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Phase II Clinical Trial of a Granulocyte-Macrophage Colony-Stimulating Factor–Encoding, Second-Generation Oncolytic Herpesvirus in Patients With Unresectable Metastatic Melanoma

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ABSTRACT

Purpose
Treatment options for metastatic melanoma are limited. We conducted this phase II trial to assess the efficacy of JS1/34.5-/47-/granulocyte-macrophage colony-stimulating factor (GM-CSF) in stages IIIc and IV disease.

Patients and Methods
Treatment involved intratumoral injection of up to 4 mL of 10^6 pfu/mL of JS1/34.5-/47-/GM-CSF followed 3 weeks later by up to 4 mL of 10^8 pfu/mL every 2 weeks for up to 24 treatments. Clinical activity (by RECIST [Response Evaluation Criteria in Solid Tumors]), survival, and safety parameters were monitored.

Results
Fifty patients (stages IIIc, n = 10; IVM1a, n = 16; IVM1b, n = 4; IVM1c, n = 20) received a median of six injection sets; 74% of patients had received one or more nonsurgical prior therapies for active disease, including dacarbazine/temozolomide or interleukin-2 (IL-2). Adverse effects were limited primarily to transient flu-like symptoms. The overall response rate by RECIST was 26% (complete response [CR], n = 8; partial response [PR], n = 5), and regression of both injected and distant (including visceral) lesions occurred. Ninety-two percent of the responses had been maintained for 7 to 31 months. Ten additional patients had stable disease (SD) for greater than 3 months, and two additional patients had surgical CR. On an extension protocol, two patients subsequently achieved CR by 24 months (one previously PR, one previously SD), and one achieved surgical CR (previously PR). Overall survival was 58% at 1 year and 52% at 24 months.

Conclusion
The 26% response rate, with durability in both injected and uninjected lesions including visceral sites, together with the survival rates, are evidence of systemic effectiveness. This effectiveness, combined with a limited toxicity profile, warrants additional evaluation of JS1/34.5-/47-/GM-CSF in metastatic melanoma. A US Food and Drug Administration–approved phase III investigation is underway.

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INTRODUCTION

The survival of patients who have stage IV metastatic melanoma with standard of care remains as it was 3 decades ago.[1-11] Historically, objective clinical responses to vaccine approaches are observed infrequently.[6-11] The 1-year survival with unresectable melanoma, treated with a wide variety of agents by meta-analysis of 42 phase II trials, is 25.5%.[12] It is clear, therefore, that new therapies for recurrent or metastatic melanoma are urgently required.

JS1/34.5-/47-/granuclocyte-macrophage colony-stimulating factor (GM-CSF; OncoVEX^{GM-CSF}, BioVex, Woburn, MA) is an immune-enhanced, oncolytic herpes simplex virus type 1 (HSV-1). It is deleted for ICP34.5, which provides tumor-selective replication, and ICP47, which otherwise blocks antigen presentation. In addition, ICP47 deletion increases US11 expression, which thereby enhances virus growth and replication in tumor cells.[13] The coding sequence for human GM-CSF is inserted, replacing ICP34.5, to enhance the immune response to tumor antigens released after virus replication.

Safety and antitumor activity, including the clearance of injected and uninjected tumors, has been demonstrated with JS1/34.5-/47-/GM-CSF in animal studies.[13] Furthermore, phase I investigation has established safety and clinical activity of JS1/34.5-/47-/GM-CSF in patients with various tumor types, including melanoma.[14]

The primary objective of this phase II clinical trial was to assess clinical efficacy of JS1/34.5-/47-/GM-CSF in patients with unresectable stage IIIc and stage IV melanoma as measured by overall tumor response rate and survival.

PATIENTS AND METHODS

Patients

The protocol was approved by the site investigational review boards and by the US Food and Drug Administration under an investigational new drug application submitted by BioVex. Signed informed consent was obtained. Eligibility criteria included histologically proven stage IIIc or IV melanoma that was not eligible for curative surgery with one or more injection-accessible (allowing ultrasound guidance) cutaneous, subcutaneous, or nodal tumors that were 0.5 to 10 cm in diameter. Additional inclusion criteria were age $\geq$ 18 years, Eastern Cooperative Oncology Group performance status of 0 or 1, life expectancy $\geq$ 4 months, recovery from prior therapy with $\geq$ 4 weeks since chemotherapy or radiotherapy, and clinical immunocompetence. In addition, total white cell count $\geq 3.0 \times 10^9$/L, platelet count $\geq 80 \times 10^9$/L, and adequate renal function (ie, serum creatinine $\leq$ 0.2 mmol/L) and liver function (ie, bilirubin $\leq$ 1.5 times the upper limit of the normal; AST, ALT, lactate dehydrogenase [LDH], and alkaline phosphatase $\leq$ 2.0 times the upper limit of the normal). Patients were excluded if they were pregnant, were lactating, were of childbearing potential and unwilling to use contraception, had participated in a clinical trial $\leq$ 1 month before entry, or had received antiviral agents or major surgery within 14 days. Also excluded were patients with bone metastases or those with tumors to be injected in mucosal regions close to an airway, major blood vessel, or spinal cord that could cause compression with tumor swelling and those who had clinically active autoimmune disease, who were positive for HIV, hepatitis B/C, or syphilis, or who required ongoing corticosteroids. Although initially excluded, the following patients were included after an amendment allowance: patients with three or fewer asymptomatic, treated cerebral metastases that had neither evidence of progression nor steroid requirement at 2 or more months post-treatment, or patients with five or fewer liver metastases that were $\leq$ 5 cm in diameter.

Treatment Plan

Patients initially received a total intratumoral (IT) injection of up to 4 mL JS1/34.5-/47-/GM-CSF at 10^6 pfu/mL to seroconvert patients who were seronegative; the initial dose was used to reduce local and systemic flu-like reactions seen in this subgroup at higher initial doses in phase I.[13] This was followed 3 weeks later by injections of up to 4 mL (ie, maximum total dose per visit) at 10^8 pfu/mL repeated every 2 weeks. Up to 10 tumors each visit (which constituted a set) were injected, starting with the largest (0.5 mL injected into tumors 0.5 to 1.5 cm; 1 mL into tumors 1.5 to 2.5 cm; 2 mL into tumors $>$ 2.5 cm) to a 4-mL maximum by using ultrasound guidance if necessary. At first injection, tumors for injection needed to be at least 0.5 cm in diameter. At least one lesion remained uninjected to monitor distant effects. Injections were made along one or multiple tracks to distribute virus through the tumor, depending on size. If, after eight doses, there was evidence of biologic activity (ie, tumor inflammatory reactions and/or stable disease [SD] or better), treatment continued up to a maximum of 24 injections.

Disease status was assessed at baseline, after six injections, then every 12 weeks by computed tomography (CT) scan and clinical evaluation. Injected tumors were swabbed to detect JS1/34.5-/47-/GM-CSF at 24 to 72 hours for the first 19 patients. Positron emission tomography (PET)/CT and ultrasound were used at the discretion of the investigator. Ophthalmology examination was performed at baseline, at treatment 6, and at the final visit.

Objectives

The primary objective was to assess overall response rate by using RECIST,[15] modified to allow biopsy to determine the status of residual pigmented or other masses and to allow early limited-disease progression, if not clinically significant, because of the possibility of delayed immune-mediated antitumor effects.[16] Partial response (PR) categorization required an overall tumor burden reduction of $\geq$ 30% from baseline (ie, sum of longest diameter of all lesions). Any new lesions must have been reduced by $\geq$ 30% from initial observation in addition to being added to the numerator for the reduction from baseline calculation. Secondary objectives included determination of median and overall survival rates and safety profile (according to National Cancer Institute Common Terminology Criteria of Adverse Events, version 3.0). For analysis of per-protocol efficacy, patients had to have received at least four injections with tumor measurements completed up to week 9.

Response rate was evaluated by using a two-stage Simon design.[17] A response rate of $\leq$ 1% was deemed clinically ineffective, whereas $\geq$ 10% was deemed compelling for additional evaluation. If no responses were observed in the first 24 patients, then JS1/34.5-/47-/GM-CSF would be assumed ineffective, and the trial would be closed. If one or more response occurred, an additional 26 patients were to be enrolled. Kaplan-Meier survival curves were used to predict median and 1-year survival rates. All results are presented on an intent-to-treat basis.

RESULTS

Patient Characteristics

Fifty patients were enrolled from January 2006 to February 2008, and the patients included 10 with stage IIIC disease and 40 with stage IV (including 20 with M1c visceral) disease. Seventy-four percent of patients had received one or more nonsurgical therapies for active disease, including dacarbazine/temolozolomide and interleukin-2 (IL-2). The median follow-up was 18 months (range, 11 to 36 months). Patients had either Eastern Cooperative Oncology Group performance status of 0 (n = 31) or 1 (n = 19). Other demographics and staging are listed in Table 1. Two patients subsequently were found to have had extensive bone metastases at enrollment; although they were included for analysis, these two patients did not meet the inclusion criteria for the study. Eight patients left study before receiving four injections because of clinically significant progressive disease (PD); however, all 50 were evaluable for toxicity and comprised the intent-to-treat population used for both safety and efficacy analyses, including survival.

Antibody Responses and Virus Shedding

All 13 patients who were HSV seronegative at baseline strongly seroconverted by week 7, which was consistent with previous findings.[14] One hundred two swabs were taken from injection sites in 19 patients at 24 to 72 hours after the first injection. Only one swab was positive at low level (ie, $<$ 10 pfu; negative after second injection), which confirmed the rare shedding seen in phase I.[14] Swab collection, therefore, was stopped. All 78 urine samples collected at 1 to 48 hours after the first dose from 13 patients were negative for JS1/34.5-/47-/GM-CSF by qualitative polymerase chain reaction.

Clinical responses. Patients received a median of six injection sets (mean, nine sets; $>$ one tumor injected on most visits). Five patients received the full course of 24 injection sets. Table 2 lists the response data by stage and other variables (Appendix Table A1, online only).

Table 1. Baseline Demographic and Clinical Characteristics

Characteristic	Patients (N = 50) No.	%
Age, years		
Median	62	
Mean	62	
Range	34-88	
Sex		
Male	22	44
Female	28	56
Ethnicity		
White	48	96
Asian	1	2
Hispanic	1	2
HSV status		
Seronegative	13	26
Seropositive	36	72
Unknown	1	2
Stage		
IIIc	10	20
IV	40	80
IV M1a	16	32
IV M1b	4	8
IV M1c	20	40
LDH		
< ULN	37	74
> ULN	13	26
ECOG PS		
0	31	62
1	19	38
Prior therapy*		
None	13	26
Chemotherapy†	25	50
Immunotherapy‡	22	44
Other§	6	12
No. of prior therapies		
1	13	26
2	8	16
≥ 3	16	32

Abbreviations: HSV, herpes simplex virus; LDH, lactate dehydrogenase; ULN, upper limit of normal; ECOG PS, Eastern Cooperative Oncology Group performance status.
*Excludes surgery, radiation, or adjuvant therapy.
†Includes regional therapy.
‡Includes vaccine, anti–cytotoxic T-cell lymphocyte-4, and cytokine therapy.
§Includes cryotherapy and imiquimod.

Table 2. Response Correlations

Variable	Overall No. of Patients (N = 50)	No. of Patients per Response Group PR (n = 5)	CR (n = 8)	CR + PR (n = 13)	% of Patients With CR + PR (n = 26)
Stage					
IIIc	10	2	2	4	40
IV M1a	16	1	4	5	31
IV M1b	4	0	1	1	25
IV M1c	20	2	1	3	15
LDH					
< ULN	37	4	8	12	32
> ULN	13	1	0	1	8
ECOG PS					
0	31	3	5	8	26
1	19	2	3	5	26
Prior therapy					
No*	13	1	3	4	31
Yes	37	4	5	9	24

NOTE. Responses noted relate to those achieved with JS1/34.5-/47-/GM-CSF treatment alone (ie, without additional surgery) and also do not include responses that occurred after additional post-protocol extended treatment.
Abbreviations: PR, partial response; CR, complete response; LDH, lactate dehydrogenase; ULN, upper limit of normal; ECOG PS, Eastern Cooperative Oncology Group performance status.
*Excludes surgery, radiation, and adjuvant therapy.

There were 13 objective systemic responses in response to virus treatment alone (all patients: complete response [CR], n = 8; partial response [PR], n = 5; 26%; stage IV patients, CR, n = 6; PR, n = 3; 22.5%). Twelve of those with objective systemic responses continued for greater than 6 months (range, 7 to 31 months [ongoing] since initiating response). One patient developed a new brain metastasis 1 month after response was confirmed. Response onset was from 2 to 10 months after the first dose. Although local responses often occurred rapidly (after as few as two injections), maximum (biopsy-confirmed) overall response has been observed as long as 12 months after first dose. In six patients (Appendix Table A1, online only; Fig 1), transient locoregional or distant progression, including the appearance of new lesions, preceded CR (n = 4) or PR (n = 2). Distant responses at uninjected sites were documented in the lung, liver, pancreas, regional and distant lymph nodes, and other soft tissue sites (Appendix Table A1; Figs 1 and 2). Two additional patients achieved a CR after additional surgery of disease, which became resectable after virus treatment (surgical CR [sCR]), one after excision of a newly identified brain metastasis at month 4, and one after additional treatment with IL-2, which the patient had previously failed after removal from the study, also at month 4. These patients were not included in the response rate numbers described above. The disease control rate (ie, CR + sCR + PR + SD maintained for > 3 months) was 50%. Four patients continued dosing past 12 months on an extension protocol (PR, n = 3; SD, n = 1). Of these, one patient with PR and one with SD achieved CR by 24 months, and one patient with PR had residual disease resected at 13 months and remains with no evidence of disease (NED). The other patient continues on therapy (Appendix Table A1). Overall, therefore, 10 patients (20%) have achieved CR without additional surgery, and 13 patients (26%) achieved NED status, with surgery in three patients to remove residual disease. There was no correlation of response with having had prior therapy, the number of prior therapies, or HSV serostatus.

Examples of patient responses. Early progression evolved into durable response. Patient 703 was diagnosed in November 2004 with a 10-mm ulcerated lesion on her posterior left shoulder, which was followed by regional progression in August 2005. After three cycles of temozolomide, she experienced progression with retroperitoneal adenopathy, at five subcutaneous sites in the left and right shoulder, in the lung, and with indeterminate lesions in the liver. She received the first injection of JS1/34.5-/47-/GM-CSF in February 2006 into one deposit in the left shoulder only. Within 2 months, this became difficult to palpate. At 3 months, the liver lesions became more distinct and numerous on CT. Treatment was continued into the minimally palpable area until 8 months, by which time all lesions had resolved, including in the liver and lung (Figs 1 and 2). Biopsy of the injection

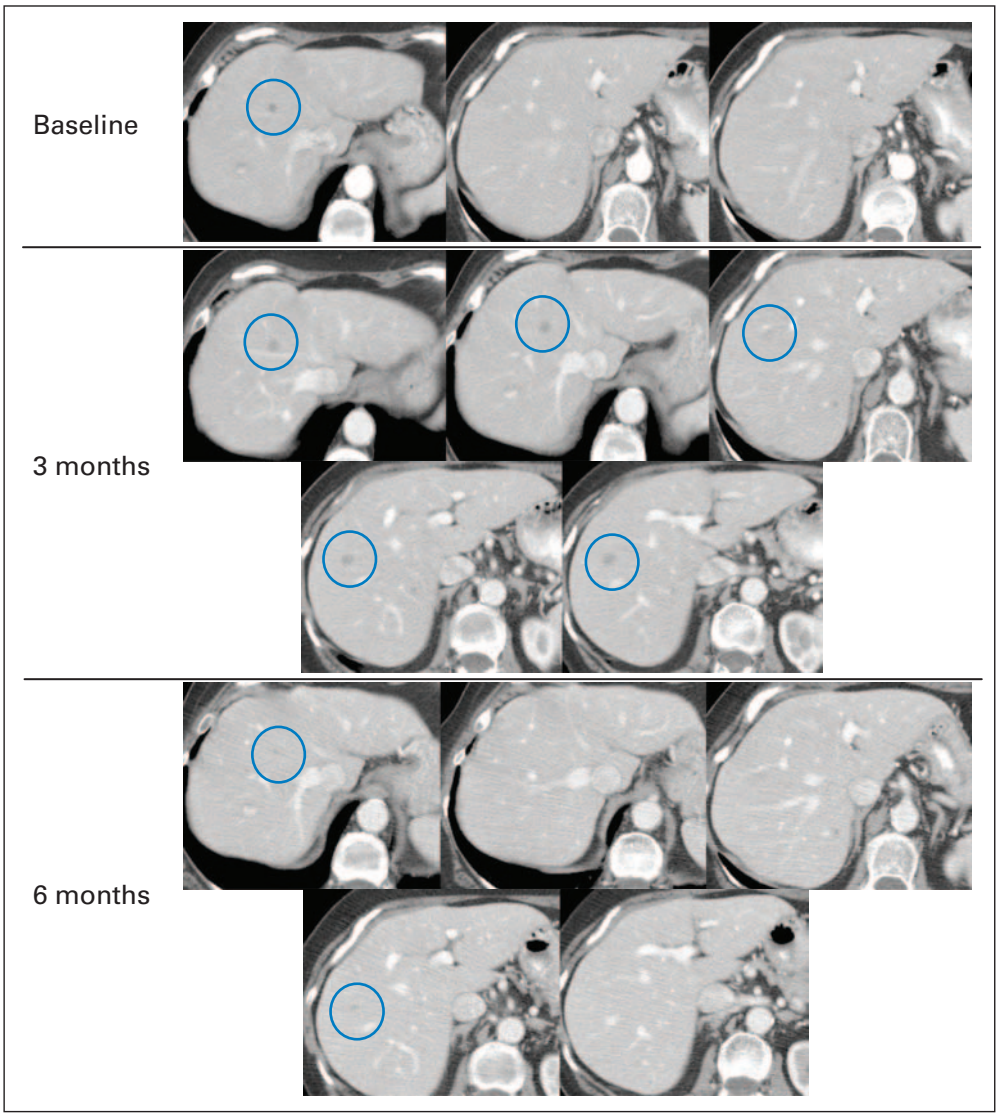

Fig 1. Example patient response. Computed tomography images of the liver at baseline, at 3 months, and at 6 months for patient 703; all slices with imageable disease are shown. Areas inside blue circles are matched sites of lesions before (baseline) and during (3-, 6-month) therapy.

site at injection 15 (the final injection) showed necrotic debris and residual ghost cells with extensive lymphocytic infiltration with follicular center formation. This patient remained off all therapy with no evidence of disease 40 months after her first dose of JS1/34.5-/47-/GM-CSF.

An example PR experienced on study was in patient 1502, which was followed with continued resolution of disease and ultimate CR on an extension protocol. Patient 1502 was diagnosed with metastatic axillary nodes in 2003 (primary unknown), which were treated with surgery, radiation, interferon alfa, GM-CSF, and NY-ESO-1 vaccine until January 2007, when progression occurred in the axilla. A 1.2-cm pancreas metastasis was noted on March 23, 2007 by PET/CT (and confirmed by fine-needle aspiration), with fluorodeoxyglucose (FDG) -avid lesions in the right chest wall and right upper arm. The patient received 24 doses of JS1/34.5-/47-/GM-CSF between May 2007 and April 2008, and all the doses were injected into the axillary mass. After the appearance of a new lesion in the chest wall, which then resolved (uninjected), the patient had achieved per-protocol PR by December 2007, and the only lesions noted by PET/CT on April 28, 2008 were the chest wall lesion (marginally reduced from baseline) and the focus of FDG uptake in the left upper arm, with no evidence of disease in the pancreas. After additional PET/CT on August 13, 2008, when the same sites of disease were noted (with marginal progression in the chest wall), the patient was transferred to an extension protocol, which by that time was in place, and injections into the chest wall mass and left upper arm were initiated on August 19, 2008. By November 13, 2008, the chest wall lesion demonstrated no FDG uptake and the left upper arm was reduced (Fig 3), and the patient had achieved a CR by May 2009 according to PET/CT.

An example of a patient with a locoregional response is shown in Figure 4. In this patient, seven of the larger nodules were injected, and all of the many other hundreds of nodules over a large area of the left calf and thigh then responded to provide a CR.

Survival. Overall median survival by Kaplan-Meier estimation was greater than 16 months for all patients as well as for the patient subset with stage IV disease (Fig 5). One-year survival rates were 58% for all patients and for all patients with stage IV disease and 40% for

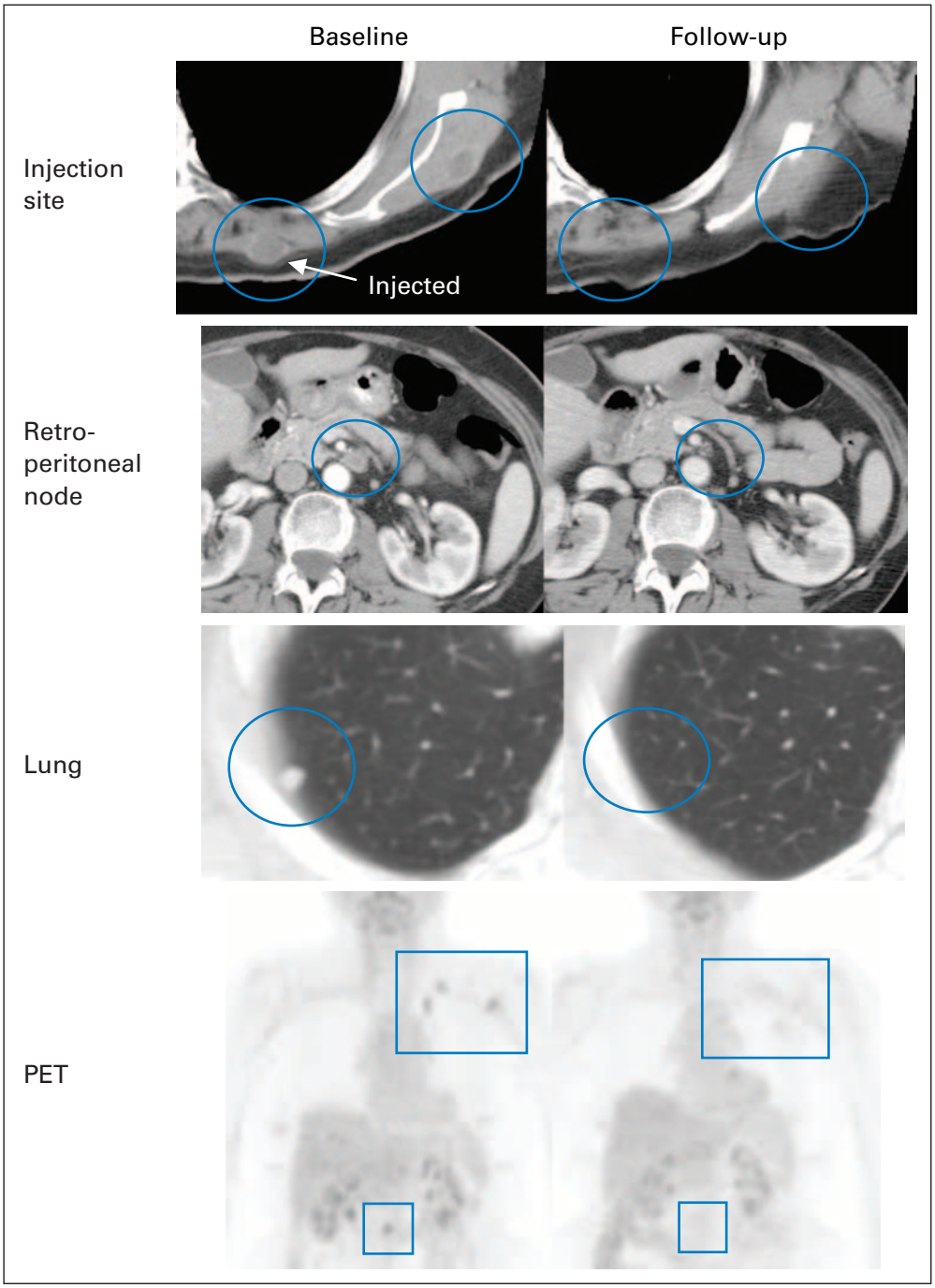

Fig 2. Example patient responses. Computed tomography images of the injection site in the left shoulder, retroperitoneal lymph node, and lungs at baseline and at 3 months, and positron emission tomography images at baseline and at 8 months for patient 703. Areas inside circles and squares are matched sites of lesions before (baseline) and after (follow-up) therapy.

patients with stage IV M1c disease. The 1-year survival rate of the patients who achieved PR, CR, or sCR (n = 15) was 93% (Fig 5).

Adverse effects. Eighty-five percent of patients had adverse effects (AEs) related to JS1/34.5-/47-/GM-CSF, all of which were grades 1 to 2. The AEs seen in three or more patients were associated with a mild influenza-like syndrome (ie, fever [52%], chills [48%], fatigue/malaise [32%], nausea [30%], vomiting [20%], and headache [20%]). Grade 3 AEs were infrequent, as six patients experienced pain (possibly disease related), and four patients each experienced fatigue and dyspnea (Table 3). There were 21 severe AEs, all of which were considered unrelated to JS1/34.5-/47-/GM-CSF. Autoimmune vitiligo was noted in three patients, two of whom achieved CR and one of whom had lesions that were responding before the patient left the study because of noncompliance issues.

DISCUSSION

A variety of tumor cell types are permissive to HSV replication and oncolysis[13,18,19] (including all human tumor cell types tested in our hands). This results in the induction of antitumor immune responses.[20] After intratumoral injections of the HSV-1 mutant G207,

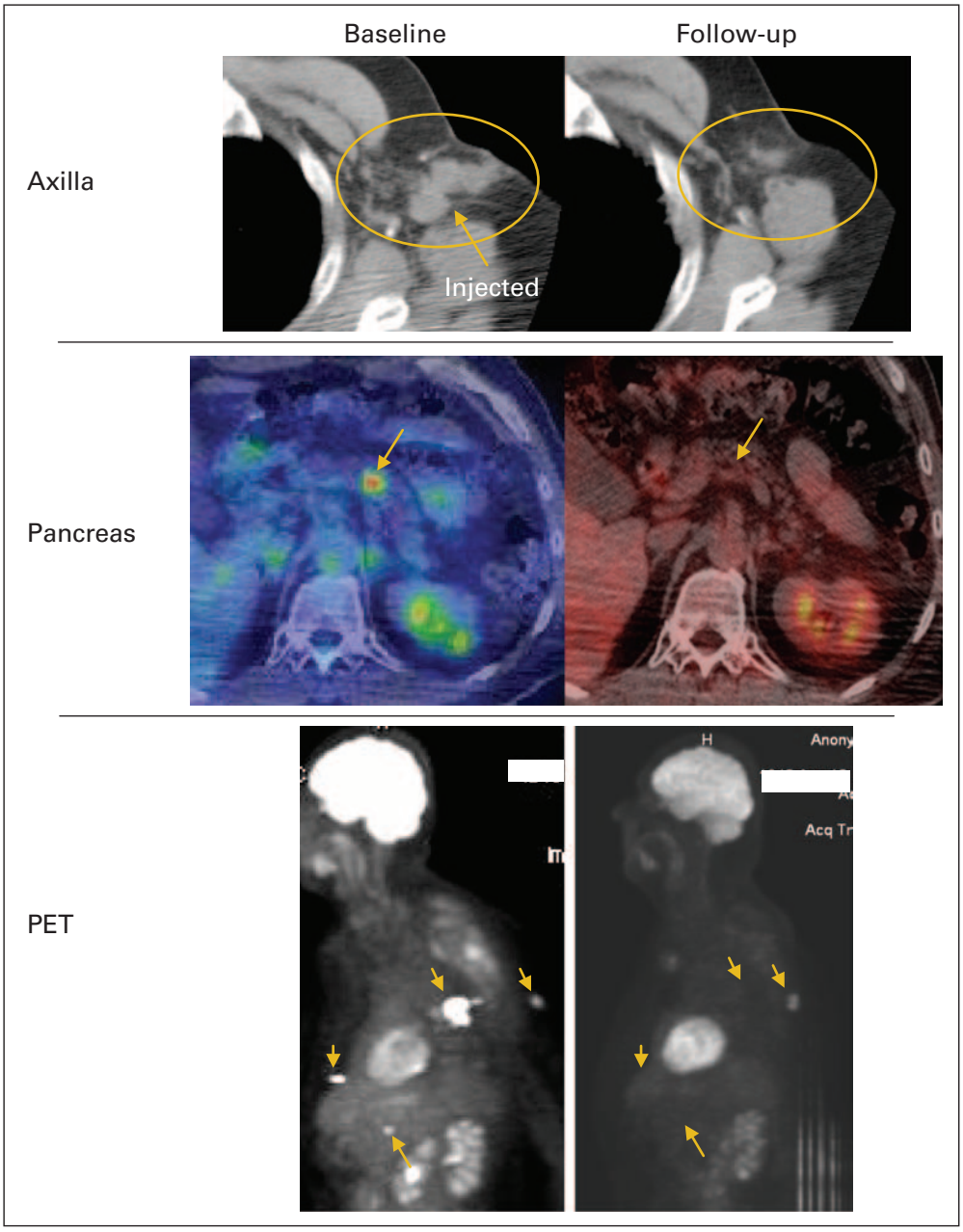

Fig 3. Computed tomography (CT) images of the injection site in the axilla at baseline and at 16 months; positron emission tomography (PET)/CT fusion images of the pancreas at baseline and at 12 months; and PET images showing the lesions in the axilla, chest wall, pancreas, and left upper arm at baseline and at 12 months for patient 1502. Arrows highlight the lesions in the axilla, pancreas, chest wall, and left upper arm.

Toda[20] showed tumor growth reduction in both injected and uninjected lesions. That the systemic effect was immune mediated was shown by the inhibition of tumor growth in athymic mice only in the injected lesion. Pre-existing HSV seropositivity did not significantly affect the therapeutic effectiveness of either the oncolytic G207 HSV[21] or JS1/34.5-/47-/GM-CSF.[13]

JS1/34.5-/47-/GM-CSF was developed to increase the oncolytic effectiveness of previous versions of oncolytic HSV and to generate enhanced systemic immune responses to the antigens released. As such, it was intended to provide a patient-specific, in situ, antitumor vaccine in addition to providing the direct oncolytic effect. JS1/34.5-/47-/GM-CSF is based on a new clinical strain of HSV-1 (ie, strain JS1), which is more effective at killing tumor cells than the HSV strains previously used. In addition to the deletion of ICP34.5, which provides tumor-selective replication as for G207, ICP47 is also deleted. ICP47 blocks antigen presentation to major histocompatibility complex class I and II molecules. Deletion of ICP47 also results in the increased and earlier expression of the HSV *US11* gene, which promotes replication in tumor cells and greatly improves tumoricidal effectiveness without decreasing tumor selectivity.[13] Finally, the virus contains the coding sequence for human GM-CSF, which stimulates the maturation, proliferation, and differentiation of dendritic cells, with the expectation of amplifying the antitumor immune response generated by lysed tumor products. Preclinical evaluation of JS1/34.5-/47-/GM-CSF confirmed GM-CSF enhancement of the immune response, enhanced surface levels of major histocompatibility complex class I molecules resulting from ICP47 deletion, and both injected and uninjected tumor responses.[13] As a safety factor, the gene for thymidine kinase remains

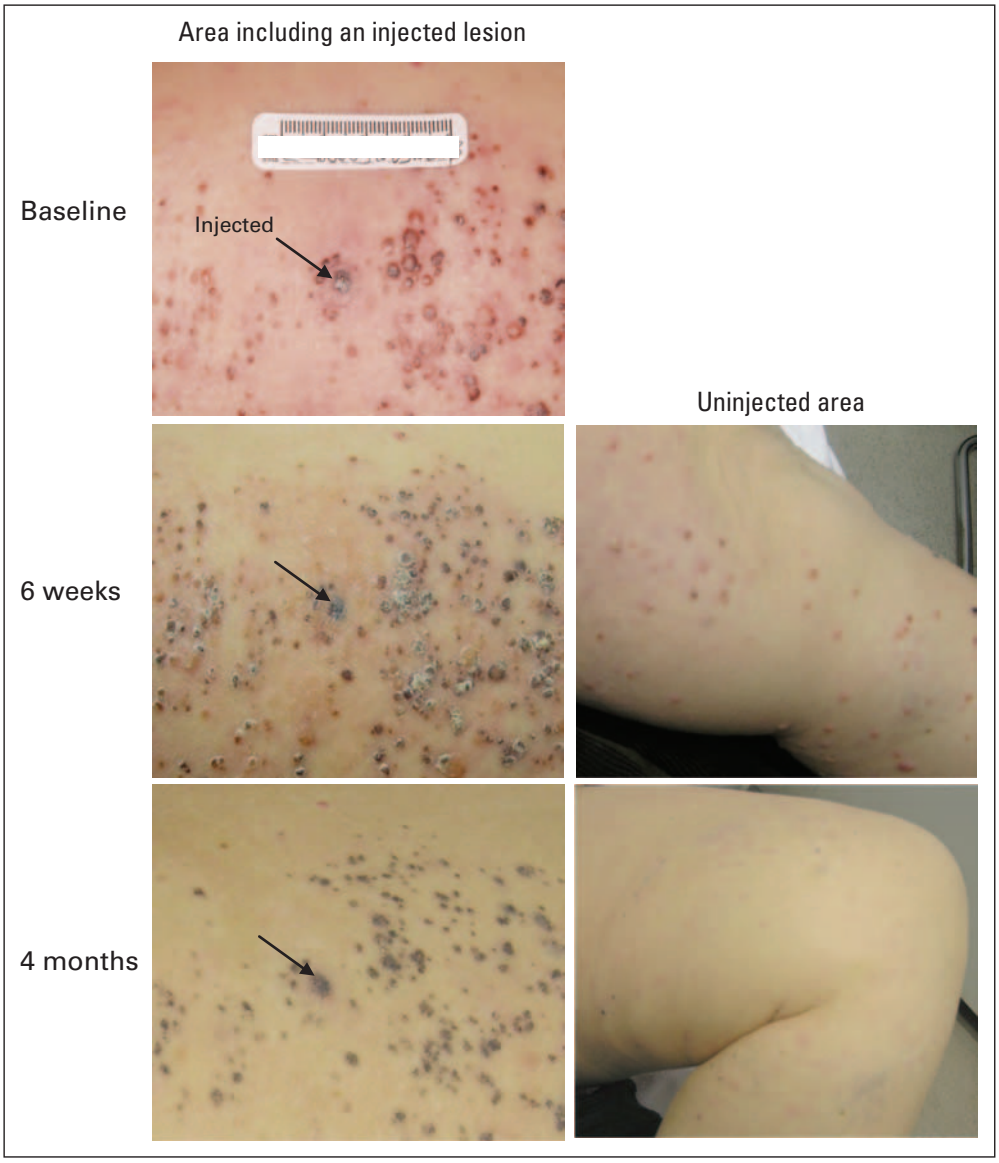

Fig 4. Photographs showing example areas of cutaneous response in patient 603 at baseline, at 6 weeks, and at 4 months. By 4 months, lesions either had completely resolved or only flat, pigmented areas remained. Representative biopsies taken at 8 months demonstrated that these contained no melanoma.

intact, which preserves sensitivity to clinically effective antiviral agents. A phase I study demonstrated JS1/34.5-/47-/GM-CSF to be well tolerated, and biologic activity was evidenced by tumor flattening, shrinkage, and necrosis in tumor types, including melanoma. Noninjected tumor effects also were seen.[14]

The data reported here support the effectiveness of JS1/34.5-/47-/GM-CSF immune-enhanced oncolytic virus therapy with a 26% objective response rate, including uninjected regional and distant soft tissue and visceral metastatic sites and, particularly, in achieving 1-year survival rates of 58%, 58%, and 40% for all patients, all patients with stage IV disease, and patients with stage IV M1c disease, respectively. Ultimately, 13 of the patients enrolled (26%) achieved NED status, 10 (20%) achieved CR through the effects of JS1/34.5-/47-/GM-CSF alone, and three patients achieved CR with additional surgery to remove residual disease. Of particular note is the durability of the responses seen, which were currently ongoing at between 16 and 40 months from first dose of JS1/34.5-/47-/GM-CSF. It is also notable that 74% of the patients enrolled had already received one or more nonsurgical prior therapies for active disease, including a number of the responding patients who had experienced failure on the only US Food and Drug Administration–approved therapies of dacarbazine/temolzolomide or IL-2. In a recently published meta-analysis of 2,100 patients with stage IV metastatic melanoma entered onto 42 phase II trials that spanned the years 1975 through 2005,[12] the 1-year overall survival rate was 25.5%, and no trial provided a survival result that was statistically different from the mean (25% in 524 patients). In the same analysis, the 1-year overall survival for only those 1,024 patients with visceral disease (ie, stage IV M1c disease) was 23.8% (40% in this study). Of the 19 patients who have been at risk for greater than 24 months from first dose, 52% remain alive. Although not directly comparable, as a better prognosis population would have been enrolled on the study reported here than in many of the studies included in Korn et al[12], the results with JS1/34.5-/47-/GM-CSF are provocative, particularly as some of the studies in Korn et al[12] excluded visceral disease or elevated LDH.

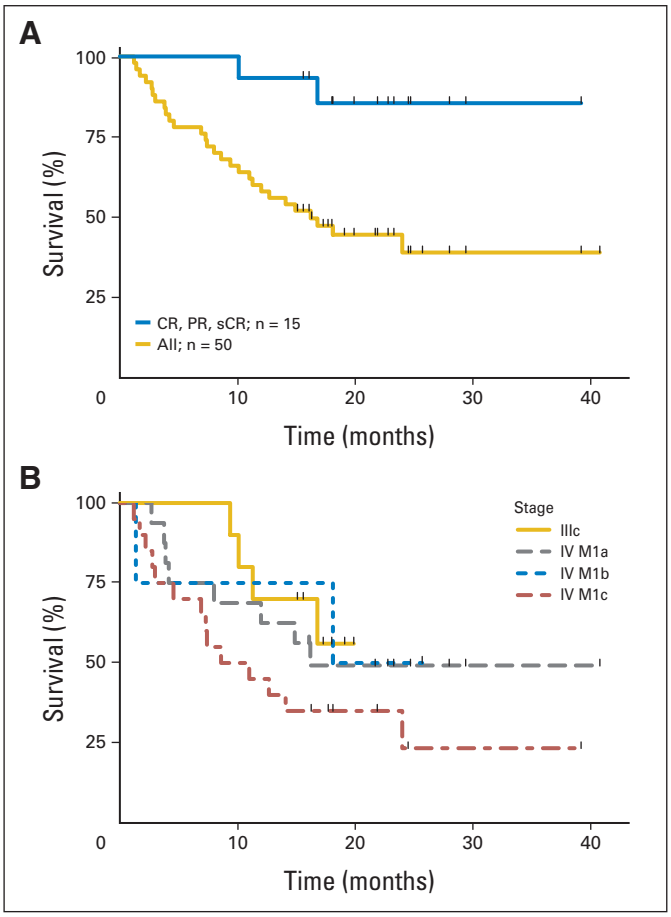

Fig 5. Kaplan-Meier survival curves. (A) Survival curves for all patients enrolled and for those who achieved complete response (CR), partial response (PR), or surgical CR (sCR). (B) Survival curves by disease stage.

Consistent with previously reported, immune-based response kinetics,[16] six patients in this trial experienced disease progression before proceeding to CR (n = 4) or PR (n = 2), which likely reflected both the latency period of immune response maturation as well as the relative kinetics of tumor growth versus immune effector activity. This included progression in both the liver and the lungs, as well as at soft tissue sites. Rather than employing standard RECIST assessment as used for evaluation of chemotherapeutic effectiveness, novel immune-related response criteria, therefore, were required.[22] Hence, a modified version of RECIST was used for response assessment, which allowed limited progression to occur before an objective response was observed. Although an immune-based mechanism is presumed to account for the distant effects seen, we have not attempted to determine any immune correlates of response in this study.

In conclusion, on the basis of the high frequency and durability of overall objective responses, the promising 1-year and overall survival rates of the patients enrolled, and the low toxicity and straightforward outpatient administration of the agent, the results of this phase II trial clearly justify that a randomized, controlled, phase III study is performed. A US Food and Drug Administration–approved, pivotal, phase III study is now underway.

Table 3. Safety Data

Adverse Event	Patients by Event Grade		
	Grades 1 and 2 (%)*	All Grade 3 (No.)	All Grade 4 (No.)
Pyrexia	52.0	0	0
Chills	48.0	0	0
Fatigue/malaise	32.0	4	0
Nausea	30.0	0	0
Pain/pain in extremity	24.0	2	0
Headache	20.0	0	0
Vomiting	20.0	0	0
Myalgia	16.0	0	0
Influenza-like illness	14.0	1	0
Depression	12.0	0	0
Diarrhea	12.0	1	0
Anemia	10.0	1	0
Anorexia	10.0	0	0
Arthralgia	10.0	1	0
Anxiety	8.0	0	0
Back pain	8.0	2	0
Gastroesophageal reflux	8.0	0	0
Rash	8.0	0	0
Abdominal tenderness	6.0	1	0
Asthenia/muscular weakness	6.0	3	0
Blood urea increased	6.0	0	0
Constipation	6.0	0	0
Cough	6.0	0	0
Decreased appetite	6.0	0	0
Dizziness/vertigo	6.0	1	0
Erythema	6.0	0	0
Insomnia	6.0	0	0
Muscle spasms	6.0	0	0
Peripheral edema	6.0	1	0
Tenderness	6.0	0	0
Tumor pain	6.0	0	0
Abdominal pain	—	2	0
Altered mental status	—	1	0
Atrial fibrillation	—	1	0
Blood amylase increased	—	1	0
Dyspnea/abnormal breath sounds	—	4	0
Chest pain	—	1	0
Chronic obstructive pulmonary disease	—	1	0
Hypertension	—	1	0
Hyponatremia	—	1	0
Hypoxia	—	2	0
Intestinal obstruction	—	1	0
Leukopenia	—	1	0
Lipase increased	—	1	0
Localized edema	—	1	0
Palpitations	—	1	0
Pleural effusion	—	1	0
Pneumonia	—	1	0
Pleural infection	—	1	0
Syncope	—	1	0
Thrombocytopenia	—	2	0

*Grades 1 and 2 events that occurred in three or more patients.

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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Appendix

Table A1. Individual Responding Patients

Patient	Stage	Baseline Disease Sites	Previous Disease	Prior Therapies*	Response	Response > 6 months	Comments
406	IIIc	Cutaneous: scalp, cheek	Primary: scalp	Radiation, interferon, cryotherapy	PR	No	Multiple new lesions appeared postbaseline, and then responded. New brain metastases were noted 1 month after PR.
408	IIIc	Cutaneous: shin	Primary: shin	None	CR	Ongoing at < 6 months	Multiple new lesions appeared postbaseline, and then responded.
601	M1a	Subcutaneous: left scapula, left chest wall; splenic flexure node	Primary: left back; recurrence: left back; in transit: left calf, left axilla (resected)	Temozolomide, vaccine, IL-2	sCR	Yes	Baseline disease responded without achieving PR; new node appeared and was treated with IL-2 (previously failed); continued response, resection.
602	M1a	Cutaneous: hand, arm	Primary: subungual (amputation), axillary nodes; multiple recurrences: right arm, chest wall, left cheek (resected)	Temozolomide, cisplatin, vinblastine, vaccine	CR	Yes	Multiple new lesions appeared postbaseline, and then responded. Biopsy was used to confirm response.
603	M1a	Cutaneous: leg	Primary: ulcerated left calf, sentinel nodes; multiple recurrences in left leg	Temozolomide, melphalan ILP, cryotherapy, infusional mustard	CR	Yes	Biopsy was used to confirm response (Fig 1).
605	M1a	Subcutaneous: right upper leg	Primary: right calf; inguinal nodes; chest wall recurrence (resected)	Immunotherapy	sCR	Yes	Brain metastases after five injections. Baseline lesions continued to respond after excision of brain metastases, and remaining lesion were resected. New lesions appeared 7 months after resection.
608	M1a	Cutaneous, subcutaneous: leg, ankle	Right second and third toes (amputated); multiple in transit recurrences in right lower leg (resected)	Cryotherapy	PR	Yes	
703	M1c	Retroperitoneal lymph node; small lung and liver lesions; left axillary node; subcutaneous lesions left scapula	Primary: ulcerated, left shoulder (resected)	Temozolomide	CR	Yes	Liver lesions progressed prior to response (Fig 1).
710	M1a	Subcutaneous: chest wall	Primary: left chest wall; bilateral axillary adenopathy (resected)	Temozolomide, interferon alfa, radiation	CR	Yes	Biopsy was used to confirm response.
714	M1b	Subcutaneous: left knee and thigh; numerous small pulmonary nodules	Primary: left ankle; left inguinal adenopathy (dissected); multiple recurrences in left lower leg (below knee amputation)	Interferon alfa	CR	Yes	Biopsy was used to confirm subcutaneous response.
716	M1a	Left axillary mass; node in pararenal space	Primary: posterior trunk; bilateral supraclavicular and axillary adenopathy (resected)	Temozolomide	CR	Yes	Biopsy was used to confirm subcutaneous response.

(continued on following page)

Table A1. Individual Responding Patients (continued)

Patient	Stage	Baseline Disease Sites	Previous Disease	Prior Therapies*	Response	Response > 6 months	Comments
901	IIIc	Cutaneous: shin, thigh	Ulcerated primary right calf, sentinel nodes; in transit: right lower leg (resected)	None	CR	Yes	Multiple new lesions appeared postbaseline, and then responded. Biopsy was used to confirm response.
902	IIIc	Cutaneous: shin; two external iliac chain nodes	Ulcerated primary: left toe; sentinel lymph nodes; multiple recurrences: lower left leg (resected)	Anti-CTLA-4, vaccine, ILP	PR	Ongoing < 6 mo	Pre-existing nodes progressed; new nodes appeared (para-aortic) postbaseline, and then responded (could have been reactive). Patient continued treatment on extension protocol.
1502	M1c	Left axillary adenopathy, left chest wall lesion, pancreatic lesion	Primary unknown; Left axillary node (resected)	Interferon alfa, GM-CSF, vaccine	PR/subsequentCR	Yes	Treatment continued on extension protocol until May 2009 when CR achieved.
1503	M1c	Cutaneous and subcutaneous lesions, left knee and thigh; external iliac nodes, inguinal nodes; pulmonary nodules; elevated LDH	Left thigh: ulcerated primary; inguinal nodes (resected)	None	PR/sCR	Yes	Residual iliac node resected at 13 months. Remained NED.
1504	M1a	Right axillary node; left axillary subdermal metastases; left axillary and subpectoral nodes	Left arm: primary (resected); extensive left axillary adenopathy (resected)	GM-CSF, radiation, imiquimod	SD	No	Patient continued on extension protocol and achieved CR (confirmed by biopsy).

Abbreviations: PR, partial response; CR, complete response; sCR, surgical complete response; IL-2, interleukin-2; ILP, isolated limb perfusion; CT, computed tomography; CTLA-4, cytotoxic T-cell lymphocyte-4; GM-CSF, granulocyte-macrophage colony-stimulating factor; NED, no evidence of disease; SD, stable disease.
*Excluding surgery.