Immunogenicity and Antitumor Effects of Vaccination with Peptide Vaccine +/- Granulocyte-Monocyte Colony-Stimulating Factor and/or IFN-α2b in Advanced Metastatic Melanoma: Eastern Cooperative Oncology Group Phase II Trial E1696

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Abstract **Purpose:** No therapy has ever shown prolongation of survival in stage IV metastatic melanoma. The association of cytokine-induced autoimmunity with improved prognosis led us to investigate the effect of multi-epitope melanoma vaccines alone and in combination with cytokines in this Eastern Cooperative Oncology Group multicenter phase II trial.

Experimental Design: Eligible patients were required to have failed prior therapies and to be HLA-A2 positive. Three HLA class I-restricted lineage antigen epitopes were administered in a factorial 2 × 2 design. Peptide vaccine alone (arm A), or combined with granulocyte-monocyte colony-stimulating factor (GM-CSF; Immunex) 250 μg/d subcutaneously for 14 of 28 days each month (arm B), or combined with IFN-α2b (Intron A; Schering-Plough) 10 million units/m^2 three times a week (arm C), or combined with both IFN-α2b and GM-CSF (arm D). The primary endpoint was immune response measured by enzyme-linked immunospot assay; secondary endpoints were clinical antitumor response, disease-free survival, and overall survival.

Results: One hundred twenty patients enrolled and 115 patients were analyzed. Immune responses to at least one melanoma antigen were observed in 26 of 75 (35%) patients with serial samples. Neither IFN-α2b nor GM-CSF significantly improved immune responses. Six objective clinical responses were documented. At a median follow-up of 25.4 months, the median overall survival of patients with vaccine immune response was significantly longer than that of patients with no immune response (21.3 versus 13.4 months; $P = 0.046$).

Conclusion: Immune response to vaccination correlates with prolonged survival in patients with metastatic melanoma and is not enhanced by immunomodulatory cytokines as tested in this trial.

There is no therapy to date that has shown a significant effect on overall survival (OS) in patients with metastatic melanoma. Only high-dose bolus interleukin-2 has been approved by the U.S. Food and Drug Administration for therapy of advanced melanoma based on durable responses induced in 4.7% to 6% of patients (1). Furthermore, in the adjuvant setting of operable high-risk melanoma, rigorous randomized phase III trials of high-dose IFN-α2b have shown both consistent relapse-free survival (RFS) and OS benefit in two of these trials (2). Granulocyte-monocyte colony-stimulating factor (GM-CSF) is another cytokine that has suggested RFS and OS benefit in a single phase II study of patients with operable resected stages III and IV melanoma compared with historic controls (3). These observations led us to design an intergroup trial of GM-CSF given in monthly cycles for treatment of patients with resectable advanced stage III and limited stage IV melanoma (4).

The common denominator of therapies of benefit in melanoma has been the augmentation of host immunity. High-dose IFN given in the neoadjuvant setting induces higher increases in the number of tumor-infiltrating CD3$^+$ and CD11c$^+$ immune cells in clinical responders compared with nonresponders (5). Similarly, high-dose bolus interleukin-2 induces inflammation at the tumor site (6), whereas GM-CSF has potent effects on myeloid dendritic cells (7, 8). These cytokines have been shown to induce autoimmunity that

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Translational Relevance

The results of the Eastern Cooperative Oncology Group multi-epitope peptide vaccine trial E1696 trial reported in this article have translational implications for the future therapy of melanoma in several respects: the results show the feasibility and immunologic efficacy of multi-epitope peptide vaccination, providing the foundation for adjuvant studies of this multi-epitope peptide vaccine that are ongoing in the intergroup. In summary, the results (*a*) show the feasibility of vaccination with rigorous immunologic assessments in the national cooperative groups, coupled with either granulocyte-monocyte colony-stimulating factor or IFN-α2b systemic therapy tested in a factorial 2 × 2 design. This study of 120 subjects who had failed prior therapy shows (*b*) that multi-epitope peptide vaccination induces immune response in a significant fraction of patients determined using complex enzyme-linked immunospot immunologic assays done in a central reference laboratory. The modulation of immune responses assessed by enzyme-linked immunospot assays of T-cell IFN-γ production is correlated with improved overall survival. Finally, this study (*c*) provides the foundation in advanced metastatic melanoma for multi-epitope peptide vaccination that has now been evaluated using a placebo-controlled design testing granulocyte-monocyte colony-stimulating factor and/or peptide vaccination in 815 patients with operable stage III to IV melanoma (Intergroup E4697).

is associated with clinical benefit (9–11). Concurrent or sequential administration of cytokines with melanoma vaccines in small studies has suggested augmented immunologic response in association with clinically significant antitumor responses (12, 13).

Vaccines have become the focus of major investigative efforts across the world, driven by the expanding understanding of the immunogenicity of melanoma antigens (14). Most previously reported vaccine studies have been conducted at single institutions, employing one or a limited panel of peptide antigens, with a variety of immunologic adjuvants, resulting in occasional clinical benefit (15–19). These limited benefits have been attributed to regulatory T cells, the low frequencies of antigen-specific T cells, and to defects in immune cell trafficking and function (20). A critical need has therefore developed for well-powered randomized studies testing the immunodominant melanoma peptide epitopes along with novel vaccination strategies to produce higher frequencies and more durable immune responses. No cooperative group trial has yet evaluated the immunogenicity of vaccines composed of multiple lineage antigens, or compared vaccination alone, versus in combination with immunomodulatory cytokines.

E1696 was designed to test immune responses of peptide-specific $CD8^+$ T-cell precursors by enzyme-linked immunospot (ELISPOT) assay against epitopes derived from three lineage-restricted antigens MART-1, gp100, and tyrosinase in patients with metastatic unresectable melanoma. The peptide epitopes were selected based on the high frequency of the anti-melanoma CTL clones derived from HLA-A2-positive tumor-infiltrating lymphocytes against antigenic sequences identified by both cDNA expression cloning and protein biochemical approaches (18, 21–28). Given the promise of two immunomodulatory biological agents available in the outpatient setting, this study was also designed to evaluate the immunomodulatory effects of GM-CSF, IFNα, or the combination of these two agents on vaccine immune response and toxicity. Finally, analyses of antitumor responses and correlations of immune response with survival outcome were explored in this multicenter randomized trial.

Patients and Methods

Patients. Eligible patients had histologically confirmed stage IV melanoma [American Joint Committee on Cancer (AJCC) 5th edition], absent brain metastases by magnetic resonance imaging or computed tomography scans within 4 weeks before randomization, and measurable disease. Prior treatments were allowed if administered >4 weeks before randomization, including IFN or GM-CSF. Patients needed to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, to have adequate hematologic (WBC $>4{,}000/mm^3$, platelet count $>100{,}000/mm^3$, and lymphocytes $>700/mm^3$), hepatic (aspartate aminotransferase, serum bilirubin, alkaline phosphatase, and lactate dehydrogenase ≤2 times institutional upper limit), and renal (serum creatinine <1.8 mg/dL) functions within 4 weeks before randomization, and to be HLA-A2 positive by serologic or genotypic analysis. Patients with HIV, other current malignancies active autoimmune disorders, or other conditions requiring chronic corticosteroid use were excluded. All patients provided written informed consent, and the study was approved by the institutional review board of each participating ECOG-affiliated institution.

Peptides. HLA-A2-restricted peptides used in this study included AAGIGILTV ($MART\text{-}1^{27\text{-}35}$; ref. 27), the enhanced gp100 peptide analogue (gp100 209, T210M) IMDQVPFSV ($gp100^{209\text{-}217}$; ref. 18), and the tyrosinase peptide analogue tyrosinase 368-376 (370D) [NSC 699048] YMDGTMSQV ($tyrosinase^{368\text{-}376}$; ref. 22). The peptides were synthesized and placed in vials containing 1 mL of a sterile 1 mg/mL solution for injections. All vaccine peptides and those used for ELISPOT assays were provided by the National Cancer Institute/Cancer Therapy Evaluation Program as Investigational New Drug 6123.

Immunization protocol. The schema of this study is illustrated in Fig. 1. After patients provided written informed consent, immunophenotypic or genotypic analysis for HLA-A2 status and radiographic studies were done. If HLA-A2 positive, additional peripheral blood was obtained for baseline immunologic assays, and patients were randomized at the ECOG operations office by telephone or through ECOG Web registration with equal weighting into one of the four treatment arms:

Arm A: Multi-epitope peptide vaccine alone. Patients were administered each multi-epitope peptide vaccine (6 injections, 3 locations) subcutaneously using rotating truncal sites close to nodal drainage groups of the four extremities on days 1 and 15 of each cycle (28 days) for up to 13 cycles (1 year). Each peptide was emulsified with Montanide ISA-51 (Seppic) and administered in two 1 mL subcutaneous aliquots. Vaccine was injected the same way in all treatment arms.

Arm B: GM-CSF + multi-epitope peptide vaccine. Patients self-administered GM-CSF (Immunex) at a fixed dose of 250 μg/d subcutaneously for 14 days followed by 14 days off every 28 days for 1 year (13 cycles) or until disease progression. We used the previously established dose (3). To ensure compliance, patients were requested to complete the E1696 Patient Diary. GM-CSF dose was permanently reduced by 50% at the beginning of the next cycle for WBC ≥ 60,000/mm^3.

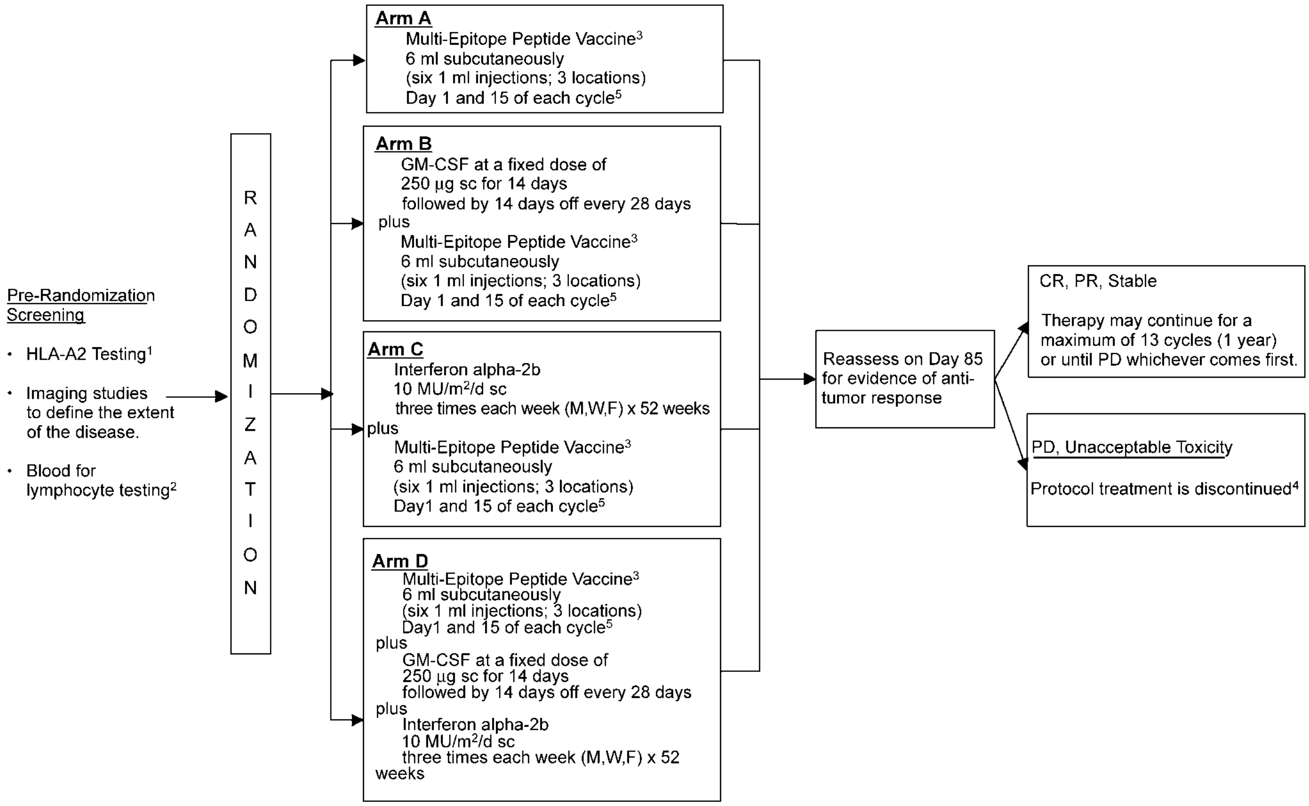
Arm C: IFN-α2b + multi-epitope peptide vaccine. IFN (IFN-α2b; Schering-Plough) was administered at 10 million units/m^2 subcutaneously three times a week (Monday, Wednesday, and Friday) for 52 weeks or until disease progression. Similar to arm B, patients were obliged to complete the Patient Diary. The dose chosen was that established for maintenance phase high-dose IFN adjuvant therapy. Criteria for IFN dose reduction were adopted from prior adjuvant studies (29).

Arm D: IFN-α2b + GM-CSF + multi-epitope peptide vaccine. Both IFN-α2b and GM-CSF were administered in the exact same schedule as in arms B and C, respectively.

The duration of therapy for all arms was 13 cycles (52 weeks). Patients who did not progress during treatment were followed every 3 to 24 months and every 6 to 60 months following study enrollment with physical exam and whole-body computed tomography scans.

HLA typing. HLA typing was done either genotypically using PCR (HLA-0201) or serologically using multiple antibodies (MA2.1, W6.32, BB7.2, and L243) for HLA-A2 with the respective isotype controls in the Immunological Monitoring Laboratory of ECOG at the University of Pittsburgh Cancer Institute. For genotypic analysis, we used PCR methods (HLA-A2 0201).

ELISPOT assays. Peripheral blood from patients was sent by overnight courier to the ECOG Core Immunological Reference Laboratory at the University of Pittsburgh Cancer Institute. Peripheral blood mononuclear cells were isolated by a Ficoll gradient centrifugation and cryopreserved. After completion of the vaccine regimen, samples from baseline and the days 43 and 85 time points were evaluated simultaneously by an IFN-γ (ELISPOT) assay (30). No *in vitro* sensitization of lymphocytes to enhance detection of low-frequency precursor T cells directed against melanoma lineage and other antigens was done, as this has been used for various intervals in multiple prior studies. We chose instead to avoid the artifacts associated with *in vitro* priming in this study where evaluation of T-cell responses was based on previously published definitions (16). The IFN-γ ELISPOT was done by carefully trained technologists, using standard operating procedures and validated reagents. Performance of different technologists involved in assay conduct was cross-compared. All plates were read by automated ELISPOT plate readers. Each assay contained wells in which normal healthy donor cells (made in large, multi-use batches) were tested for spontaneous and phorbol 12-myristate 13-acetate/ionomycin-stimulated cytokine release, serving as an assay control. Medium-only wells served as controls for nonspecific spots from other reagents.



1. Only HLA-A2+ patients are eligible for this study.
2. Blood for Assessment of Lymphoid Immune Response will be taken pre-randomization, at days 43, 85, day 1 of cycles 6, 9, and 12, and optionally at progression to test the effect of vaccination on the number of cytokine-producing peripheral blood T-cells, the frequenccy of peptide specific CD8+ T-cell precursors, and the generation of anti-peptide antibodies.
3. Patients will be administered each multi-epitode peptide vaccine subcutaneously in two contiguous 1 ml aliquots.
4. All patients will be followed for response until progression and for survival until death.
5. A cycle of therapy is defined as 28 days.

Fig. 1. Schema for ECOG phase II trial E1696.

Patient cells stimulated with OKT3 served as patient cell function controls over and above percent recovery and percent viability measures. Recall responses to flu peptide from $CD8^+$ cells and nonspecific responses to Ova were also included. Wells were plated in triplicate and SDs were measured. All samples were assayed on the same plate on the same day to avoid interassay variation. Several internal controls were used to test for spontaneous release of IFN-γ as well as stimulation to test for the ability to mount an immune response. For example, test wells were plated with the responders alone, responders + antigen-presenting cells (no peptide), and responders + Ova protein to test for spontaneous IFN-γ production in the absence of a stimulus. In addition, test wells were plated with responders + OKT3, and a set of wells with flu peptide to insure an immune response could be elicited. All reagent lot numbers were tested before patient assays for quality assurance.

Evaluation of clinical response and statistical considerations. Objective antitumor response was evaluated in measurable disease by computed tomography or magnetic resonance imaging scans and by clinical measurement of cutaneous lesions using the standard ECOG tumor response criteria (ECOG Policies and Procedures Manual).

The primary endpoint of the study was peptide-specific immune response by treatment (IFN versus no IFN or GM-CSF versus no GM-CSF) in a 2 × 2 factorial design with IFN on one axis and GM-CSF on the other axis. ELISPOT assays were the sole measure of treatment-induced immune modulation in this study. For an increase of immunologic response from 5% in non-IFN or non-GM-CSF treatment arms to 30% in IFN or GM-CSF treatment arms, 18 fully evaluable patients per treatment arm, and a two-sided significance level of 10%, the power to detect such a difference using Fisher's exact test was 81%. The secondary endpoints of the study were assessment of progression-free survival (PFS) and OS for patients in all four treatment arms.

In analyzing the time-to-event data, the method of Kaplan-Meier was used and significant differences observed were tested by the log-rank test. The Cox proportional hazards model was used for a multivariate time-to-event analysis. Fisher's exact test was used to compare proportions. All *P* values were reported for two-sided tests.

Results

Accrual. This study accrued 120 patients in seven major institutions of ECOG between September 22, 2000 and May 14, 2003. One patient randomized to arm B never received any therapy due to insurance issues. Four patients were ineligible. For E1696, 115 cases were included in the analysis of clinical outcomes, whereas toxicity data were summarized for all 117 treated cases. Seventy-five patients had ELISPOT data taken at baseline and at least one later time point (day 43 or 85). The stage breakdown for 72 of 75 patients with full staging data was M_{1a} [15 (20.8%)], M_{1b} [30 (41.7%)], and M_{1c} [27 (37.5%)].

Patient characteristics and immunologic response. Table 1 displays the on-study characteristics of the 115 treated patients who were analyzable for immune response. Seventy-five patients had ELISPOT data at baseline and for at least one later time point. Of these 75 patients, 66 patients had ELISPOT data at day 43 and 54 patients at day 85. Table 2 summarizes immune responses by ELISPOT data for each lineage-restricted melanoma antigen. The immune response rate to at least one of the peptides [overall immune response rate (OIRR)] was 21.2% at day 43 and 37% at day 85. The OIRR at day 43 or 85 was 34.7% (95 confidence interval, 24-46.6%). The immune response rate to gp100 was the highest compared with MART-1

Table 1. On-study characteristics (*N* = 115)

	***n* (%)**
Gender	
Male	69 (60)
Female	46 (40)
Race (*n* = 110)	
White	110 (100)
Performance status (*n* = 113)	
0	74 (65.5)
1	39 (34.5)
Depth of invasion (*n* = 80)	
I	1 (1.3)
II	8 (10.0)
III	22 (27.5)
IV	32 (40.0)
V	17 (21.3)
Primary tumor site (*n* = 106)	
Head and neck	19 (17.9)
Upper limb	5 (4.7)
Lower limb	17 (16)
Trunk	46 (43.4)
Anagenital	2 (1.9)
Ocular	8 (7.6)
Mucosal	4 (3.8)
Other	5 (4.7)
Histologic type (*n* = 79)	
Amelanotic	6 (7.6)
Melanotic	73 (92.4)

Sites of distant metastasis

Visceral involvement	**Measurable (*n*)**	**Nonmeasurable (*n*)**
Lung	70	11
Pleura	1	1
Liver	24	5
Brain/central nervous system	0	1
Bone	0	6
Other visceral	18	6

Nonvisceral involvement	**Measurable (*n*)**	**Nonmeasurable (*n*)**
Distant nodes	29	5
Soft tissue	23	6
Skin	19	2
Other nonvisceral	12	4
AJCC M stage (*n* = 111)		
M_{1a}	20 (18)	
M_{1b}	47 (42.4)	
M_{1c}	44 (39.6)	

	***n* (%)**
Age, y	
Median (range)	62 (23-83)
Breslow thickness, mm (*n* = 81)	
Median (range)	2.5 (28-24)
Prior treatment	
Surgery	109
Chemotherapy	24
Radiotherapy	22
Immunotherapy	44
Hormonal therapy	1
Gene therapy	1
Other	9

NOTE: Accrual by institution >5%. (*a*) University of Pittsburgh, 23.3%; (*b*) University of Wisconsin, 10.00%; (*c*) Mayo Clinic Rochester, 10.0%; (*d*) University of Pennsylvania, 8.3%; (*e*) Johns Hopkins University, 7.5%; (*f*) Beth Israel Deaconess Medical Center, 6.7%; (*g*) Emory University, 6.7%.

Table 2. Immune response by peptide (n = 75)

Day	gp100, %	MART-1, %	Tyrosine, %
Day 43	12.3 (8/65)	9.1 (6/66)	5.1 (3/59)
Day 85	28 (14/50)	5.6 (3/54)	10.6 (5/47)
Overall response to any of three peptides			
Day 43		21.2 (14/66)	
Day 85		37 (20/54)	
Day 43 or 85		34.7 (26/75)	

and tyrosinase. There was a marginally significant association between the OIRR and the disease stage (AJCC 6th edition) on study enrollment (46.7% and 43.3% in patients with M_{1a} and M_{1b} disease, respectively, versus 20.7% in patients with M_{1c} disease; P = 0.112). Neither IFN nor GM-CSF significantly increased the immune response rate. OIRR was 16 of 37 (43.2%) with IFN treatment in comparison with 9 of 36 (25%) without IFN treatment. By GM-CSF treatment, OIRR was 13 of 34 (38.2%) with GM-CSF and 12 of 39 (30.8%) without GM-CSF.

Clinical antitumor responses. Of the 115 analyzable patients, 2 patients had a complete response (CR), 4 had a partial response (PR), 30 had stable disease, and 73 had progressive disease, whereas 6 patients were nonevaluable. Table 3A and B summarizes the baseline characteristics of the 6 patients with CR or PR and the correlation of clinical and immunologic response. There was no significant difference across treatment arms in terms of best overall response, defined as non-progression (CR + PR + stable disease). As expected, only AJCC staging of disease on study enrollment was significantly correlated with best overall response [13 of 20 (65%) for M_{1a} patients versus 12 of 47 (25.5%) for M_{1b} patients versus 11 of 44 (25%) for M_{1c} patients; P = 0.04].

Survival. The median follow-up for the 115 analyzable cases was 25.4 months (range, 19.7-45.3) to final analysis. For the entire study cohort, the median PFS was 2.8 months (95% confidence interval, 2.7-3.0) and the OS was 11.9 months (95% confidence interval, 9.5-14.6). Figure 2 (*1* and *2*) show the Kaplan-Meier plots for PFS and OS by treatment status, respectively. There was no significant difference in OS by IFN and/or GM-CSF treatment status (data not shown).

Associations between immune and clinical data. The immune response data were associated with clinical data. Of the 75 patients with available ELISPOT data, 73 patients were clinically analyzable. Of these 73 patients, 25 patients had an immune response at any of the two time points studied (day 43 or 85) and to at least one of the three peptides administered.

There was a significant difference in OS by immune response status. Immune responders lived longer than the nonimmune responders (median OS, 21.3 versus 10.8 months; P = 0.033). The Cox model was used to evaluate the significance of immune response status for OS while adjusting for age, sex, performance status, AJCC stage at diagnosis, primary disease information (including Breslow depth, nodal involvement, and metastatic involvement), and ulceration status. Due to missing data on primary disease information, the total sample size for this analysis was smaller (n = 44). As expected, AJCC stage at diagnosis was the most significant predictor of OS (P = 0.002). Immune response status was marginally significant (P = 0.073). No other covariates were significant. The Kaplan-Meier plot for OS by immune response status is displayed in Fig. 3. No significant difference in PFS by immune response status was noted (data not shown). The data regarding immune response in relation to clinical response have been summarized in Table 3B.

Toxicity. Table 4 summarizes treatment-related toxicities based on 117 evaluable patients. No treatment-related deaths occurred. Grade ≥3 treatment-related toxicity rates were 41.9%, 48.3%, 83.3%, and 48.7% for arms A to D, respectively. As expected, arm C had a significantly higher rate of toxicity with lymphopenia (9 of 30), neutropenia (7 of 30), and fatigue (11 of 30) being the most frequent.

Discussion

This study is the first large, randomized, multi-institutional trial of multi-epitope peptide vaccination for patients who have failed prior therapy for metastatic unresectable melanoma. The

Table 3.

(A) Baseline characteristics of six clinical responders (CR and PR)

	Clinical response	Immune response	Diagnosis	Stage	Age	Gender	Metastatic Sites
1	CR	Yes	A	M_{1a}	47	M	Soft tissue
2	CR	No	C	M_{1a}	50	F	Distant node
3	PR	Yes	D	M_{1c}	68	F	Lung, soft tissue, other
4	PR	Yes	A	M_{1b}	51	F	Lung
5	PR	Missing	C	M_{1c}	74	M	Distant node, other
6	PR	Yes	D	M_{1b}	65	F	Lung

(B) Correlation of clinical response and immunologic response to peptide vaccination

Immune response	CR	PR	Stable disease	Progressive disease	Unevaluable
No	1	1	15	29	2
Yes	1	2	9	13	0

study addresses laboratory as well as traditional clinical endpoints that were considered critical to future vaccine development. Previous large randomized multicenter melanoma vaccine trials either could not evaluate therapeutic effect because they were conducted in the adjuvant setting (31–33) or they have not generally employed current immunologic monitoring against defined immunogenic peptides (34). We tested the immune response to lineage antigen-derived peptides administered either alone or in combination with two immunomodulatory agents whose efficacy has been or is being tested in the adjuvant clinical arena. We have shown the feasibility and safety of multicenter vaccine studies in conjunction with laboratory immunologic analyses for patients with metastatic melanoma.

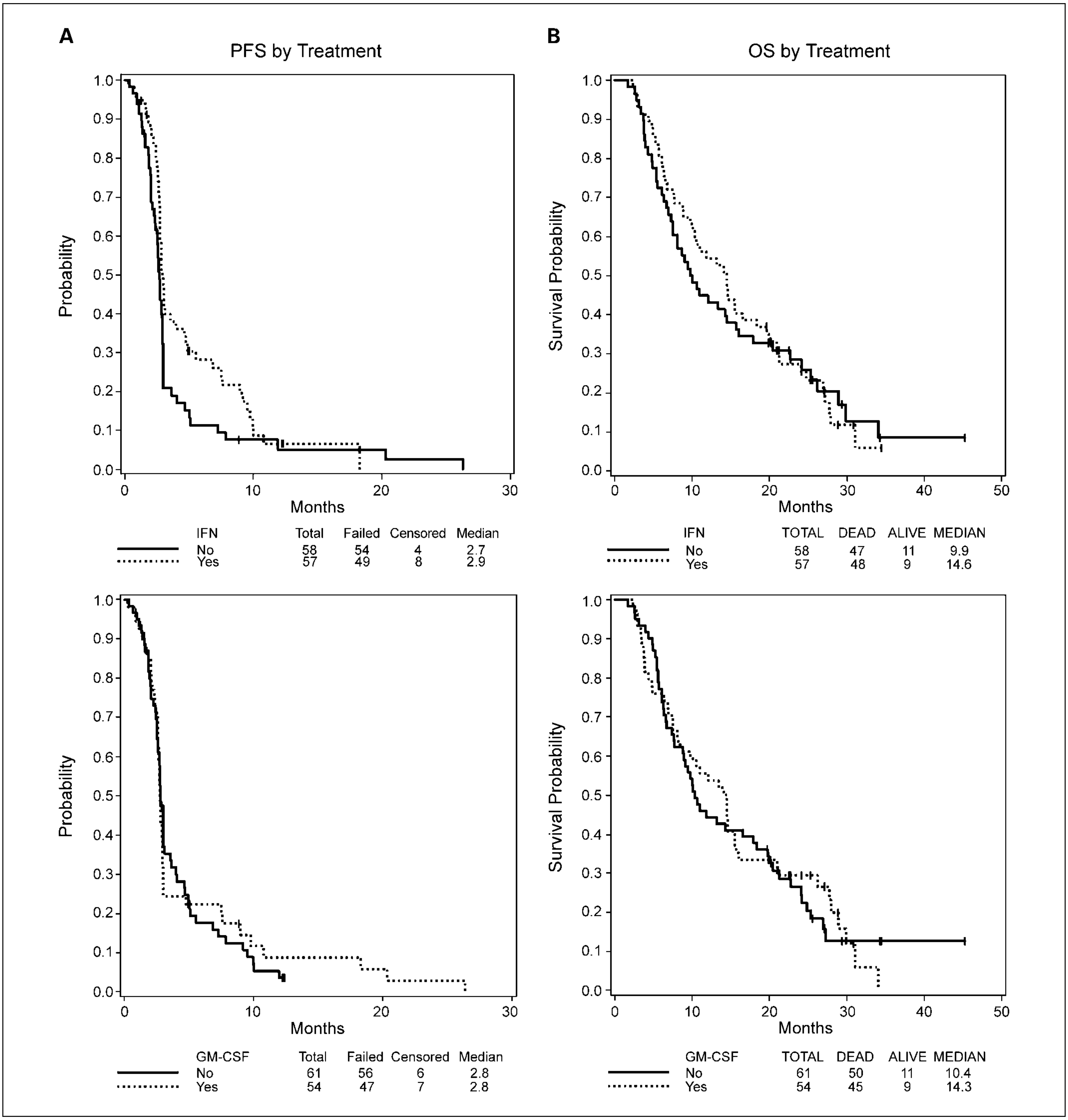


Fig. 2. PFS and OS by treatment (*n* = 115) and PFS by treatment (*n* = 115). *1, A,* progression of survival of patients according to treatment with IFN-α2b; *B,* progression of survival of patients according to treatment with GM-CSF. OS by treatment (*n* = 115). *2, A,* OS by treatment with IFN-α2b; *B,* OS by treatment with GM-CSF.

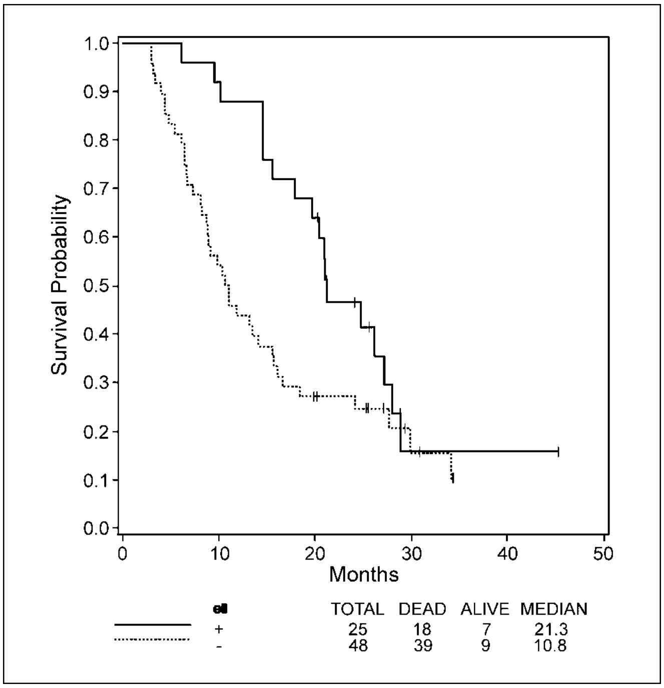


Fig. 3. OS by ELISPOT response ($n = 73$). OS according to immune response to one or more of the three peptide vaccine antigens measured by ELISPOT.

The primary endpoints of this study were to define the immune response rate to the multi-epitope peptide vaccination and to determine the influence of immunomodulatory agents on peptide-specific immune response. The OIRR was lower compared with prior multi-epitope peptide vaccination studies in patients with unresectable metastatic melanoma (15, 16). This inferior immune response rate may be a laboratory artifact and could be attributable to the *in vitro* sensitization of lymphocytes to enhance detection of low-frequency melanoma antigen-specific precursor T cells used in other studies.

This study was large enough to have adequate statistical power to confirm observations from smaller vaccine studies previously reported in metastatic melanoma. First, the objective response rate (CR + PR) observed in this study was similar to reported results (20) suggesting limited epitope spreading, with insufficient duration and/or magnitude of immune responses induced to achieve tumor regression. Second, gp100$^{209-217}$ induced higher rates of vaccination response than the other two lineage-specific peptides, and this high immunogenicity has been reported previously in other studies (35, 36). There are two possible explanations for this observation: a higher immunogenicity of the gp100$^{209-217}$ peptide analogue has been noted and an increased abundance of gp100-positive melanoma cell lines has been described (37). However, no enhancement of immunogenicity was observed with the modified tyrosinase peptide analogue used in this study. Third, the survival of HLA-A2-positive patients enrolled into this trial was longer than historically anticipated (38, 39). HLA-A2-positive patients have been noted to have improved outcome in the setting of operable high-risk disease (40). The prolonged OS observed in this series of patients, all of whom were HLA-A2 positive, will be of interest to compare with patients other series of patients with metastatic melanoma according to MHC type (41).

This is the first study large enough to have adequate power to assess the immunomodulatory effects of cytokines on antigen-specific immune responses and antitumor responses of patients with refractory advanced metastatic melanoma. In this study, neither IFN-α nor GM-CSF significantly augmented the immunologic response to peptide vaccination of patients with active measurable metastatic melanoma. This may be explained by counter-regulatory mechanisms that may be triggered by the systemic administration of immunomodulatory cytokines. Alternatively, and given the effect of IFN-α and GM-CSF on tumor-infiltrating immune cells (5, 8), it may be that the most relevant assessment of tumor immunity and vaccination would have focused on tumor-infiltrating rather than peripheral blood antigen-specific lymphocyte responses.

The most promising finding that emerges from this study is that patients with immune response to at least one of the vaccine peptides lived longer than patients without detected immune responses, as has been noted previously in smaller vaccine studies (38), and is remarkable for several reasons. First, it suggests that therapies that augment host immune response may improve the outcome of metastatic melanoma. Second, this finding suggests that immune response to vaccination may be used as a marker of improved prognosis in patients with metastatic melanoma. Our results concord well with the recent results of a separate adjuvant study on the prognostic significance of autoimmunity in patients with resected high-risk melanoma who receive high-dose IFN and suggest that autoimmunity is an intermediate marker of benefit from high-dose IFN-α2b through the induction of a more specific immunity to lineage and other antigens of melanoma (9). Third, understanding the molecular mechanisms regulating the development of antigen-specific immune response to vaccination may lead to further improvement of treatment for metastatic melanoma.

In summary, this large multicenter randomized phase II study of vaccination with lineage peptide antigens of melanoma administered in conjunction with two different immunomodulatory cytokines has shown that large studies with complex intermediate immunologic endpoints are feasible in the cooperative groups using a central reference immunologic monitoring laboratory. Neither of the cytokines tested in this study was capable of significantly augmenting the immunologic or therapeutic response to vaccination. However, immune response to vaccination was associated with improved OS. This provides support for the pursuit of these same peptide vaccines in the adjuvant setting, which has been accomplished in the E4697 intergroup trial that enrolled 815 patients to rigorously test the benefit of vaccination versus placebo using the exact same peptides and schedule tested in E1696. A better understanding of the mechanisms that regulate immune responses to melanoma vaccines is necessary before larger phase III randomized vaccine trials are conducted.

Disclosure of Potential Conflicts of Interest

J.M. Kirkwood: commercial research grant, Schering Plough, Berlex; speaker's bureau, Schering Plough.

Table 4. Treatment-related toxicity (*n* = 117)

Toxicity type	**Arm A (*n* = 31)**			**Arm B (*n* = 29)**			**Arm C (*n* = 30)**			**Arm D (*n* = 27)**		
	Grade			**Grade**			**Grade**			**Grade**		
	3	**4**	**5**	**3**	**4**	**5**	**3**	**4**	**5**	**3**	**4**	**5**
Hemoglobin	2	—	—	—	—	—	—	—	—	—	—	—
Leukocytes	1	—	—	—	—	—	3	—	—	—	—	—
Lymphopenia	2	—	—	5	—	—	9	—	—	7	—	—
Neutrophils	—	1	—	1	—	—	7	—	—	1	—	—
Transfusion: pRBCs	—	—	—	1	—	—	—	—	—	1	—	—
Supraventricular arrhythmias	1	—	—	1	—	—	1	—	—	—	—	—
Vasovagal episode	—	—	—	—	—	—	—	—	—	1	—	—
Cardiac-ischemia	—	—	—	—	—	—	—	—	—	—	1	—
Edema	1	—	—	—	1	—	—	—	—	1	—	—
Hypertension	1	—	—	—	—	—	—	—	—	—	—	—
Hypotension	—	—	—	—	—	—	—	—	—	2	—	—
Pericardial effusion/pericarditis	—	—	—	—	—	—	—	1	—	—	—	—
Thrombosis/embolism	1	—	—	—	—	—	—	—	—	1	—	—
Fatigue	2	—	—	—	—	—	10	1	—	4	—	—
Rigors/chills	—	—	—	—	—	—	—	—	—	1	—	—
Weight loss	—	—	—	—	—	—	—	—	—	1	—	—
DIC	—	—	—	—	—	—	—	—	—	—	1	—
PTT	1	—	—	—	—	—	—	—	—	—	—	—
PT	1	—	—	—	—	—	—	—	—	—	—	—
Injection site reaction	—	—	—	—	—	—	1	—	—	2	—	—
Rash/desquamation	—	—	—	—	—	—	—	—	—	1	—	—
Wound-infectious	—	—	—	—	—	—	—	—	—	1	—	—
Anorexia	—	—	—	—	—	—	2	—	—	—	—	—
Colitis	—	—	—	—	—	—	—	—	—	1	—	—
Constipation	—	—	—	—	—	—	—	—	—	1	—	—
Dehydration	1	—	—	—	—	—	—	—	—	1	—	—
Ileus	—	—	—	1	—	—	—	—	—	—	1	—
Nausea	1	—	—	—	—	—	—	—	—	1	—	—
Stomatitis	—	—	—	—	—	—	—	—	—	1	—	—
Vomiting	1	—	—	—	—	—	1	—	—	2	—	—
Diarrhea without prior colostomy	—	—	—	—	—	—	2	—	—	1	—	—
Central nervous system hemorrhage	—	1	—	—	—	—	—	—	—	—	—	—
SGPT	—	—	—	—	—	—	1	—	—	—	—	—
Infection with unknown ANC	1	—	—	—	—	—	—	—	—	—	—	—
Infection without neutropenia	1	—	—	—	—	—	—	—	—	—	—	—
Infection-other	—	—	—	—	—	—	1	—	—	—	—	—
Lymphatics	2	—	—	—	—	—	—	—	—	—	—	—
Hyperglycemia	—	—	—	1	—	—	—	—	—	—	—	—
Hypokalemia	—	—	—	1	—	—	—	—	—	—	—	—
Hyponatremia	1	—	—	—	—	—	—	—	—	—	—	—
Hypophosphatemia	—	—	—	—	—	—	—	—	—	1	—	—
Joint, muscle, bone-other	—	—	—	—	—	—	1	—	—	—	—	—
Confusion	1	1	—	—	—	—	—	—	—	—	—	—
Dizziness/lightheadedness	—	—	—	—	—	—	1	—	—	3	—	—
Hallucinations	1	—	—	—	—	—	1	—	—	—	—	—
Depression	—	1	—	—	—	—	2	—	—	—	—	—
Neuropathy-motor	1	1	—	—	—	—	1	—	—	—	—	—
Neuropathy-sensory	—	—	—	—	—	—	1	—	—	—	—	—
Syncope	—	—	—	—	—	—	3	—	—	1	—	—
Vertigo	—	—	—	—	—	—	—	—	—	1	—	—
Ocular-other	—	—	—	—	—	—	—	1	—	—	—	—
Abdominal pain	—	—	—	—	—	—	—	—	—	1	—	—
Arthralgia	—	—	—	1	—	—	—	—	—	1	—	—
Bone pain	1	1	—	3	—	—	—	—	—	—	—	—
Headache	—	—	—	—	—	—	2	—	—	—	—	—
Myalgia	—	—	—	1	—	—	—	—	—	1	—	—
Neuropathic pain	1	—	—	—	1	—	—	—	—	—	—	—
Pleuritic pain	—	—	—	1	—	—	—	—	—	—	—	—
Pain-other	2	—	—	—	—	—	—	—	—	1	—	—
Dyspnea	—	1	—	1	2	—	2	—	—	—	1	—
Hypoxia	1	—	—	—	—	—	—	—	—	—	—	—
Pneumonitis/pulmonary infiltrates	—	—	—	1	—	—	—	—	—	—	—	—
Pulmonary-other	1	—	—	—	—	—	—	—	—	—	—	—
Worst degree	8	5	—	11	3	—	22	3	—	13	3	—

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