

A phase I trial of immunotherapy with intratumoral adenovirus-interferon-gamma (TG1041) in patients with malignant melanoma

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Aims: Interferon-gamma (IFN- γ) has been shown to upregulate MHC class I and II expression, and to promote generation of specific antitumor immune responses. We hypothesized that intratumoral administration of an IFN- γ gene transfer vector facilitates its enhanced local production and may activate effector cells locally. We conducted a phase I dose-escalation study of a replication-deficient adenovirus-interferon-gamma construct (TG1041) to determine safety and tolerability of intratumoral administration, in advanced or locally recurrent melanoma. **Methods:** Patients were enrolled at four successive dose levels: 10^7 infectious units (iu) ($n=3$), 10^8 iu ($n=3$), 10^9 iu ($n=3$), and 10^{10} iu ($n=2$) per injection per week for 3 weeks. TG1041 was injected in the same tumor nodule weekly in each patient. Safety, toxicity, local and distant tumor responses and biologic correlates were evaluated. **Results:** A total of 11 patients were enrolled and received the planned three injections per cycle. One patient with stable disease received a second cycle of treatment. A maximum tolerated dose was not reached in this study. No grade 4 toxicities were observed. Two grade 3 toxicities, fever and deep venous thrombosis were observed in one patient. The most frequently reported toxicities were grade 1 pain and redness at the injected site ($n=8$), and grade 1 fatigue ($n=5$) patients. Clinical changes observed at the local injected tumor site included erythema ($n=5$), a minor decrease in size of the injected lesion ($n=5$) and significant central necrosis by histopathology ($n=1$). Systemic effects included stable disease in one patient. Correlative studies did not reveal evidence of immunologic activity. **Conclusion:** Weekly intratumoral administration of TG1041 appears to be safe and well tolerated in patients with advanced melanoma.

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Interferon-gamma (IFN- γ) belongs to a family of proteins called interferons, originally believed to be produced as a defensive response by virally infected cells.¹ Extensive research over the past several years has demonstrated that IFN- γ plays a critical role in a multitude of immunologic processes, and is vital to the promotion of tumor surveillance in immunocompetent hosts. IFN- γ upregulates MHC class I and class II molecules in a wide variety of cells.^{2–5} IFN- γ treatment of various nonantigenic tumors has been demonstrated to make them highly antigenic, and this correlates with an increase in expression levels of IFN- γ -inducible genes that regulate MHC class I presentation.^{6,7} IFN- γ is capable of

upregulating the expression of tumor-associated antigens *in vitro* and *in vivo*,^{8–10} thereby increasing the susceptibility of tumors to MHC-restricted CD8+ cytotoxic T-cell-mediated lysis. IFN- γ also activates macrophages to nonspecifically lyse neoplastic cells through various mechanisms.^{11–14} IFN- γ may correct antigen-processing defects by upregulating the transporter associated with antigen processing (TAP-1).¹⁵ This may be important in tumor surveillance, especially since melanoma cells have been shown to consistently underexpress peptide transporters.¹⁶

Interleukin-12 (IL-12) has potent antitumor activity, and IFN- γ is required for robust IL-12 secretion by antigen-presenting cells (APC).^{17–19} Recent work has focused attention on inhibition of angiogenesis by IFN- γ .²⁰ Members of the CXC chemokine family that are inducible by IFN- γ and lack the ELR motif are potent inhibitors of angiogenesis.²¹ These include IFN- γ -inducible protein-10 (IP-10)²² and monokine induced by IFN- γ (MIG).²³

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Despite promising *in vitro* evidence of potent antitumor activity of IFN- γ , trials involving systemic administration of IFN- γ have been generally unsuccessful.^{24,25} Exceptions to this include significant disease responses in chronic myelogenous leukemia²⁶ and ovarian cancer.²⁷ A possible explanation for this lack of clinical efficacy may relate to the relative importance of local expression of IFN- γ . In a study of combination systemic IFN- γ and IL-2 in melanoma patients, only one of 12 patients (a responder) showed upregulation of HLA class I antigens; indeed, pharmacokinetic studies conducted in these patients demonstrated peak systemic levels of IFN- γ to be 5- to 10-fold less than the optimal concentrations required to elicit class I expression.²⁸

One approach to obviate this defect of systemic administration of IFN- γ would be to utilize gene therapy. Intratumoral gene therapy permits the targeted delivery of a gene to a specific site and the expression of that gene in a controlled fashion. Viral vectors in general have the advantage of allowing relatively efficient transfer of the desired gene into the cell, and the expression of the gene within that cell. TG1041 is an adenovirus vector carrying the nucleotide sequence for the human gene for IFN- γ (Fig 1). The vector is derived from a type 5 (group C) adenovirus, with deletion of the E1, E3 and E4 regions. The vector is replication deficient, and the deletions of both the E1 and E4 regions separately ensure its inactivation. The cDNA for human IFN- γ has been inserted into the E1 region in an expression cassette, which also includes the RSV promoter, as well as intron 2 of the β -globulin gene, to increase efficiency of transcription. Preclinical work established sustained expression of human IFN- γ in several different established cell lines, including human primary tumor cells (P Leroy, Transgene, unpublished data). In addition, antitumor effect was demonstrated by a delay in tumor appearance in B6D2 mice injected with B16F0 cells infected with the study drug, as compared to a control group ("empty" adenoviral vectors) (P Slos, Transgene, unpublished data). Other workers have introduced IFN- γ genes through retroviral vectors into tumor-bearing mice, and demonstrated high expression of MHC class I antigens.²⁹

On the basis of these preclinical and clinical data and background, we conducted a phase I dose-escalation study of TG1041 in patients with metastatic or locally

recurrent melanoma. The primary objectives of this phase I study were to determine the safety and tolerability of intratumoral injection with adeno-IFN- γ , and to determine the maximum tolerated dose. Secondary objectives included determination of any local or distant antitumor effects, and of the biologic and immunologic effects of tumor transduction with the study vector.

Patients and methods

Study vector

The study vector, TG1041, was supplied by Transgene, Inc., Rockland, MA. The product was provided deep-frozen in 2-ml glass ampoules. The viral suspension was diluted in a sterile solution of NaCl (9 g/l) to obtain the desired therapeutic dose, with total volume not exceeding 0.2 ml. The suspension was prepared in a laminar airflow hood immediately prior to administration. The study drug was administered in a 1-ml syringe via the intratumoral route, with tumor localization by physical examination.

Definition of infectious units: Virus is serially diluted on to LCA1 cells (293 cells transfected with plasmid pTG5606 that carries a recombinant gene coding for open reading frames (ORF 6 and 6/7 of the E4 region of Ad5) in a culture of 70–90% confluence. After 16–20 hours of incubation, the cells are treated with acetone/methanol then washed with PBS + FBS. Areas of adenoviral infection are detected by incubation with the antibody 72K-B6-8 that is specific for DNA binding protein (DBP), an early adenovirus protein. A fluorescent secondary reagent (goat anti-mouse Ig or rabbit anti-mouse Ig) is used to reveal areas of anti-DBP binding. An area of fluorescence on the cell monolayer is considered an infectious unit.³⁰

Eligibility criteria

Eligible patients had a histologic diagnosis of malignant melanoma with either metastatic disease or locally recurrent disease not amenable to curative therapy, with cutaneous or nodal sites of disease accessible for intratumoral administration. No systemic therapy was permitted within the preceding 3 weeks; and eligible

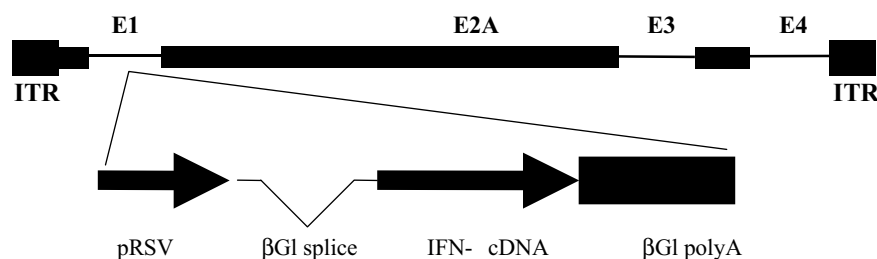


Figure 1 Schematic diagram describing the structure of the adenovirus gene therapy vector TG1041.

patients had to be aged ≥ 18 years; not to be pregnant and use adequate contraception during study; ECOG performance status of 0–2; minimum life expectancy of 3 months; adequate hematologic, hepatic and renal function; and provide a written informed consent. Patients were excluded if they had a known history of human immunodeficiency virus infection or other active systemic infections, uncontrolled central nervous system metastases, serious concomitant systemic medical disorders, concomitant systemic corticosteroid therapy, or were participating in another experimental protocol during the study period. Prior biologic therapy was permitted.

Study design and treatment plan

This was a phase I single-center dose-escalation study of intratumoral administration of adeno-IFN- γ , conducted at the James P. Wilmot Cancer Center, University of Rochester, Rochester, NY, and approved by the Institutional Review Board. Dose escalation was planned to be carried out in cohorts of at least three patients to be treated successively at four dose levels, 1×10^7 , 1×10^8 , 1×10^9 and 1×10^{10} iu, or until the maximum tolerated dose was reached. Each patient was to receive three doses of study drug at weekly intervals (days 1, 8 and 15). Observed toxicities were graded according to the World Health Organization Criteria. Dose-limiting toxicity was to be defined as the level at which grade 4 toxicity believed to be related to treatment is observed, or the level at which more than two patients experience grade 3 treatment-related toxicity. The maximum tolerated dose (MTD) was defined as the dose level immediately below that of dose-limiting toxicity.

Each patient received intratumoral injections into the same tumor site on days 1, 8 and 15. No dose modifications were permitted at the assigned dose level. Any patient who developed grade 3 or 4 toxicity on the assigned dose was to receive no further therapy on protocol. Additional cycles of three injections were allowed at the investigator's discretion if there was evidence of tumor response.

Evaluation during study

Clinical: Patients were evaluated with a complete history, physical examination, complete blood count (CBC), liver functions tests (LFTs), serum creatinine, pregnancy test (for females of childbearing potential) and HLA typing by serology, prior to study entry. Patients were observed closely for 4 hours after the initial dose (day 1), and for 2 hours after subsequent doses (days 8 and 15). Clinical evaluation, CBC, LFTs and serum creatinine were repeated on days 8, 15 and 36 (end of study).

Immunology: Immunologic evaluation prior to therapy included a delayed-type hypersensitivity panel, and measurement of cellular and humoral immune responses to both melanoma antigens and adenoviral antigens. Measurement of cellular and humoral immune responses to both melanoma antigens and adenoviral antigens was repeated on days 15 and 36. Analysis of circulating IFN-

γ , IL-6 and β -2 microglobulin was performed pre-injection and at 6, 24 and 48 hours after injection on days 1 and 15.

Virology: Blood samples were obtained for viral particle detection by culture and PCR assays prior to injection, 1 hour postinjection (day 1 only), 24 hours postinjection and on day 36. Urine samples for viral particle detection were obtained prior to each injection, 6 hours post-injection (days 1 and 15 only), 24 hours post-injection and on day 36.

Tumor sites: The lesion to be injected was measured with a caliper whenever possible, and measurements were conducted by the same health-care provider pre-therapy on day 1 and post-therapy on days 15 and 36. Assessment of all other sites of disease was performed pretherapy and on day 36 using computed tomography or magnetic resonance imaging techniques, as clinically indicated.

Enzyme-linked immunospot (ELISPOT) assays

Peripheral blood mononuclear cells (PBMC) were collected and cryopreserved. For ELISPOT assays, PBMC were thawed, allowed to recover in the complete assay medium overnight, washed, counted in a trypan blue dye to determine cell recovery and viability, and used in direct ELISPOT assays for IFN- γ production in response to melanoma or control peptides. In the assay, PBMC were plated in wells of the ELISPOT plate at 100,000 cells per well, except when available cells were few and it was necessary to plate 50,000 cells per well. As APC, T2 cells were used (1 T2 for 20 PBMC); these were pulsed with a single melanoma peptide (experimental) or pulsed with a control peptide (Flu matrix or HIV reverse transcriptase). APC were added to triplicate wells, and the plates were incubated at 37°C. for 20 hours. The assay was then developed and the number of spots/well was read in a Zeiss image analyzer/microscope. The data are presented as mean spot counts from triplicate wells. The "background" spots (spots counted in wells containing unpulsed T2 cells plus PBMC) were subtracted from the number of spots in experimental or control peptide wells. The frequency of IFN- γ -producing cells was then calculated using these corrected values. Internal controls for the assay were PBMC obtained from a normal donor and stimulated with PHA. The priority was set to test the following peptides, depending on the availability of PBMC: gp100 280–288, Mart-1/Melan-A 26–35, Tyrosinase 368–376, Flu matrix 58–66 and HIV reverse transcriptase 476–484 (obtained from Dr Pedro Romero, Lausanne, Switzerland). All peptides were pulsed on T2 cells during a 2-hour incubation at the concentration of 20 μ g/ml. T2 cells were washed to remove an excess of the peptide prior to their addition to PBMC in wells of the ELISPOT plates.

Enzyme-linked immunosorbent assay (ELISA)

Blood samples were drawn at the time points specified in red top or serum separation tube and allowed to clot for at least 30 minutes, but no longer than 1 hour, before centrifugation. Tubes were centrifuged at 1000 rpm for 15

minutes; the serum was aliquoted into cryovials and samples were stored at -20°C until assays were run. Human β -2 microglobulin ELISA kits were purchased from R&D Systems (Minneapolis, MN). Human IL-6 ELISA kits were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Human IFN- γ ELISA kits were purchased from both R&D Systems and Amersham Pharmacia Biotech. The ELISA assays were run in accordance with the manufacturer's instructions.

Results

Patient characteristics

A total of 11 patients with histologically confirmed locally recurrent or metastatic malignant melanoma were enrolled in the trial between June 1999 and April 2001 (Table 1). There were six men and five women, with a median age of 69 years (range, 45–81 years). At diagnosis, four patients were stage IV, four were stage III, one was stage II, one was stage I and one had ocular melanoma. At study entry, these patients had a median Eastern Cooperative Oncology Group (ECOG) performance status of 1 (range, 0–2) and 8/11 (72%) patients had ≥ 3 sites of disease. Study patients had received a median of four previous therapies (range, 1–11), including immunotherapy with interferon- α , and high-dose Il-2, chemotherapy with various regimens including dacarbazine and cisplatin-based therapies, surgery and external beam radiation.

Toxicity

All 11 patients were assessable for toxicity evaluation (Table 2). The study drug was well tolerated, and each of the patients enrolled received the full three doses at their

assigned dose level. Patient 006 enrolled at dose level 2 received an additional cycle of three doses. No patients died as a result of administration of adeno-IFN- γ . No grade 4 or dose-limiting toxicities were observed.

Patient 011, enrolled at dose level 4, and the site of injection was in the patient's left axilla. This patient experienced two grade 3 toxicities: fever of 40.3°C on day 15, and right lower extremity deep venous thrombosis on day 19. The patient was hospitalized primarily for observation of fever, and treated empirically with a broad-spectrum antibiotic. The fever resolved within the next 12 hours, and the patient was discharged 1 day later, without antibiotics. No organisms were cultured from blood or urine. Interestingly, cytokine levels obtained on day 15 demonstrated a marked increase in serum IFN- γ levels by ELISA approximately 6 hours after injection of dose 3. Of note, only one other patient (005) had detectable serum levels of IFN- γ , obtained 48 hours after dose 3. On day 23, patient 011 developed right leg swelling and cramping, and was found to have a right lower extremity deep venous thrombosis. He was admitted for intravenous anticoagulation. The patient was discharged on day 27 on oral dicoumarol; no pulmonary or other complications were observed. It is unclear whether the development of deep venous thrombosis could be related to study drug, or whether it was a complication of the patient's underlying advanced malignancy.

The most frequently reported toxicities were grade 1 pain and/or redness at the injected site in 8/11 (72%) patients, and grade 1 fatigue in 5/11 (45%) patients. Other toxicities reported, of unknown relation to study drug, included grade 2 upper respiratory tract illness ($n=1$), grade 1 fever ($n=1$), grade 1 rash ($n=1$), grade 1 headache ($n=2$), grade 1 or 2 nausea ($n=5$), grade 2

Table 1 Patient characteristics at study entry

Patient	Age	Gender	Stage at diagnosis	PS ^a	Prior therapies	Location of injected lesion	Size of injected lesion (cm)
001	45	F ^b	III	0	Surgery, IFN- α , IL-2, paclitaxel+carboplatin+topotecan (3 cy ^c), temozolamide (2 cy)	Left submandibular mass	4.5 ×
002	48	M ^d	IV	1	DTIC+carmustine+cisplatin (3 cy)	Left postauricular mass	2.4 × 2.2
003	80	F	III	0	Surgery, IFN- α	Right axillary nodule	2.1 × 2.1
004	62	F	I	0	Surgery, IFN- α	Left axillary mass	4.0 × 3.0
005	69	F	IV	2	DTIC(2 cy), XRT ^e	Left inguinal mass	4.4 × 14.0
006	70	M	IV	1	DTIC (3 cy)	Left thigh mass	2.9 × 2.9
007	81	M	Ocular	2	Surgery	Neck mass above cricoid cartilage	2.5 ×
008	72	F	III	0	Surgery, IFN- α	Right axillary nodule	3.8 × 3.2
009	54	M	IV	1	Surgery, XRT, IL-2	Right posterior cervical mass	2.3 × 3.1
010	69	M	II	0	Surgery, XRT, IFN- α	Right gluteal mass	3.4 × 3.1
011	50	M	III	1	Surgery, BCNU+DTIC+cisplatin (2 cy), IFN- α , GMCSF, BCNU+DTIC+cisplatin (2 cy)	Left axillary nodule	2.5 × 3.3

^aPS=performance status (ECOG).

^bF=female.

^cCy=cycles.

^dM=male.

^eXRT=external beam radiation.

Table 2 Nonhematologic toxicity

Grade of toxicity	Toxicity	Dose level (iu) ^a	Number of patients
1	<i>Injection site</i>		
	Tenderness	10 ⁷ , 10 ⁸ , 10 ⁹ , 10 ¹⁰	8
	Erythema	10 ⁷ , 10 ⁹ , 10 ¹⁰	5
	<i>Genera:</i>		
	Myalgia	10 ⁷	1
	Headache	10 ⁸ , 10 ¹⁰	2
	Fever	10 ⁷	1
	Nausea	10 ⁷ , 10 ⁸ , 10 ¹⁰	3
	Fatigue	10 ⁷ , 10 ⁸ , 10 ⁹ , 10 ¹⁰	5
	Diarrhea	10 ⁷ , 10 ⁹	2
	Rash	10 ⁷	1
	Oropharyngeal candidiasis	10 ⁸	1
	Somnolence	10 ⁸	1
	Facial flushing	10 ⁹ , 10 ¹⁰	2
	Insomnia	10 ⁹ , 10 ⁷	2
2	Nausea	10 ⁸ , 10 ⁹	2
	Emesis	10 ⁸	1
	Upper respiratory illness	10 ⁹	1
	Constipation	10 ⁸	1
	Lower extremity edema	10 ⁸	1
3	Fever	10 ¹⁰	1
	Deep venous thrombosis	10 ¹⁰	1

^aiu=infectious units.

emesis ($n = 1$), grade 1 diarrhea ($n = 2$), grade 2 constipation ($n = 1$), food aversion ($n = 1$), grade 2 lower extremity edema ($n = 1$), oropharyngeal candidiasis ($n = 1$), grade 2 anemia ($n = 1$), somnolence ($n = 1$), dry mouth ($n = 1$), dry eyes ($n = 1$), facial flushing ($n = 2$), and insomnia ($n = 2$). A majority of these toxicities were transient and resolved on completion of study course.

Biologic correlates

ELISPOT assays: Only HLA-A2+ patients ($n = 7$) were tested. Patient 006 had two cycles of therapy, and specimens from both cycles were tested. 5/7 patients (003, 004, 008, 009 and 010) had detectable melanoma peptide-specific T cells in the circulation before therapy. In these patients, the general trend was for the absence of or lower frequency of melanoma-peptide-specific T cells after therapy. The possible exception was patient 003, in whom gp100-specific T cells increased from 1/25,000 to 1/16,000 and tryosinase-specific T cells increased from undetectable to 1/8333 cells. Additionally, in patient 001, gp-100+ T cells increased from undetectable to 1/8333 cells and Melan-A/Mart-1⁺ T cells from undetectable to 1/10,000 cells on day 15.

IL-6: Baseline serum levels of IL-6 were consistently elevated in all patients on study, as compared to normal controls, with a median value of 3.65 pg/ml (range, 1.18–

15.93 pg/ml). Two patients demonstrated an increase in serum IL-6 levels while on study: patient 006, from 4.58 to 31.16 pg/ml, and patient 011, from 15.12 to 71.32 pg/ml (Fig 2). In both these patients, the increase in IL-6 level was observed at the 6-hour time point on day 15.

β -2 microglobulin: Baseline serum levels of β -2 microglobulin ranged from 1.5 to 5.7 mcg/ml with a median level of 2.7 mcg/ml. No significant trends relative to therapy were observed.

IFN- γ : Systemic levels of IFN- γ were undetectable at all time points in all but two patients. Patient 005 had a serum IFN- γ level of 30.1 pg/ml 48 hours after the final injection of the vector, on day 17. Patient 011 had serum IFN- γ levels of 47.3 pg/ml 6 hours postinjection on day 15, and this coincided with a rise in body temperature to 40.3°C.

Clinical efficacy

Although clinical response was not a primary end point of this phase I study, several interesting clinical observations were made at the injected site as well as distant sites of disease. Erythema, local tenderness or signs of inflammation at the injected site, were reported in seven of 11 patients. A minor decrease in size (<25%) of the injected lesion was observed in five patients, primarily on day 7 on study. Patient 006 was noted to have minor decrease in size of injected nodule as well as stable disease at distant subcutaneous nodules and liver metastases after the completion of cycle 1. Subsequently, he completed a second cycle of study drug. During the first cycle, inflammation was noted at non-injected distal subcutaneous melanoma nodules as well as minor response or stable disease at non-injected nodules. However, this patient developed progression of disease immediately on completion of cycle 2. Patient 007 completed a cycle of 3 intratumoral injections of study drug to a midline neck mass following which an increase in the size of the mass was noted. The patient subsequently underwent surgical resection of both the injected anterior midline neck mass as well as a noninjected right anterior cervical mass, performed for purposes of locoregional control. Histopathologic examination of these masses showed significant central coagulative necrosis with a rim of persistent melanoma in the injected nodule, and persistent melanoma with no evidence of necrosis in the noninjected nodule. This patient remains disease-free 40 weeks after completion of study and subsequent surgical resection. Patient 010 had a 30% decrease in injected lesion on day 15 with stable distant disease, but eventually developed progression of disease by day 36.

Systemically, one patient on study had stable disease and 10 had progression of disease. Median survival of patients treated at the first two dose levels was 206 days (range, 65–553 days). Three of five patients treated at dose levels 3 and 4 were alive at time of data analysis (range, 605+ to 870+ days). Survival for the other two patients was 426 (patient 008) and 182 days (patient 011).

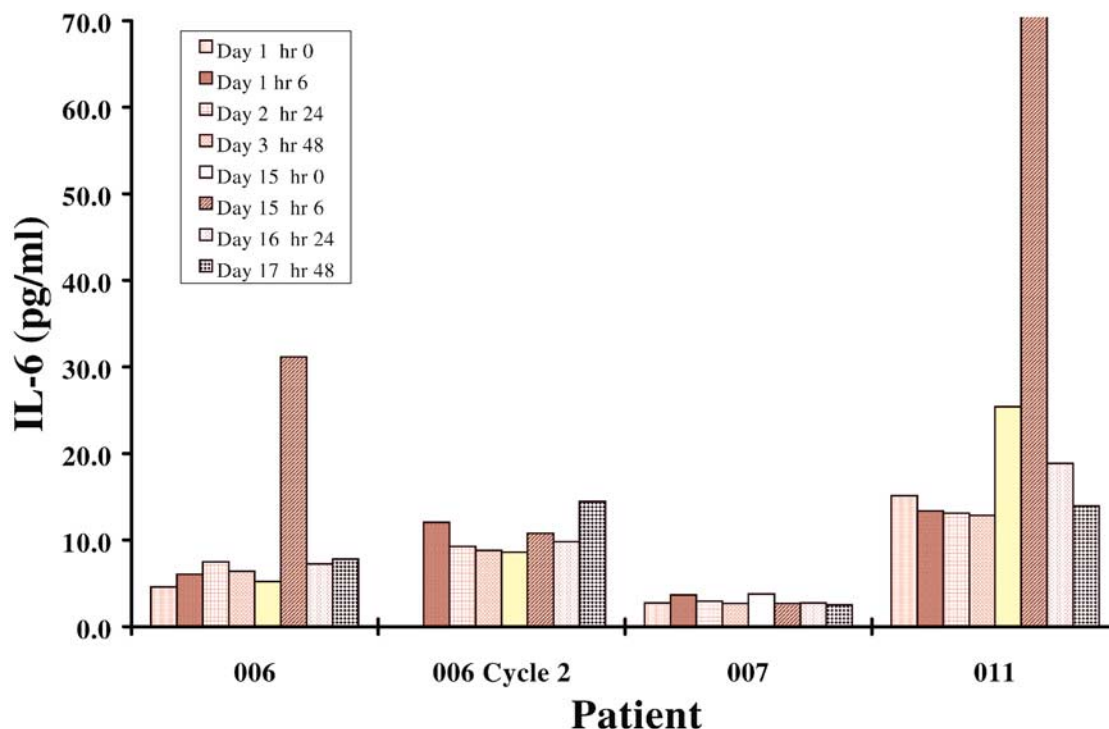


Figure 2 Serum IL-6 levels at different time points for patients 006 (cycles 1 and 2), 007 and 011. A significant increase in serum IL-6 levels was seen in patient 006 (cycle 1) and in patient 011, both at 6 hours postinjection on day 15. Patients 006 (cycle 2) and 007 are representative of other patients who did not manifest such an increase.

Discussion

In this phase I study, we have demonstrated that weekly intratumoral injection of the TG1041 gene therapy vector is both safe and well tolerated. No grade 4 toxicities were observed, and no patients died on study. Two grade 3 toxicities were reported in the same patient at dose level 4; it is unclear whether one of these (deep venous thrombosis) could be directly related to study drug. Toxicities reported in this trial were primarily grade 1 or 2, transient and resolved on completion of study. After enrollment of patient 011, the manufacturer decided to change to a new vector using a CMV promoter instead of the RSV promoter used in TG1041, because of increased cellular expression with the new vector.³¹ Owing to availability of a further developed product, the phase I study was terminated without completely enrolling the requisite patients for dose level 4. Therefore, a maximum tolerated dose was not reached in this study of 11 patients. It is likely that tolerance of this study drug was related to low systemic levels of IFN- γ , obviating many of the toxicities observed with systemic administration of IFN- γ .³²

Malignant melanoma is poorly responsive to most therapies, including chemotherapy and immunotherapy. We did not see any complete or partial responses in this population of patients with advanced or locally recurrent melanoma, the majority of whom had received multiple previous therapies. However, the following observations suggest preliminary evidence of clinical activity: (i) minor

decreases in size of injected tumor nodules in five patients, (ii) local signs of inflammation in eight patients, (iii) significant coagulative necrosis in injected nodule in one patient not seen in an adjacent noninjected nodule, (iv) inflammation of distant nodules in one patient, and (v) stabilization of disease in one patient while on study. It appears possible that intratumoral injection of TG1041 and subsequent local immunomodulation may lead to induction of a systemic response, although whether clinical benefit can be derived from such a response remains to be seen.

In this study, we also analyzed immunologic correlates in patients who were HLA-A2 positive. This included an analysis of T cells specific for peptides for Mart-1/Melan-A residues 26–35, tyrosinase residues 368–376, and gp100 residues 280–288. We were able to test for immunologic reactivity by ELISPOT in seven patients prior to and following treatment. In several patients, high levels of background secretion of IFN- γ were noted. These background IFN- γ levels did not appear to be related to EBV reactivity of patients' T cells (data not shown). In general, the patients manifested poor responses to the influenza peptide. As a group, melanoma patients have been observed to have poor responses to influenza peptides (T Whiteside, unpublished data). In our experience, the overall sensitivity of ELISPOT is 1/100,000 cells; we consider frequencies equal or lower than 1–25,000 to be borderline. However, we considered changes in frequencies from undetectable ELISPOT reactivity to frequency of less than 1/25,000 to be of probable biological

significance. Using our criteria, we were able to detect melanoma peptide-specific T cells in the circulation before therapy in five of seven patients. A general trend toward a lower frequency of melanoma-specific T cells following therapy was noted in these patients with the exception of patient 003. This patient may have been a true immunologic responder. Overall, however, these results portend a general decrease in frequency of T cells responsive to peptides rather than the hoped for increase. This is not surprising since in the majority of melanoma immunotherapy clinical trials, there has been no correlation between a detectable melanoma-specific cellular immune response in the periphery and a clinical response at the site of the tumor (reviewed in Romero et al³³). It is also possible that exposure of T cells from melanoma patients, to IFN- γ -transduced melanoma cells may actually decrease reactivity, due to the absence of requisite costimulatory signals such as B7.1, or CD40L. If this is the case, expression of additional costimulating molecules or cytokines may be helpful in eliciting a more vigorous T-cell response. Another possibility is that the study drug induced tolerance, since changes in tumor nodules were not seen at later time points.

Other immunologic correlates measured in our study patients included serum levels of IL-6 and β -2 microglobulin. IL-6 is an autocrine growth factor in advanced stage melanoma, and serum levels may be surrogate markers of progressive disease.³⁴ Other workers have found increased IL-6 to correlate with patient response, including minor responses.³⁵ In our study, all patients had markedly elevated serum IL-6 levels, as compared to normal donor controls (data not shown). Two patients demonstrated an increase in serum levels of IL-6 while on study drug. Patient 006 demonstrated an increase during cycle 1, when a minor local response, inflammation of distant subcutaneous nodules and stable systemic disease were observed, but not in cycle 2, when no response was seen. Patient 011 demonstrated an increase in serum IL-6 levels at time points that coincided with detectable serum IFN- γ levels. In healthy subjects, IFN- γ has been shown to increase significantly plasma IL-6 levels.³⁶ It is possible that the documented rise in IL-6 levels in these two patients might again indicate some systemic effect of locally injected TG1041. Serum β -2 microglobulin has previously been shown to be a marker of biological activity of interferon, although it appears to have little efficacy in predicting clinical outcome.³⁷ Levels of serum β -2 microglobulin were elevated in our study population, and fluctuated during study course, but no statistically significant trends were observed.

It is possible that a different dose schedule may enhance clinical efficacy of the study drug. Evidence for this hypothesis comes from a recent study of intratumoral delivery of IFN- γ using a retroviral vector in melanoma patients, administered daily for 5 days every 2 weeks³⁸. In this study, patients were enrolled into two treatment arms; one receiving a single cycle of therapy ($n=9$) and the second receiving six cycles ($n=8$). All patients enrolled in the multiple-cycle arm manifested either stable disease or a response, as opposed to only one patient in the single-

cycle arm. Interestingly, anti-MAGE-A1 and tyrosinase antibody were significantly elevated from baseline in patients who received multiple injections. It is possible that an increased frequency of administration of TG1041 may be clinically and immunologically more efficacious.

Another approach to enhance the clinical efficacy of TG1041 would be to administer it in combination with another immunologic agent, such as IL-2. The effects of these two agents on the immune system appear to be complementary. As previously discussed, IFN- γ enhances immunogenicity of the target cell, whereas IL-2 promotes the cellular immune response and may assist in the expansion of cytolytic precursors.³⁹⁻⁴¹ Indeed, *in vitro* studies have established a synergistic effect between human recombinant IL-2 and IFN- γ .⁴²⁻⁴⁴ In one study, pulmonary metastases were significantly reduced in mice treated with this combination, and better response rates were obtained when IFN- γ preceded the IL-2.⁴⁵ Additionally, IL-2 enhances the cytotoxic effect of IFN- γ by providing a costimulatory signal necessary for the development of cytotoxic T cells.⁴⁶ A phase I/II study of combination biologic therapy with intratumoral TG1042 and high-dose IL-2 in patients with metastatic melanoma and advanced renal cell carcinoma is planned.

In summary, weekly intratumoral administration of adenovirus-IFN- γ appears to be safe and well tolerated at the dose levels tested in this study population of patients with locally recurrent or advanced malignant melanoma. However, intratumoral adenovirus-IFN- γ treatment in malignant melanoma does not appear to be of major clinical interest at the doses investigated. Further testing utilizing a schedule or in combination with other biologic therapies may be of benefit, once phase I dose-finding studies with the new vector have been safely completed.

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