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ORIGINAL REPORT

Phase I Trial of Interleukin-12 Plasmid Electroporation in Patients With Metastatic Melanoma

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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The Acknowledgment and Appendix are included in the full-text version of this article; they are available online at www.jco.org. They are not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose
Gene-based immunotherapy for cancer is limited by the lack of safe, efficient, reproducible, and titratable delivery methods. Direct injection of DNA into tissue, although safer than viral vectors, suffers from low gene transfer efficiency. In vivo electroporation, in preclinical models, significantly enhances gene transfer efficiency while retaining the safety advantages of plasmid DNA.

Patients and Methods
A phase I dose escalation trial of plasmid interleukin (IL)-12 electroporation was carried out in patients with metastatic melanoma. Patients received electroporation on days 1, 5, and 8 during a single 39-day cycle, into metastatic melanoma lesions with six 100-μs pulses at a 1,300-V/cm electric field through a penetrating six-electrode array immediately after DNA injection. Pre- and post-treatment biopsies were obtained at defined time points for detailed histologic evaluation and determination of IL-12 protein levels.

Results
Twenty-four patients were treated at seven dose levels, with minimal systemic toxicity. Transient pain after electroporation was the major adverse effect. Post-treatment biopsies showed plasmid dose proportional increases in IL-12 protein levels as well as marked tumor necrosis and lymphocytic infiltrate. Two (10%) of 19 patients with nonelectroporated distant lesions and no other systemic therapy showed complete regression of all metastases, whereas eight additional patients (42%) showed disease stabilization or partial response.

Conclusion
This report describes the first human trial, to our knowledge, of gene transfer utilizing in vivo DNA electroporation. The results indicated this modality to be safe, effective, reproducible, and titratable.

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INTRODUCTION

The promise of gene therapy has not been realized, in part because of the limitations of current delivery methods.[1] Viral vectors, probably most commonly utilized for gene delivery, have had issues with host immune response, systemic toxicity, and integration into the host genome.[2-4] Plasmid DNA-based vectors avoid these particular problems, but are handicapped by the lack of efficient delivery methods.[5-7] In vivo electroporation, which utilizes an electric charge to facilitate entry of macromolecules into the cell, can be a reproducible and highly efficient method to deliver plasmid DNA.[8,9] Electroporation has also been used to deliver antitumor agents such as bleomycin[10,11] (electrochemotherapy). In mice, intratumoral electroporation of interleukin (IL)-12 plasmid resulted in complete tumor regression rates of 80% after three cycles of treatment.[12,13] Encouragingly, 100% of cured mice were resistant to further challenge with B16.F10 melanoma cells. No comparable tumor regression was seen in athymic mice after intratumoral plasmid IL-12 electroporation arguing for a role of T-cell immune responses in tumor regression.[13] No significant organ, laboratory, or symptomatic toxicity was associated with the electrically mediated delivery of plasmid (p)IL-12 in mice.[14]

Melanoma is the leading cause of skin cancer death.[15] Metastatic melanoma is generally treated with systemic chemotherapy or immunotherapy.[16-20] Several approaches have been utilized to enhance the effectiveness of immunotherapy using gene therapy with a variety of cytokines including IL-12.[19-24] This cytokine stimulates both adaptive and innate immunity.[22-24] In animal melanoma

models, the administration of pIL-12 resulted in inhibition of tumor growth as well as regression of established tumors.[12,13] Clinical phase I and II trials of systemic IL-12 (rhIL-12) protein have been reported; responses were observed in melanoma and other tumors.[25-27] Systemic rhIL-12 protein can cause significant toxicity.[25] Local delivery of IL-12 seems to be less toxic, while retaining biologic activity, in preclinical studies.[8,12,13,28-30] Recently, phase I trials have been reported with direct intratumoral injection of IL-12 plasmid DNA,[31] liposome encapsulated Semliki forest virus expressing IL-12,[32] and with IL-12 producing fibroblasts.[33] All of these were well tolerated; however, a limitation of these trials has been undocumented efficiency of delivery and lack of durable systemic clinical responses.

We report here the first human trial to our knowledge of the delivery of a DNA plasmid designed to express a therapeutic protein by in vivo electroporation.

PATIENTS AND METHODS

Trial Design

This trial was approved by the scientific review committee, institutional review board, and the institutional biosafety committee at the H. Lee Moffitt Cancer Center (Tampa, FL) as well as the National Institutes of Health Office of Biotechnology Activities and the Food and Drug Administration, Center for Biologics Evaluation and Research. The study was conducted in two segments. Patients received treatment for one cycle only, which spanned 39 days. This cycle consisted of three plasmid injection/electroporation treatments on days 1, 5, and 8, with up to four accessible lesions injected each day.

Patients

Eligible patients had pathologically documented metastatic melanoma, stages IIIB/C or IV with at least two subcutaneous or cutaneous lesions accessible for electroporation. Patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of no more than 2, have adequate renal, hepatic and bone marrow function (creatinine $< 1.5\times$ upper limit of normal, bilirubin and AST within normal limits, and an absolute neutrophil count $> 1{,}500/mm^3$). Patients with electronic pacemakers, defibrillators, or a history of significant cardiac arrhythmia or seizure within the last 5 years were excluded from the study.

Plasmid

The plasmid pUMVC3-hIL-12-NGVL3[31] was produced under Good Manufacturing Practices (GMP) conditions at the recombinant DNA production facility at the City of Hope Center for Biomedicine and Genetics (Duarte, CA) and supplied in sterile vials at a final concentration of 1.6 mg/mL and stored at −80°C. Before use, plasmid was thawed to 4°C and diluted in sterile saline to the required concentration.

Electroporation

Lidocaine was applied topically to or injected around each tumor site, and all patients were offered intravenous analgesic (morphine sulfate, 1 mg) and/or anxiolytic (lorazepam 1 mg) medications before electroporation. Plasmid solution was injected using a 25-gauge needle into the tumor nodule to a depth no greater than 3/8 inch. A sterile applicator containing six needle electrodes arranged in a circle was inserted into the tumor and six pulses at field strength of 1,300 V/cm and pulse duration of 100 μs were applied using a Medpulser DNA EPT System Generator (Inovio Biomedical Inc, San Diego, CA). Treatments were performed on days 1, 5, and 8.

Dose Escalation

Dose escalation was performed by increasing plasmid concentration. Plasmid was dispensed at concentrations of 0.1, 0.25, 0.5, 1.0, and 1.6 mg/mL. For cohorts 1 through 5, the plasmid injection volume was calculated using the formula $P = V/4$, where P is the plasmid injection volume and V is the estimated tumor volume. Tumor volume was estimated using the formula $V = ab^2/2$ where a is the longest diameter and b is the next longest diameter perpendicular to a in any dimension. Patients in cohorts 6 and 7 received a total dose of 3.8 or 5.8 mg/treatment divided among two to four tumor sites selected irrespective of tumor volume. Dose-limiting toxicity (DLT) was prespecified for this study as any hematologic toxicity of grade 3 or greater, or nonhematologic toxicity of grade 2 or greater as defined by the National Cancer Institute Common Terminology Criteria for Adverse Events, version 2.0.

Tumor Pathology, Lymphocytic Infiltrate Assessment, and IL-12 Measurement

Patients underwent fine-needle aspiration (FNA) at an accessible disease site before treatment and FNA and excisional biopsy on days 11, 22, and 39 on electroporated lesions depending on their number and size. Excisional biopsies were bisected and one half processed for pathology and the other half for cytokine analysis. FNA biopsies were used for cytokine analysis. Histopathology specimens were sectioned at 5 μm and stained with hematoxylin and eosin, and in selected specimens immunohistochemistry was performed with anti-CD3, CD4, CD8, and CD56 antibodies using the avidin-biotin method (Vectastain ABC Kit, Elite Series, Vector Laboratories, Burlingame, CA). IL-12 and IFN-γ levels in FNA and excisional biopsy samples were determined by enzyme-linked immunosorbent assay (ELISA) using the manufacturer's instructions (R&D Systems, Minneapolis, MN).

Response Evaluation

Overall response was evaluated by a modification of Response Evaluation Criteria in Solid Tumors (RECIST).[41] Progressive disease (PD) was defined by the presence of new lesions or a 20% or greater increase in the longest diameter of an existing measurable lesion, and stable disease (SD) by an increase less than 20% in the largest diameter of a given lesion with no new distant sites of disease seen. A complete response (CR) was considered to be present only if a patient had distant (nonelectroporated) sites of disease at the start of treatment and if all sites of disease regressed completely with no evidence of disease with complete radiologic, clinical, pathologic, and laboratory evaluation. Local responses (necrosis) after the electroporation treatments were graded by our study pathologist in a blinded fashion after examining the entire biopsy.

RESULTS

Patient Characteristics

Twenty-four patients were enrolled onto seven cohorts (Table 1) between December 2004 and February 2007. All patients received treatment as planned, as shown in Table 1.

Adverse Events

In vivo electroporation was associated with minimal systemic toxicity. No hematologic abnormalities were observed. The most frequent adverse event related to treatment was transient pain during the electroporation procedure (13 patients had grade 1 and 11 had grade 2 pain) and bleeding around the treatment site (13 patients had grade 1 and 11 grade 2 hemorrhage). We found local infiltration of 1% lidocaine around tumor lesions together with lifting the lesions off the underlying soft tissues during the electroporation procedure was effective at minimizing pain. Because no DLT was noted in cohorts 1 to 5, the experimental plan was amended to add two additional cohorts, 6 and 7, where plasmid dosage was fixed at 3.8 and 5.8 mg/treatment, respectively, and divided among the tumors selected for injection. No DLT was noted at these levels, either. The maximum administered dose in the trial was 5.8 mg administered as a fixed dose.

Table 1. Patient Characteristics and Treatment Response

Cohort	Patient	Age	Sex	AJCC Stage	LDH	IL-12 Plasmid		Electroporation		Distant Disease Sites	Objective Response	
						Concentration (mg/mL)	Lesion Volume (mL)	No.	Site		Overall Response	Duration (months)
1	1	35	M	IVA	382	0.1	0.56	3	Leg	SQ, LN	PD	
	2	54	M	IVC	927	0.1	3.9	4	Trunk	SQ, LN	PD	
	3	69	M	IVC	923	0.1	4.4	2	Trunk	SQ	PD	
2	4	55	M	IVC	1,974	0.25	4.98	4	Trunk	Multiple sites	PD	
	5	66	M	IVB	368	0.25	4.03	3	Trunk	Multiple sites	SD	4
	6	43	M	IVA	483	0.25	2.98	2	Trunk, arm	SQ	PD	
3	7	50	M	IIIC	541	0.5	1.16	4	Trunk, arm	SQ	*	> 18
	8	61	M	IIIC	356	0.5	0.82	4	Leg	SQ	PD	
	9	80	M	IVA	449	0.5	0.13	4	Trunk, arm	SQ	CR	> 20
4	10	68	M	IVA	514	1	0.07	3	Trunk	SQ	SD	> 20
	11	64	F	IVC	908	1	1.2	3	Leg	SQ, LN	PD	
	12	70	M	IIIC	370	1	0.96	3	Trunk	—	PD	
5	13	61	M	IIIC	418	1.6	0.57	4	Arm	—	PD	
	14	76	F	IIIC	565	1.6	0.27	4	Leg	SQ	CR	> 16
	15	83	M	IIIC	465	1.6	0.04	4	Arm	SQ	PD	
6	16	56	M	IIIC	400	1.6	FV	4	Trunk	SQ	SD	4
	17	79	F	IIIB	470	1.6	FV	3	Leg	—	SD	> 4
	18	56	F	IIIC	584	1.6	FV	4	Leg	SQ	PD	
7	19	72	M	IIIC	507	1.6	FV	2	Leg	LN	PD	
	20	41	M	IIIB	433	1.6	FV	4	Leg	—	SD	4
	21	26	M	IVA	358	1.6	FV	4	Leg	SQ	SD	4
	22	62	M	IVA	480	1.6	FV	2	Trunk	SQ	PD	
	23	85	M	IVA	572	1.6	FV	4	Leg	SQ, LN	SD	> 6
	24	63	M	IVC	1,380	1.6	FV	3	Neck	Liver, lung	PD	

Abbreviations: AJCC, American Joint Committee on Cancer; LDH, lactate dehydrogenase; IL, interleukin; lesion volume, cumulative volume of lesions treated; M, male; SQ, subcutaneous; LN, lymph node; PD, progressive disease; F, female; SD, stable disease; CR, complete response; FV, fixed volume; em, no distant disease.
*Patient 7, overall response was a CR 5 after following treatment with plasmid IL-12 delivered with electroporation; however, the patient was treated with dacarbazine after completion of the IL-12 study and before the CR. Therefore, the response can not be definitively attributed to either therapy.

Plasmid Expression

Levels of measured IL-12 increased as the plasmid dose was escalated, as demonstrated in Figure 1. The highest level of expression obtained was 2,813 pg/g of tumor on a day-11 sample from cohort 5 (patient 13). Mean (standard deviation) day-11 IL-12 levels were highest in cohorts 5 and 6 (1,124 ± 1,470 picogram/gram [pg/g] and 870 ± 1,216 pg/g, respectively), representing as much as an 18-fold increase over the median baseline IL-12 measurement for the entire study group (Fig 1). Mean IL-12 levels generally were lower on days 21 and 39 than day 11, but mean IL-12 levels remained higher than baseline at day 39 in cohorts 5 and 7. To evaluate IL-12 activity, interferon-γ (IFN-γ) levels were measured in the biopsy samples. Levels of IFN-γ generally increased and peaked at days 11 and 21 (Appendix Fig A1, online only). The highest level of expression obtained was 10,383.92 pg/g of tumor on a day 21 sample from cohort 5 (patient 15). Mean day-21 IFN-γ levels were highest in cohort 5 (4,195.6 ± 5,366.63 pg/g) and mean day 11 IFN-γ levels were highest in cohort 7 (2,587.4 ± 4,225.43 pg/g) representing a 7- to 60-fold increase over the median baseline IFN-γ levels measurement for the entire study group (Appendix Fig A1). No increased levels of IL-12 or IFN-γ were observed in serum samples.

Tumor Necrosis and Lymphocytic Infiltration

Biopsies of injected lesions were graded for percentage of tumor necrosis and degree of lymphocytic infiltrate in a blinded fashion (Table 2). Seventy-nine skin punch biopsies were examined. Tumor necrosis levels in the sample ranged from 0% to 100%. Sixty lesions (76%) were observed to have greater than 20% necrosis with 19 (24%) and 25 (32%) having 50% to 99% and 100% necrosis respectively. The lymphocytic infiltrate ranged from a sparse peritumoral infiltrate to dense aggregates of tumor-infiltrating lymphocytes associated with tumor cell necrosis (Fig 2).

Clinical Response

There was evidence that both injected lesions and distant noninjected lesions regressed after the treatment regimen. Nineteen of the 24 patients enrolled onto this study had additional sites of disease outside the treated lesions, and these patients could therefore be evaluated for distant responses. In 10 patients (53%) there was evidence of a systemic response resulting in either stable disease or objective regression of untreated lesions. In addition, in three of these patients (15%), all of the distant lesions regressed completely in either the absence of any other systemic antitumor therapy (two patients) or after treatment with dacarbazine (one patient). Patient 9 (cohort 3) had an 8.4-mm ulcerated high mitotic rate posterior shoulder melanoma with one positive axillary lymph node. After surgery, he received 10 months of interferon alfa 2B therapy. Two months after terminating interferon alfa 2B therapy, he started developing rapidly progressing cutaneous metastasis with more than 50 nodules on his right chest and shoulder. After treatment with pIL-12 delivered with electroporation, no new lesions developed, and over a period of 18 months, all lesions flattened

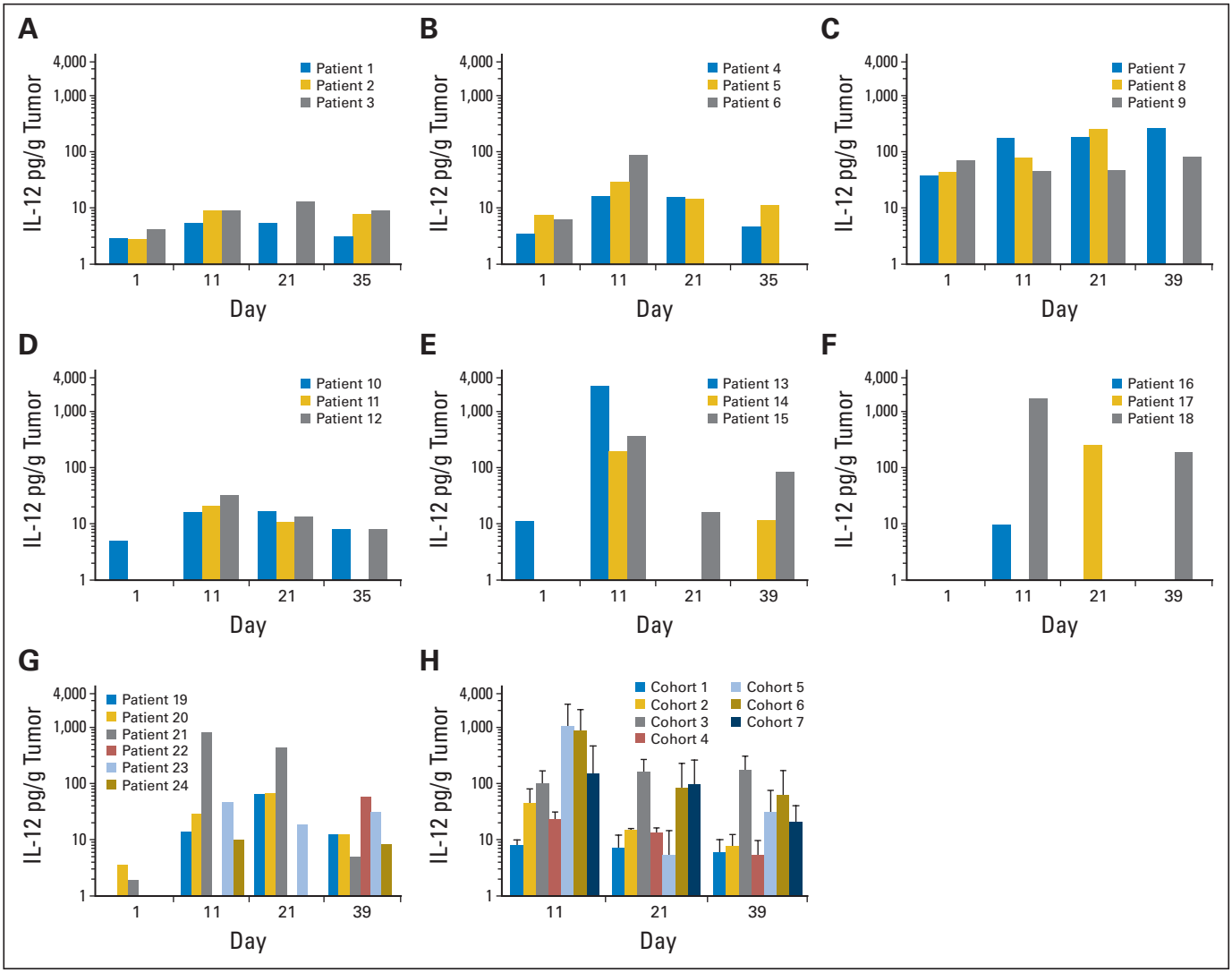

Fig 1. Interleukin (IL)-12 expression measured by enzyme-linked immunosorbent assay in samples obtained from electroporated tumors pre- and postelectroporation. Each panel represents a single cohort with samples from an individual patient depicted with individual bars. The time and type of biopsy specimen is as described in the x-axis labels and the quantity of IL-12 is depicted in a logarithmic scale on the y-axis. (A) Cohort 1, (B) cohort 2, (C) cohort 3, (D) cohort 4, (E) cohort 5, (F) cohort 6, (G) cohort 7, and (H) mean and standard deviation of IL-12 levels for each cohort. Note that cohort 7 (the maximally administered dose) has six patients whereas all other cohorts have three patients.

out and faded (Fig 3A-3F). The sites of regressed lesions were biopsied at 7 and 18 months (Fig 2D), did not demonstrate evidence of melanoma, and showed only residual pigmentation. In addition, the patient had no evidence of systemic disease by positron emission tomography (PET) or computed tomography (CT) imaging at 20 months post-treatment. Patient 14 (cohort 5) had progressive cutaneous lesions in the right lower extremity (Fig 4A-4B) after multiple surgeries and hyperthermic isolated limb perfusion with melphalan. Six months after the electroporation delivery of pIL-12, the cutaneous lesions started regressing and developing hypopigmentation (halo effect) around them, and this effect persisted and the lesions have regressed further (Fig 4C-4D). A sample pigmented lesion was biopsied and showed only residual melanin pigment without any evidence of tumor. PET imaging, which had previously revealed positive results in the left calf, showed no uptake at 17 months post-treatment and continued to show no evidence of noncutaneous disease. Patient 7 (cohort 3), had an interesting post-treatment history with a rapidly progressing cutaneous metastases from a primary flank tumor that had been widely resected and irradiated after a local resection. After completing day-39 resection, the patient received dacarbazine therapy. Five months post-electroporation, after having received four cycles of dacarbazine, he had complete regression of all lesions and on a follow-up CT scan had no evidence of disease. At a further follow-up exam, now 24 months after completion of electroporation, he is radiologically and clinically free of disease. Patient 23 (cohort 7) had progressive disease in the thigh and supraclavicular lymph nodes after participating in an autologous tumor vaccine trial. After pIL-12 delivery with electroporation, this patient had partial regression of local thigh lesions as well as regression of a distant supraclavicular lymph node site. In six other patients, uninjected lesions remained stable, with no new lesions developing, during a period of 4 to 20 months after the end of protocol therapy (one from cohort 2, one from cohort 4, two from cohort 6, and two from cohort 7). A statistically significant correlation was

Table 2. Histologic Grading of Electroporated Lesions

		Lesion Histology and Lymphocytic Infiltrate					
		Day 11		Day 22		Day 39	
Cohort	Patient	Necrosis (%)	Lymph	Necrosis (%)	Lymph	Necrosis (%)	Lymph
1	1	–	–	0	0	20	+
	2	50	++	20	+	30	+
	3	100	0	–	–	–	–
2	4	–	–	–	–	10	+
	5	100	+	100	0	15	+++
	6	80	+++	0	0	30	+
3	7	20	++	100	0	10	+
	8	0	0	20	+	60	+
	9	50	++	100	++	88	+
4	10	20	++	90	+++	0	+++
	11	90	+	50	+	100	0
	12	75	+++	100	++	30	++++
5	13	25	++	100	+	13	+
	14	90	++	100	+	0	+
	15	100	++++	100	++	–	–
6	16	50	+	75	+	0	0
	17	80	++	0	0	0	0
	18	100	++	100	+	65	+
7	19	100	++++	100	+++	90	+
	20	50	++	90	++	10	+
	21	100	0	10	+	5	0
	22	100	+++	100	+	–	–
	23	100	+	100	0	10	+
	24	100	+	60	0	30	0

NOTE. Lymphocytic infiltration scale: −, not measured; 0, absent; +, few lymphocytes at periphery of tumor; ++, more lymphocytes surrounding tumor; +++, lymphocytes surrounding and partially infiltrating tumor nodule; ++++, lymphocytes extensively infiltrating tumor nodule and surround individual cells.

seen between tumor necrosis at day 39 (Table 2) and distant clinical responses (objective CR + PR + SD) by Fisher's exact test ($P = .069$), but no correlation was seen between lymphocyte infiltration and clinical response.

DISCUSSION

This study evaluated the toxicity profile, tolerability, and efficacy of IL-12 plasmid delivered by electroporation. It is the culmination of several years of preclinical studies aimed at improving the effectiveness of in vivo gene transfer. Intratumoral plasmid IL-12 delivered by electroporation in the B16.F10 melanoma model can achieve local regression rates similar to those seen with electrochemotherapy or other plasmid delivery approaches, but with greater protection against tumor rechallenge, suggesting induction of a systemic antitumor immune response even in poorly immunogenic models.[8,12,13] In cell culture and in animal models, electroporation greatly increases both the efficiency of gene transfer and the therapeutic efficacy of the gene-based treatment, which are interrelated.[8]

IL-12 has been evaluated as a potential immunotherapeutic agent.[22-24] Delivery of IL-12 in the form of recombinant protein caused significant toxicity. This toxicity was reduced or eliminated by delivering the *IL-12* gene.[31,34-39] Comparison of efficacy across differing modalities of gene transfer in clinical trials is more difficult given the varying patient populations, small sample sizes, differing end points, and lack of quantitative expression data in these studies. Despite these caveats, when compared with other techniques of gene delivery, electroporation seems to produce a greater magnitude of clinical benefit in this aggressive and often fatal disease. Although intratumoral plasmid injection has resulted occasionally in local tumor response after treatment, it has only rarely resulted in regression of disease at distant sites and has not resulted in documented durable complete responses at distant sites. Local intratumoral injection of IL-12 plasmid in a recent phase I study using the same plasmid as our electroporation study resulted in local tumor regression in five of 12 patients, but no change was seen in nontreated distant lesions.[31] In this study, 11 of 12 patients had metastatic melanoma, as in our study. In another earlier trial, IL-12 plasmid was also injected directly into melanoma tumors.[36] Four of nine patients had regression of injected tumors, and one patient had a mixed distant response but no patient experienced a distant complete response. Several other immunomodulatory gene therapy trials have been conducted in melanoma; for example, one of 51 patients on the liposomal B7-1-β2 macroglobulin (Allovectin, Vical Inc, San Diego, CA) phase II trial had a distant partial regression,[39] but no patient had a distant complete regression. Similarly, in the phase I plasmid IL-2 (Leuvectin, Vical) direct-injection trial, no distant CRs were seen.[40]

In the current study, extensive tumor sampling with measurement of IL-12 levels, tumor histopathology, and analysis of lymphocytic infiltrate was performed. A dose-proportional increase in IL-12 protein expression compared with pretreatment was seen in all patients with no significant IL-12 spillage into circulation and a correlative increase in tumor levels of IFN-γ. Most (76%) electroporated

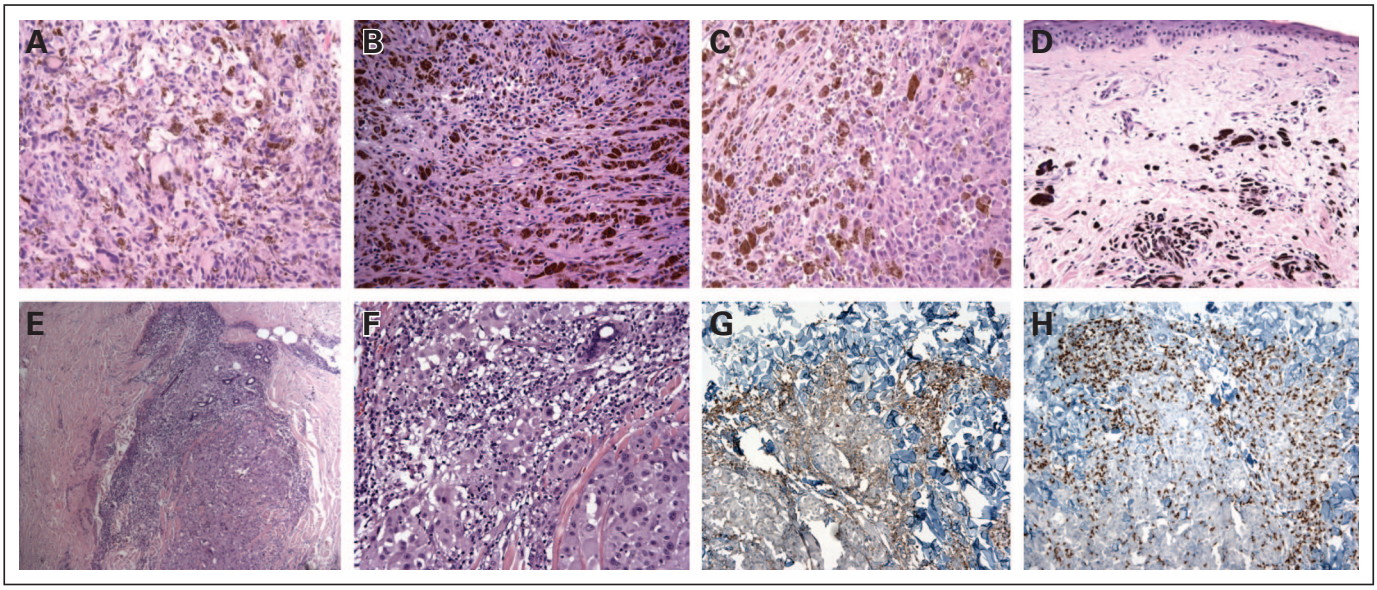

Fig 2. Histologic appearance of electroporated lesions. (A-C) Hematoxylin and eosin–stained tumor samples on patient 9 (cohort 3). (A) Melanoma lesion immediately pre-electroporation (magnification = 200×), (B) on day 22 (magnification = 200×), (C) on day 39 (magnification = 200×), and (D) pigmented nodule with residual melanosis without viable melanoma excised from the chest 18 months after the electroporation procedure was performed (magnification = 200×). (E-H) Patient 10 (cohort 4). (E) A 50× magnification with hematoxylin and eosin staining with a central viable melanoma tumor surrounded by necrotic tumor removed on day 22, Panel F shows a section from the same tumor at a higher magnification (magnification = 200×) showing inflammatory infiltrates. (G, H) Sections from the same patient with CD4 and CD8 immunoperoxidase staining respectively on day (magnification = 200×).

lesions demonstrated necrosis (> 20%) at the time of follow-up biopsy or excision performed between 3 and 31 days after the last injection. Because IL-12 has been established to upregulate both adaptive and innate immunity, we also examined lymphocytic infiltrate in the treated tumors. Electroporated tumors demonstrated $CD4^+CD8^+$ lymphocytic infiltrate in the treated lesions. The experimental regimen was found to be safe and well tolerated, with minimal systemic toxicity and with transient pain associated with the administration of

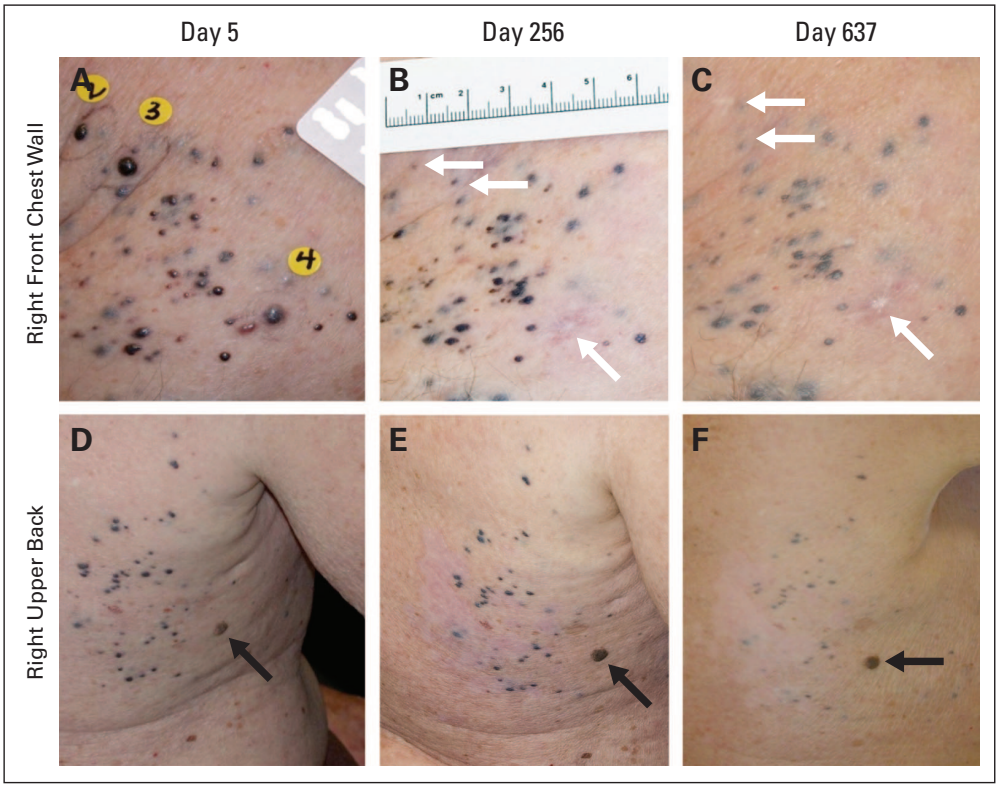

Fig 3. Cutaneous lesions in (A-F) patient 9 from cohort 3 and (G-J) patient 14 from cohort 5. (A-C) Right front chest wall. (D-F) Right upper back. A and D were photographed on day 1 (pretreatment), B and E on day 256, and C and F on day 637. Note that the electroporated lesions (2, 3, 4 in panel A) were resected and the sites are shown by white arrows. The nonelectroporated lesions gradually flatten and fade away. (D-F) The seborrheic keratosis (shown by the black arrows) persists whereas the metastatic melanoma lesions flatten and fade with time.

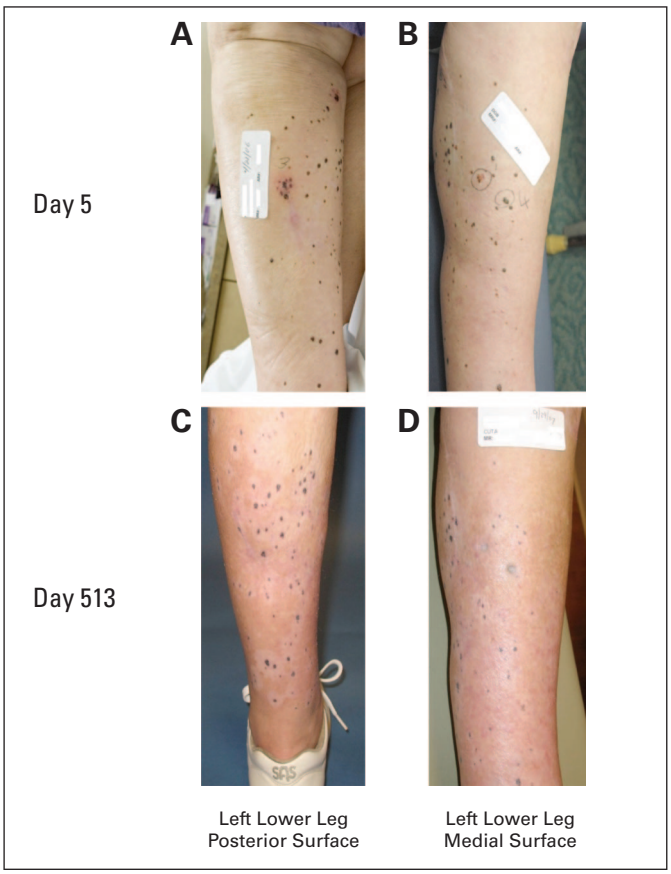

Fig 4. Cutaneous lesions in patient 14 from cohort 5. A and B were photographed on day 5 after the first electroporation treatment, and C and D on day 513. (A, C) The left lower leg posterior surface. (B, D) The medial surface. Note the depigmentation seen around lesions in C and D.

the electrical pulse being the major adverse reaction experienced by patients.

On the basis of preclinical data, we anticipated that augmented innate and adaptive immunity and tumor necrosis at the site of treatment could result in regression of distant tumors; Four of 19 patients who had distant disease had evidence of distant responses including three CRs in patients with progressive metastatic disease. Of these patients, two patients had not had any subsequent systemic therapy and one patient had received dacarbazine after pIL-12 therapy. All three CRs occurred in the setting of patients with disseminated progressive cutaneous lesions. These responses occurred over a span of 6 to 18 months with hypopigmentation and gradual volume loss occurring at sites distinct from the electroporated sites, which argues for immune system involvement in this effect. None of these patients have developed any new evidence of distant disease to date. In addition to these four patients, six patients had SD lasting from 4 to 20 months at distant sites. On the basis of these favorable clinical responses, a confirmatory phase II trial is planned.

On balance, this study suggests that electroporation-mediated plasmid delivery is a powerful new tool for effective gene transfer with implications for the clinical arena. In the future, electroporation could have applications beyond the use described herein to transfer combinations of genes or knock down the expression of a given gene(s), or to produce spatially and/or temporally distinct patterns of gene expression without the safety and biohazard considerations implicit in viral vectors.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Glossary Terms

Cytokines: Cell communication molecules that are secreted in response to external stimuli.

IFN-γ (interferon gamma): Cytokine that is produced by activated T cells and natural killer cells, its primary action is the activation of macrophages.

ELISA (enzyme-linked immunosorbent assay): ELISA is used to detect the presence of an antibody or an antigen in a sample.

ELISpot: Enzyme-linked immunospot that is exquisitely sensitive to assay minute amounts of mediators that are produced by cells. Typically, cells are deposited on a membrane coated with an antibody specific for a given protein. The protein of interest is captured directly around the secreting cell and is detected with an antibody specific for a different epitope. Coupled with colorimetry, the cells are visualized by specialized plate readers. Thus, the molecule is assayed before it is diluted in the supernatant, captured by receptors of adjacent cells, or degraded.

Immunohistochemistry: The application of antigen-antibody interactions to histochemical techniques. Typically, a tissue section is mounted on a slide and is incubated with antibodies (polyclonal or monoclonal) specific to the antigen (primary reaction). The antigen-antibody signal is then amplified using a second antibody conjugated to a complex of peroxidase-antiperoxidase (PAP), avidin-biotin-peroxidase (ABC) or avidin-biotin alkaline phosphatase. In the presence of substrate and chromogen, the enzyme forms a colored deposit at the sites of antibody-antigen binding. Immunofluorescence is an alternate approach to visualize antigens. In this technique, the primary antigen-antibody signal is amplified using a second antibody conjugated to a fluorochrome. On UV light absorption, the fluorochrome emits its own light at a longer wavelength (fluorescence), thus allowing localization of antibody-antigen complexes.

Plasmid: A circular, double-stranded unit of DNA that transcribes RNA within a cell independent of the chromosomal DNA.