Phase II Study of Direct Intralesional Gene Transfer of Allovectin-7, an HLA-B7/β2-Microglobulin DNA-Liposome Complex, in Patients with Metastatic Melanoma[1]

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ABSTRACT

Cutaneous melanoma is one of the most rapidly increasing cancers in the United States. Because of the lack of effective treatment options and toxicities of most chemotherapeutic and radiation regimes, immunotherapies such as vaccination therapy represent an attractive approach for patients with advanced melanoma. The purpose of this study was to evaluate the response rate, time to progression, and survival of patients with metastatic melanoma treated by direct intratumoral injection with Allovectin-7 (a plasmid DNA encoding the genes *HLA-B7* and β*2-microglobulin* complexed with a cationic lipid mixture, DMRIE/DOPE. Fifty-two patients with metastatic melanoma were enrolled in this Phase II study. Therapy consisted of six intratumoral injections of 10 μg of Allovectin-7 over a 9-week period. Treatment was well tolerated. Treatment-related adverse events were mild to moderate, the most frequent of which were ecchymosis, pruritus (and/or discomfort at the injection site), and pneumothoraces. Regression of the injected lesion was observed in 18% of patients, including one complete response, three partial responses, and five minor responses. An overall response rate of 4% (two partial responses) was documented, and nine patients (18%) maintained stable disease for at least 11 weeks. Six patients remained alive 25.1 to 39.4 months from their first injection, including two patients with local (injected tumor) responses and one patient with an overall disease partial response. This study demonstrates that intratumoral administration of Allovectin-7 in metastatic melanoma is safe and can produce both responses in injected lesions and in overall disease. Clinical trials optimizing patient selection and combining Allovectin-7 with other modalities of therapy are currently ongoing in an effort to improve response rates.

INTRODUCTION

Cutaneous melanoma has one of the most rapidly increasing incidence rates of any cancer in the United States (1–3). Surgical excision of early stage melanomas can be curative, but the outlook for patients with advanced (stage III and IV) melanoma remains poor. Currently, there are no consistently effective therapies for advanced disease.

The role of the immune system in malignant melanoma has long been an area of interest. Although the immune system may play a central role in holding potential tumor cells in check, the majority of tumors arise in apparently immunocompetent hosts. This observation suggests that tumor cells are able to escape host immunosurveillance mechanisms. The objective of immunotherapy is to stimulate the immune system to recognize and destroy cancerous cells by modifying them and/or the host response to the tumor cells. Immunotherapies for melanoma have included the administration of nonspecific immunomodulating agents, such as *Bacillus Calmette-Guerin*, cytokines, IFNs, and IL-2.[3] Each has shown promise in animal and human studies (4–9). High-dose systemic IL-2 was Food and Drug Administration-approved for metastatic melanoma because of the induction of durable complete remissions, albeit in a small percentage of patients. Severe toxicity, often requiring intensive care support, limits the use of IL-2 to specialized centers (8, 10, 11).

More recently, research has focused on identifying tumor-specific antigens to serve as immunotherapy targets to improve efficacy and tolerability in patients (12, 13). Recognition of foreign antigens by the immune system requires presentation of antigen peptide fragments in the context of MHC class I or class II molecules. However, many tumor cells, including melanoma cells, frequently demonstrate absent or reduced levels of MHC class I expression on their cell surfaces that can limit the recognition and lysis of these tumor cells by class I-restricted T cells (14–16). Such loss of MHC class I expression is believed to be one mechanism by which tumor cells evade immune recognition and rejection (16). In experimental tumor models, variation in the expression of MHC class I antigens has been shown to exert a decisive influence on local tumor growth and metastases (17).

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[3] The abbreviations used are: IL, interleukin; CR, complete response; PR, partial response; SD, stable disease; MR, minor response; LDH, lactate dehydrogenase; KPS, Karnofsky performance status; HLA, human leukocyte antigen.

It is possible to reintroduce MHC class I expression in tumors via DNA-based therapy. Preclinical studies have demonstrated that direct gene transfer of an allogeneic MHC class I gene into murine tumor cells results in the induction of a specific cellular immune response and the subsequent rejection of tumor cells (17–21). The first human study using direct gene transfer to modify MHC class I antigen expression by tumor cells was conducted in 1993 by Nabel *et al.* in a Phase I clinical trial (22). Five patients with cutaneous melanoma tumors were treated by direct intratumoral injection. The delivery procedure was safe; DNA was successfully transfected into tumor cells, and a melanoma-associated antigen-specific immune response was induced. Furthermore, one patient experienced a partial remission at both injected cutaneous and noninjected visceral sites.

Allovectin-7 consists of a DNA plasmid containing the genes for an allogeneic MHC class I protein, HLA-B7 and β2-microglobulin. Allovectin-7 is complexed with a cationic lipid mixture, DMRIE/DOPE (1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide/dioleoyl-phosphatidyl-ethanolamine) that aids in uptake of the DNA by tumor (23). HLA-B7 antigen is infrequently expressed in the United States population (about 20% of the United States Caucasian population) and thus allows for a potential allogeneic immune response in most patients (24). Loss of or mutations in β2-microglobulin have also been reported as possible mechanisms for deficient MHC class I expression in tumor cells (25). Thus, the β2-*microglobulin* gene was also included in Allovectin-7 to allow for expression of the complete MHC class I complex on the tumor cell surface. All together, Allovectin-7 provides several potential immune-stimulating functions: expression of a foreign and highly immunogenic cell surface protein (*i.e.,* HLA-B7 in HLA-B7 antigen-negative patients), an increased antigen-presentation signal (antigen presented in the context of HLA-B7), and replacement of β2-microglobulin for increased surface expression of the patient's own MHC-class I molecules.

Three Phase I studies have been conducted assessing the safety and efficacy of various dosing schedules of Allovectin-7 in patients with metastatic melanoma, colon cancer, and renal cell carcinoma (22, 24). In each study, Allovectin-7 was demonstrated to have an excellent safety profile with no dose-limiting toxicity found. In addition, plasmid uptake and expression, tumor-associated antigen-specific immune responses, and objective clinical responses were documented in all of the trials.

The 10-μg dose of Allovectin-7 was chosen for Phase II studies primarily because responses were seen at this dose level in the prior Phase I studies, and a clear dose response was not observed. The first Phase II study was an open-label, multicenter study evaluating repeat administration of Allovectin-7 (4×10 μg) over an 8-week period. Thirty-eight patients were enrolled with 25 patients considered evaluable for tumor response assessment (26). A total of seven (28%) patients had a decrease of ≥25% in at least one tumor lesion. Of these, five patients had shrinkage of their injected tumor (local response), whereas two had regression of at least one noninjected lesion.

These encouraging results prompted further development of this agent. Thus, this second Phase II study was initiated to test a more frequent injection schedule in a greater number of patients. The results of this study are presented in this manuscript.

PATIENTS AND METHODS

Patient Eligibility. Adult patients (≥18 years of age) with metastatic melanoma recurrent or unresponsive to standard therapy or who refused standard therapy were eligible for this study. Patients were required to give informed consent and could not have received radiation, chemotherapy, or glucocorticoid therapy within 3 weeks of enrollment or mitomycin C or nitrosourea within 6 weeks of enrollment. Inclusion criteria also required patients to have a KPS ≥80% and adequate hematological, renal, and hepatic function. Patients were excluded if they had brain metastases, any tumor greater than 5 cm × 5 cm, or active infection requiring parenteral antibiotics. Active autoimmune disease, uncontrolled diabetes mellitus, uncontrolled hypertension, New York Heart Association class 3 or 4 heart disease, and HIV seropositivity were other exclusionary factors. Given that the effects of gene therapy on embryogenesis are unknown, pregnant patients were excluded, and the use of contraception was required for patients of child-bearing potential. Patients with major surgery within 2 weeks of study entry or intrathoracic or intra-abdominal surgery within 4 weeks were also excluded. Concomitant treatment with anticancer drug therapy, immunosuppressive drugs, or any other experimental therapy was not permitted.

Dose Regimen. All of the patients were to receive six intratumoral injections with 10 μg of Allovectin-7 over a 9-week period, with a follow-up evaluation at week 11. The first series of injections was to be given at weeks 1, 2, 3, and 4 followed by a 4-week observation period. If a patient showed no signs of clinically progressive disease after this observation period, they were eligible to receive the final two injections at weeks 8 and 9. Patients diagnosed with stable or responding disease at the week-11 evaluation were eligible to receive an additional course of treatment. Allovectin-7 was administered by intratumoral injection into a single accessible tumor lesion. The lesion selected for injection was required to be ≥1 cm in longest diameter but ≤5 cm × 5 cm in size and accessible to direct needle injection. For visceral lesions, the study drug was injected with the aid of sonographic or computed tomography scan guidance. Injections were administered into the same lesion on subsequent weeks. Tumor nodules were injected in multiple locations to maximize the contact between Allovectin-7 and the tumor cells.

Study Drug. Allovectin-7 consists of plasmid DNA encoding the genes for HLA-B7 heavy chain and β2-microglobulin inserted into a simplified eukaryotic expression vector (pBR322). Expression of both genes is driven by the Rous Sarcoma Virus-Long Terminal Repeat promoter. The two genes are separated by cap-independent translational enhancer, an internal ribosomal entry site that permits coexpression of both genes from a single promoter. The plasmid is complexed with a cationic lipid mixture, DMRIE/DOPE: 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide/dioleoyl-phosphatidyl-ethanolamine.

Clinical Response Evaluations. Evaluation of tumor burden was performed by radiographic scans (computed tomog-

raphy, magnetic resonance imaging, and X-rays) and physical examination. Tumor sizing of the injected lesion was performed at each injection with a full disease assessment performed before the study and at week 11. Patients were evaluated approximately every 4 months thereafter. Response to treatment was determined for both local (injected tumor) and overall disease using standard outcome measures for clinical trials (CR, PR, SD, and progressive disease). Tumor measurements were recorded in millimeters and consisted of the product of the longest perpendicular diameters as determined by radiographic or physical examination. A local response to treatment was defined as a decrease of ≥25% in the product of the bidimensional diameters of the injected lesion. A CR was defined as the disappearance of all of the clinical evidence of tumor. PR required a ≥50% decrease in the size of all of the measurable lesions with no new lesions appearing, and SD was defined as <25% decrease or <25% increase in the size of tumor lesions with no new lesions appearing. MRs were defined as tumor regression ≥25% but <50%. Any response to treatment (either MR, PR, or CR) required a confirmatory staging at least 4 weeks later. Patients demonstrating clinically progressive disease were taken off study.

Toxicity Assessments. Safety was assessed at each injection with a follow-up evaluation 2 weeks after the last injection. Safety assessments included physical examinations and laboratory hematology and biochemistry testing. Patients were also evaluated for performance status, vital signs, and adverse events. All of the toxicities were graded according to the WHO Common Toxicity Criteria Grading Scale.

Definition of an Evaluable Patient. Patients were considered evaluable for toxicity after receiving a single injection of Allovectin-7. Patients were considered evaluable for response if they received at least two of the six planned injections with Allovectin-7 and underwent a post-treatment tumor evaluation within 4 weeks of the last injection.

RESULTS

Clinical Sites. Five clinical sites enrolled patients for this Phase II clinical study: Arizona Cancer Center, British Columbia Cancer Agency, University of Washington Medical Center, Northern California Melanoma Clinic, and University of Colorado Cancer Center.

Patient Demographics. Fifty-two adult patients (25 males and 27 females) with a median age of 49 years (range, 29–84 years) were enrolled. A summary of patient characteristics at study entry is provided in Table 1. Nineteen (37%) patients presented with disease limited to s.c. or nodal sites; 12 (23%) had visceral disease limited to the lung, whereas 21 (40%) patients presented with visceral disease involving other organs. Seven patients (13%) were HLA-B7-antigen positive. LDH values were elevated in 20 of the 52 patients (range, 1.1 to 4.9 × upper limit of normal). Of the 52 patients, 33 had previously received chemotherapy, 15 patients had undergone prior radiation therapy, and 37 patients had received prior immunotherapy (34 of whom received IL-2 or IFNα therapy). Four patients had no prior therapy for their disease upon study entry. Tumors chosen for injection included skin (56%), lymph nodes (17%), lung (13%), and other visceral sites (13%), four of which included liver lesions. The median size of the injected lesion at baseline was 6.1 cm^2 (range, 0.7–24 cm^2).

Table 1 Summary of patient characteristics at study entry

Characteristics		No. of Patients
Patients enrolled		52
Age (yr)		
Median	49	
Range	29–84	
Gender		
Male		25
Female		27
Ethnicity		
Caucasian		50
Hispanic		2
KPS		
100		25
90		20
80		7
Serum LDH		
Normal levels		32
Elevated levels		20
Time from diagnosis		
<2 years		16
2–5 years		21
>5 years		15
Mean	4.6 yr	
Median	3.3 yr	
Range	0.3–18.1 yr	
Extent of disease		
s.c./nodal		19
Visceral (lung mets only)		12
Visceral		21
Prior therapy		
Chemotherapy		33
Radiation		15
IL-2/IFN-α immunotherapy		34
Other immunotherapy		3
No therapy		4
Site of injection		
Skin		29
Lymph node		9
Lung		7
Other[a]		7
Size of injected lesion (cm^2)		
<5		22
5–10		12
>10		18
Median	6.1	
Range	0.7–24	
HLA-B7 Typing		
Negative		45
Positive		7

[a] Other injection sites: liver four, adrenal one, muscle two.

Treatment and Response Rate. Of the 52 patients enrolled, 51 were evaluable for clinical response assessment. One patient was inevaluable because no post-treatment tumor evaluation was performed. Tumor response was assessed by the treating oncologist.

A local (injected) tumor response rate of 18% (9 of 51) was observed, with one CR, three PRs, and five MRs. Maximum tumor response was achieved at a median of 11 weeks. One patient developed a PR in the injected tumor after retreatment. Stable injected lesions were observed at week 11 in 12 of 51 (24%) patients. Fig. 1 displays the best local tumor response in the 21 patients with stable or responding injected lesions.

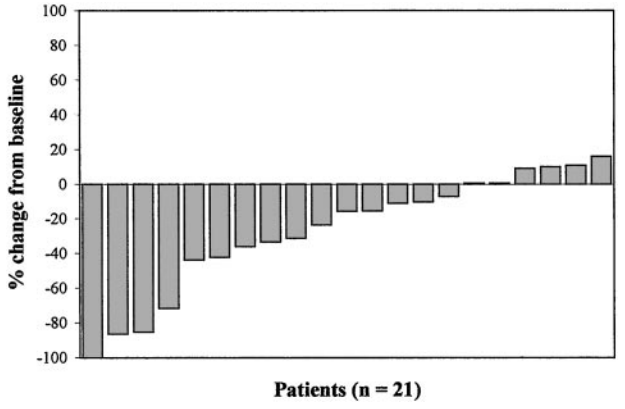

Fig. 1 Maximum response in injected lesions (percentage change from baseline) in patients with a stable or responding (CR, PR, or MR) tumor.

An overall disease response rate of 4% (2 of 51) was observed. Two patients developed a PR in overall disease. A 34-year-old male presented with a single site of disease in a mediastinal lymph node, a KPS of 90%, and a normal LDH level. After two cycles of therapy, the 12.8-cm^2 lymph node regressed 85% to 1.5 cm^2. This patient has received no further melanoma therapy and remains in a stable PR 26 months after initiating Allovectin-7 therapy. The second PR occurred in an 84-year-old woman with disease limited to skin lesions, a KPS of 80%, and a normal LDH level. A 1-cm^2 skin lesion on her right leg was injected that underwent an 86% reduction after the first cycle of therapy. Several other small skin nodules (<1 cm^2) also regressed significantly, but objective measurements were not obtained. This patient received a second cycle of therapy and remained in PR for 27.7 months, until the time of her death from unrelated causes.

Nine patients remained stable after their initial cycle of therapy. In addition, regression of noninjected tumors (range, -21% to -78%) was documented in four patients in the face of progressive overall disease.

Responses were correlated to gender, site, and size of injected lesion, tumor burden, LDH value, and HLA-B7 status (Tables 2 and 3). Patient numbers were too small for meaningful statistical analysis. Of those patients developing a local response (nine patients), six were female. Of those patients developing either a local response or a response in overall disease, almost all had s.c. or nodal lesions as the site of injection (8 of 9 and 2 of 2, respectively). The extent of disease was also evaluated. Locally responding patients were more likely to have s.c. or nodal involvement (4 of 9), followed by metastases limited to lung (3 of 9). Neither patient developing a response in overall disease presented with visceral disease. The majority of responders also had normal LDH values at baseline (7 of 9 and 2 of 2, respectively). The remaining two patients who developed a local response had LDH values of only 2.2 and 2.3 $\times$ upper limit of normal.

Duration of Response and Overall Survival. The median time to progression for all of the evaluable nonresponders was 2.3 months. Patients with stable overall disease had a median time to progression of 5.3 months (range, 2.3–14.9 months). Neither patient who developed a response in overall disease progressed. One patient died of unrelated causes at 27.7 months, before progression occurred. The second patient remains stable at last observation (26 months after treatment).

Six patients remain alive at last observation (range, 25.1 to 39.4 months from first injection) including two patients who developed local responses and one patient with a PR in overall disease. Nonresponding patients had a median time to death of 7.6 months.

Toxicity. No patient was required to discontinue treatment or have therapy delayed because of unacceptable toxicity. Thirty-one of the 52 patients entered on study completed all six injections of a cycle according to schedule. Of the 21 patients who failed to complete a single cycle of therapy, 19 withdrew because of progressive disease and 2 patients requested to be taken off study.

Forty-nine patients experienced a total of 312 adverse events, of which 53 were judged by the investigator to be treatment-related. Adverse events were further classified as study drug-related (24 events) or procedure-related (29 events). All of the study drug-related toxicities were mild to moderate (no grade 3 or higher) and included pruritus and erythema at the injection site and general aches and pains.

Procedure-related toxicities included mild to moderate pneumothoraces, ecchymoses, and pain at injection sites. One patient was hospitalized twice after pulmonary injections with grade 2 pneumothoraces requiring chest tube placement. In both occasions, the patient recovered quickly (4 days and 2 days, respectively). A summary of all of the toxicities considered by the investigators to be related to treatment during the study is listed in Table 4.

DISCUSSION

The lack of effective therapies for patients with advanced melanoma and the toxicities associated with standard chemotherapeutic regimens and nonspecific immunotherapies make targeted therapy using DNA-based gene transfer an attractive approach for investigation. Many DNA-based therapies aim to replace defective genes to restore normal cellular functions. However, tumorigenesis is most often characterized by multiple genetic abnormalities and thus would require replacing or altering several genes. In addition, replacement gene therapy theoretically requires treating all of the tumor cells in a given patient to ensure that no cells remain with malignant potential. Immune-based gene therapies circumvent this limitation by inducing a host immune response that recognizes and destroys cancerous cells specifically. In such a scenario, nontransfected tumor cells could be subject to immune attack based on locally high concentrations of immunostimulating cytokines or by virtue of shared tumor antigens now recognized by the immune effector cells. Allovectin-7, a novel nonviral vector encoding the genes for an allogeneic MHC class I antigen, HLA-B7, and β2-microglobulin has been developed for this purpose.

This trial confirms that patients with smaller tumor burden, as determined by tumor measurements and LDH, and disease limited to cutaneous/nodal involvement are most likely to respond to single agent Allovectin-7 therapy. This is consistent with preclinical studies of cancer vaccines, where tumor burden

Table 2 Patient features and local response rates

Parameter (no. enrolled)	Response					
	CR[a]	PR	MR	Total response	SD	Total patients
Sex						
Male (25)	1	1	1	3	5	8
Female (27)		2	4	6	7	13
Site of injection						
Skin (29)	1	2	2	5	10	15
Lymph node (9)		1	2	3		3
Lung (7)					1	1
Liver (4)			1	1	1	2
Other (3)						
Extent of disease						
s.c./nodal (19)	1	2	2	5	4	9
Visceral (lung mets only) (12)		1	1	2	3	5
Visceral (21)			2	2	5	7
Serum LDH						
Normal levels (32)		3	4	7	7	14
Elevated levels (20)	1		1	2	5	7
HLA-B7 typing						
Negative (45)	1	3	4	8	11	19
Positive (7)			1	1	1	2

Table 3 Patient features and overall disease response rates

Parameter (no.)	Response		
	CR	PR	SD
Sex			
Male (25)		1	4
Female (27)		1	5
Site of injection			
Skin (29)		1	3
Lymph node (9)		1	4
Lung (7)			1
Liver (4)			1
Muscle (2)			
Adrenal (1)			
Extent of disease			
s.c./nodal (19)		2	5
Visceral (lung mets only) (12)			1
Visceral (21)			3
Serum LDH			
Normal levels (32)		2	6
Elevated levels (20)			3
HLA-B7 typing			
Negative (45)		2	7
Positive (7)			2

is a major determinant of tumor rejection and antitumor responses (27). This trial also confirms the excellent toxicity profile observed with Allovectin-7 therapy. Toxicities were mainly mild to moderate, limited to the injection and/or biopsy procedure of visceral lesions, and resolved rapidly.

The observed response rate in this trial is also in agreement with prior single institution and multicenter trials using different intratumoral injection schedules. A composite of all of the evaluable patients (90 patients) with stage III or IV metastatic melanoma treated with Allovectin-7 found a correlation between tumor response and site of disease (24, 26, 28). Patients with cutaneous and/or nodal disease demonstrated the best responses to Allovectin-7 therapy, followed by patients with disease limited to lung metastases. Thirty percent of all of the evaluable patients with cutaneous and/or nodal disease developed a local response (CR, PR, or MR), and more significantly, 15% also developed an overall disease response (overall CR or PR). A local response rate of 30% and an overall disease response rate of 10% were reported in patients with disease limited to lung metastases. In contrast, only 16% of patients with visceral disease had local responses with 3% of patients developing an overall disease response.

The mechanism accounting for the clinical responses observed with Allovectin-7 is still not completely defined, although several possible mechanisms have been proposed. Allo-immune responses against injected tumor cells expressing the allo-antigen, HLA-B7, may explain responses in HLA-B7 antigen-negative patients. Alternatively, reintroducing HLA-B7 into patients whose tumors have lost the ability to present antigens because of deficient MHC class I expression may also lead to responses in HLA-B7 antigen-positive patients. The higher rate of regression of injected tumors observed in HLA-B7 antigen-negative patients (one CR, three PR, and four MR) compared with HLA-B7 antigen-positive patients (a single MR) suggests that the generation of an allo-immune response is the predominant mechanism accounting for the observed clinical responses.

Other possible mechanisms for responses include deficient MHC class I expression in tumor cells because of deletion of or mutations of the β*2-microglobulin* gene. Reintroducing β2-microglobulin may increase surface expression of the patients' own MHC class I molecules and restore antigen presentation. Yet another possibility is that unmethylated bacterial CpG motifs in the backbone of the Allovectin-7 plasmid may be responsible for inducing an immune response with antitumor activity. CpG motifs in bacterial DNA have been reported to trigger direct B-cell activation by inducing B cells to proliferate and secrete immunoglobulin (29). Bacterial CpG motifs have also been shown to suppress IgE synthesis while promoting IgG, IL-12, IL-18, and IFN-α, IFN-β, and IFN-γ production, all of

Table 4 Adverse events reported during the trial possibly or definitely related to Allovectin-7 study drug or injection procedure

Adverse events	No. of patients	Severity (WHO grade)[a]			
		1	2	3	4
Total events (53)	25	46	6	1	0
Study drug-related (24)					
General pain	5	5	2		
Pruritus at injection site	4	4	1		
Erythema at injection site	2	2	1		
Paresthesia	2	2			
Asthenia	1	2			
Discharge at injection site	1	2			
Rash	1	1			
Edema at injection site	1	1			
Hematoma	1	1			
Procedure-related (29)					
Pneumothorax (pulmonary injections)	5	11	2		
Pain/discomfort at injection site	9	9		1	
Ecchymosis at injection site	6	6			

[a] Toxicity grades: 1, mild; 2, moderate; 3, severe; 4, life threatening.

which foster a Th1 response and enhance cell-mediated immunity in gene-vaccinated animals (30). On the basis of these observations, an adjuvant role for bacterial CpG motifs has been proposed. Because we did not treat any patients with empty or noncoding plasmid DNA constructs, the role of CpG motifs in inducing clinical responses remains unknown.

The consistent and reproducible tumor responses seen in Phase II trials of Allovectin-7 in stage III and IV melanoma patients are encouraging. Our experience with *in vivo* DNA-based immunotherapy leads us to conclude that this approach is safe in accessible nodules, well tolerated, and can induce both local and overall disease responses in melanoma. Furthermore, the ease of manufacture (*in vitro* manipulation of tumor cells is not required), routine treatment administration (performed in the clinic with minimal discomfort), and the excellent toxicity profile suggest that Allovectin-7 may offer advantages over current modalities of therapy in select subsets of patients with melanoma.

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