward systemic toxicity (16–18). Preclinical studies have shown that adenovectors with TNF-α under control of a constitutive promoter, while successful in producing partial and complete tumor regression, result in significant systemic toxicity (25, 26). To circumvent systemic toxicity, a gene therapy approach targeted by radiotherapy has been proposed as an approach to optimize the therapeutic index of TNF-α. A radiation-inducible promoter (16) allows for locally targeted induction of high TNF-α levels within the tumor volume with little TNF-α secreted into the systemic circulation. Moreover, an additive or synergistic antitumor interaction between TNF-α and ionizing radiation, based principally on attacking the tumor vasculature, is facilitated by this strategy (11, 27). Indeed, TNFerade (up to 4 × 10^11 pu repeated up to six times over a 5-week period) was well tolerated without significant systemic or local toxicities.
Interestingly, plasma concentration of TNF-α did not exceed 15 pg/mL in any patients at any time point (assay normal <8.3 pg/mL). Serum TNF-α levels, while elevated in some patients (assay range normals < 8.3 pg/mL), were significantly below the peak plasma concentrations of TNF-α (approximately 10,000–25,000 pg/mL) at the maximal tolerated dose observed in clinical studies using infusions of the recombinant protein (9, 10). These data support the lack of systemic toxicity observed in this study. Some of the toxicities observed (fever, chills, flu-like symptoms, and fatigue) are consistent with systemic TNF-α exposure but could also be explained by the adenovector itself or by a combination of radiation and the adenovector. The low serum TNF-α levels suggest that the cause of the fevers is not TNF-α secreted by the vector. It is possible that the time intervals selected for TNF-α serum measurements were not frequent enough to detect subtle increases in serum levels, recognizing that the half life of the recombinant protein after intravenous injection is short (<0.5 hours; ref. 9). The dose-response relationship found, however, indicates that at least some of the adverse events observed are drug-related.

Preoperative and/or postoperative wound complications were observed in 5 patients, consisting of wound infection and wound dehiscence postoperatively (patient 3), wound abscess postoperatively (patient 5), cellulitis and need for wound debridement pre and postoperative (patient 10), a preoperative superficial wound infection and postoperative wound infection (patient 11), and gangrenous necrosis requiring amputation postoperatively (patient 14). All of the wound complications resolved with therapy, and only the superficial wound infection, postoperative wound infection (patient 11), and the gangrenous necrosis (patient 14) was attributed by the investigators as being possibly related to study drug. Although none of these events were considered dose-limiting toxicities, the fact that the two grade 4 events were reported in patients treated at the highest dose level does suggest some concern about the effect of TNRFerade and radiation on postoperative wound healing. An increased incidence of postoperative wound infections has been reported when patients received preoperative radiotherapy (28–31). Wound complications have been reported in 22–51% of patients with sarcoma of the extremity that received preoperative radiotherapy (28–31). Of note, when isolated limb perfusion with TNF-α, interferon γ, and melphalan was combined with resection and high-dose postoperative ionizing radiation, delayed healing of resection wounds and major wound complications, including amputations, were reported (32). Upon comparison with data reported previously, the data from the present

### Table 3: Individual patient demographics and tumor response

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Indication</th>
<th>Histology</th>
<th>Radiation (Gy)</th>
<th>Tumor measurements (computed tomography scan)</th>
<th>Response</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 x 10^6 pu</td>
<td>Palliation</td>
<td>High-grade sarcoma, round cell liposarcoma</td>
<td>50</td>
<td>25</td>
<td>10</td>
<td>PR</td>
</tr>
<tr>
<td>2</td>
<td>Pre-op.</td>
<td>Myxoid liposarcoma, mixed type, myxoid and round cell components</td>
<td>50</td>
<td>103</td>
<td>75</td>
<td>MR</td>
</tr>
<tr>
<td>3</td>
<td>Pre-op.</td>
<td>Myxoid liposarcoma</td>
<td>50</td>
<td>328</td>
<td>230</td>
<td>MR</td>
</tr>
<tr>
<td>4</td>
<td>Pre-op.</td>
<td>Malignant fibrous histiocytoma; pleomorphic-storiform type</td>
<td>50</td>
<td>338</td>
<td>330</td>
<td>SD</td>
</tr>
<tr>
<td>5</td>
<td>Pre-op.</td>
<td>Intermediate grade spindle cell sarcoma, consistent with leiomysarcoma</td>
<td>50</td>
<td>238</td>
<td>264</td>
<td>SD</td>
</tr>
<tr>
<td>6</td>
<td>Palliation</td>
<td>Malignant fibrous histiocytoma</td>
<td>36</td>
<td>42</td>
<td>51</td>
<td>SD</td>
</tr>
<tr>
<td>4 x 10^11 pu</td>
<td>Pre-op.</td>
<td>Spindle cell sarcoma</td>
<td>50</td>
<td>72</td>
<td>136</td>
<td>PD</td>
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<tr>
<td>8</td>
<td>Pre-op.</td>
<td>Myxoid liposarcoma</td>
<td>45</td>
<td>128</td>
<td>108</td>
<td>SD</td>
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<td>9</td>
<td>Pre-op.</td>
<td>Dedifferentiated liposarcoma</td>
<td>45</td>
<td>46</td>
<td>37</td>
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<td>10</td>
<td>Pre-op.</td>
<td>Myxoid liposarcoma</td>
<td>50.4</td>
<td>78</td>
<td>32</td>
<td>MR</td>
</tr>
<tr>
<td>11</td>
<td>Pre-op.</td>
<td>Malignant spindle cell sarcoma</td>
<td>50.4</td>
<td>46</td>
<td>37</td>
<td>MR</td>
</tr>
<tr>
<td>12</td>
<td>Pre-op.</td>
<td>High grade pleomorphic sarcoma, consistent with malignant fibrous histiocytoma</td>
<td>50</td>
<td>675</td>
<td>442</td>
<td>MR</td>
</tr>
<tr>
<td>13</td>
<td>Pre-op.</td>
<td>Extraskeletal myxoid chondrosarcoma</td>
<td>50</td>
<td>271</td>
<td>218</td>
<td>MR</td>
</tr>
</tbody>
</table>

Abbreviations: Pre-op, preoperative; PR, partial response; MR, CR, complete response; SD, stable disease.

* Complete disappearance of all tumor tissue upon pathological examination.
Phase I study suggest that wound complications are not increased by the addition of TNFerade injections to preoperative radiotherapy. Long-term follow-up will be required to assess the late effects of TNFerade and radiotherapy in these patients.

Pathological complete responses as a result of radiation alone are very rare in extremity soft tissue sarcomas of the size seen in the present study, with baseline tumors of 328 cm$^2$ and 338 cm$^2$, respectively (Table 3). In the current study, 6 of 11 patients who underwent resection showed $\geq$95% necrosis (2 complete response, 4 patients showed 95% necrosis), which prognostically carries an improved long-term survival (33). When compared with patients who achieve less tumor necrosis, Eilber et al. (33) reported a pathological complete response (>95% necrosis) of 14% in patients with extremity soft tissue sarcoma treated with neoadjuvant chemoradiation, whereas a 15% pathological complete response (100% necrosis) was reported by Cormier et al. (34). Hew et al. (35) noted that 35% of patients had >80% necrosis when treated with preoperative chemoradiation. The present study provides preliminary evidence that TNFerade in combination with radiation represents a potential alternative to isolated limb perfusion (36). The apparent lack of a clear relationship between response and (vector) particle units may be due to the relatively small number of patients in this study, the multiple histologic subtypes of sarcomas, or the possibility that the maximum effective vector dose was reached.

Data from this study also demonstrate that computed tomography scans, performed 2 weeks after completion of TNFerade and concurrent radiotherapy, appear to underestimated the actual antitumor effects of TNFerade and ionizing radiation. For 9 of 11 patients who had surgery, computed tomography scans showed less response than histologic assessment (Table 3). For example, patient 4 showed SD on computed tomography scan but was classified as a complete pathological response after histologic assessment of the resected tissue. These observations indicate that in patients with soft tissue sarcoma, computed tomography scan might underestimate the true response rate of TNFerade and ionizing radiation, in particular if recorded shortly after completion of treatment.

This study demonstrates that preoperative administration of repeated intratumoral injections of TNFerade in combination with radiation is feasible and well tolerated. In addition to a favorable safety profile, these data provide support for antitumor effects, which are greater what would be expected for radiotherapy alone in patients with soft tissue sarcoma. These data also provide support for the hypothesis that gene transfer, using a radiation-inducible promoter, in combination with radiotherapy, may represent an effective way to capitalize on the antitumor activity of TNF-$\alpha$ without the systemic toxicity. This is supported by results of a recently completed Phase I study in patients with solid tumors (37). The results justify Phase II studies in patients with soft tissue sarcoma. Due to the effects of radiotherapy alone on efficacy and safety assessments, it is critical that Phase II studies be randomized and controlled.

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