A Randomized Phase II Trial of Multiepitope Vaccination with Melanoma Peptides for Cytotoxic T Cells and Helper T Cells for Patients with Metastatic Melanoma (E1602)

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Abstract

Purpose: This multicenter randomized trial was designed to evaluate whether melanoma helper peptides augment cytotoxic T lymphocyte (CTL) responses to a melanoma vaccine and improve clinical outcome in patients with advanced melanoma.

Experimental Design: One hundred seventy-five patients with measurable stage IV melanoma were enrolled into 4 treatment groups, vaccinated with 12 MHC class I-restricted melanoma peptides to stimulate CTL (12MP, group A), plus a tetanus peptide (group B), or a mixture of 6 melanoma helper peptides (6MHP, group C) to stimulate helper T lymphocytes (HTL), or with 6 melanoma helper peptide (6MHP) alone (group D), in incomplete Freund’s adjuvant plus granulocyte macrophage colony-stimulating factor. CTL responses were assessed using an in vitro-stimulated IFN-γ ELIspot assay, and HTL responses were assessed using a proliferation assay.

Results: In groups A to D, respectively, CTL response rates to 12 melanoma peptides were 43%, 47%, 28%, and 5%, and HTL response rates to 6MHP were in 3%, 0%, 40%, and 41%. Best clinical response was partial response in 7 of 148 evaluable patients (4.7%) without significant difference among study arms. Median overall survival (OS) was 11.8 months. Immune response to 6 MHP was significantly associated with both clinical response (P = 0.036) and OS (P = 0.004).

Conclusion: Each vaccine regimen was immunogenic, but MHPs did not augment CTL responses to 12 melanoma peptides. The association of survival and immune response to 6MHP supports further investigation of helper peptide vaccines. For patients with advanced melanoma, multipeptide vaccines should be studied in combination with other potentially synergistic active therapies.

Introduction

Therapeutic cancer vaccines using defined tumor antigens show promise for melanoma and other cancers (1-5).

Melanoma cells express peptides presented by MHC class I molecules, recognized by CD8+ cytotoxic T lymphocytes (CTL), or by MHC class II molecules, recognized by CD4+ helper T lymphocytes (HTL). Common melanoma peptides are from melanocytic differentiation proteins (MDP) and cancer-testis antigens (CTA; refs. 6-13). MDPs are expressed in 70% to 90% of metastatic melanomas and may be downregulated with progression (14-17); several CTAs are expressed in 30% to 60% of melanomas, and their expression may increase in more advanced melanomas (18, 19). Thus, there is rationale for vaccines targeting both MDPs and CTAs (20).

Most peptide vaccines have targeted only CTL; however, natural immune responses to pathogens induce both HTLs and CTLs. The present trial tests vaccines targeting both. A mixture of 12 melanoma peptides (12MP) restricted by HLA-A1, A2, or A3 has induced CTLs in up to 70% to 100% of patients (20-22). A mixture of 6 melanoma helper peptides (6MHP) has been immunogenic in 80% of patients and associated with clinical activity (22, 23), and a tetanus helper peptide has been immunogenic and safe. We proposed to test, in advanced melanoma, whether the
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**Translational Relevance**

This article reports results of a 4-arm randomized multicenter phase II clinical trial testing whether multipepptide melanoma vaccines that stimulate CD4⁺ helper T lymphocytes (HTL) would augment CD8⁺ cytotoxic T lymphocyte (CTL) responses for patients with advanced melanoma. Each vaccine regimen was immunogenic, but melanoma helper peptides (MHP) did not augment CTL responses to 12 melanoma peptides. However, there was a strong and significant association between survival and HTL responses to vaccines containing a mixture of 6MHP, whereas a similar association was not observed with HTL responses to a tetanus helper peptide. These data suggest that HTL responses to melanoma antigens have clinical relevance and deserve further investigation, but require consideration of different vaccine preparations or adjuvants. The low rates of objective clinical responses may be enhanced by combination with immune checkpoint blockade or other combination immune therapies.

Immunogenicity and immune protection of a 12MP melanoma vaccine would be augmented by addition of melanoma-specific helper epitopes (6MHP).

**Materials and Methods**

**Patients**

Eligible patients had histologically proven American Joint Committee on Cancer Stage IV melanoma with measurable disease, HLA-A1, A2, or A3 expression, age 18 years or more, Eastern Cooperative Oncology Group (ECOG) performance status 0 and 1, adequate organ function, and lactate dehydrogenase (LDH) ≤ 2 times the upper limits of normal (ULN). Exclusions included: pregnancy; other therapy within 4 weeks; allergy to vaccine components; other cancers within 5 years, untreated or progressive brain metastases; steroids; immunosuppression; or severe autoimmune disease. Patients provided informed consent, and the study was approved by the institutional review boards and the US Food and Drug Administration and registered at ClinicalTrials.gov (NCT00071981).

**Vaccines**

Patients received vaccines composed of multiple peptides from MDP and CTA, including 12MP (20; Arms A–C) restricted by HLA-A1, A2, or A3, to stimulate CTLs, 6MHP (23; Arms C and D), or a tetanus helper peptide (tet; 24, Arm B) to stimulate HTLs. Peptide sequences and details of Good Manufacturing Practice preparation and compliance (25) are provided in Supplementary Text.

Each vaccine was administered as a 2 mL stable water-in-oil emulsion, prepared by the two-syringe method, containing 100 mcg (12MP) and/or 200 mcg (6MHP, tet) of each peptide, plus 110 mcg granulocyte macrophage colony-stimulating factor (GM-CSF; Berlex/Genzyme) plus 1 mL.

**Trial design**

This was an open-label, multicenter phase II study, with randomization equal to vaccination with 12MP, 12MP/Tet, 12MP/6MHP, or 6MHP (Supplementary Fig. S1), through the ECOG web-registration program, using permuted blocks within strata with dynamic balancing within main institutions and affiliate networks. Patients were stratified by HLA status (HLA-A1⁺ only, HLA-A2⁺ only, HLA-A1⁺ and HLA-A2⁺, other) and sentinel immunized node (SIN) biopsy to be conducted (yes, no).

The primary objective was to determine the CTL response to 12MP. Secondary objectives were: (i) to determine the HTL response to 6 MHP; (ii) to estimate whether addition of 6MHP to 12MP augments CTL responses to 12MP; (iii) to obtain preliminary data on whether booster vaccination may maintain immune responses, (iv) to estimate rates of clinical response and survival, and (v) to obtain preliminary data on whether cellular immune responses may correlate with clinical outcome.

The primary endpoint was the CTL response, with the main comparisons of CTL response rates in arms A, B, and C versus D. We expected CTL response rates less than 10% in arm D and at least 40% in arms A to C. The sample size of 40 evaluable patients in each arm provided at least 85% power to detect a difference of at least 30% in CTL response rate, based on a 2-sided type 1 error of 0.05 using Fisher exact test. Secondary endpoints included HTL responses in peripheral blood mononuclear cells (PBMC), clinical response, and outcome. We expected HTL responses to be higher in arms B, C, and D (at least 30%) than A (5%), with the main comparisons between HTL immune response rates in arms B, C, and D versus A. There would be at least 82% power to detect a difference of at least 25% using Fisher exact test with a 2-sided type I error of 0.05.

**Clinical endpoints**

Tumor response was evaluated per RECIST v1.0 at weeks 8 and 12, then every 12 weeks, with survival follow-up thereafter. Objective response rate (ORR) was defined as complete response (CR) + partial response (PR) rates. Disease control rate (DCR) was defined as CR+PR+SD (stable disease at 8 weeks). Overall survival (OS) was defined as the time from randomization to death from any cause. Patients still alive
were censored at the date last known alive. Progression-free survival (PFS) was defined as time from randomization to disease progression or death, censoring cases without progression at the date of last disease assessment.

**Correlative studies**

Peripheral blood (100 mL) was collected pretreatment, at weeks 1, 3, 5, 7, and 11, and at the first and third of each set of booster vaccines. PBMCs were isolated from 90 mL of blood and viably cryopreserved, and serum was collected from 10 mL blood. Lymphocytes were isolated from the SIN (26). CTL response was determined by IFN-γ ELISPOT assay conducted 14 days after 1 in vitro sensitization, as described (in vitro-stimulated ELISPOT assay; ref. 20). CTL response required at least a 2-fold increase over background and 30 responding cells per 100,000 (details in Supplementary Text) HTL response was evaluated in a 5-day tritiated thymidine proliferation assay, as described in ref. (23) and in Supplementary Text, and required at least a 4-fold increase in proliferation. Cytokine [interleukin (IL)-12 p70, IL-1b, IL-2, IL-5, IL-6, IL-10, IL-17, TNFα, IFN-γ, GM-CSF], and chemokine (CCL3, CCL4, CCL5, CXCL9, CXCL10) production was measured by Luminex in supernatants collected from PBMC after 2-day or 5-day culture with helper peptides or mitogens [phytohaemagglutinin (PHA) and phorbol myristate acetate (PMA)]. Treatment samples (week 3–7) were compared with pretreatment samples with paired Student t tests.

**Toxicity assessment and stopping rules**
The trial was monitored continuously by the ECOG Data Monitoring Committee for treatment-related adverse events, using NCI Common Terminology Criteria for Adverse Events version 3.0. Protocol treatment was to be discontinued for unexpected treatment-related grade 4 hematologic, grade 3 nonhematologic, grade 2 allergic, or grade 1 ocular events, or for disease progression requiring other therapy. If 3 of the first 9 patients in any arm experienced dose-limiting toxicities (DLT), we would consider closing that arm. If the true DLT rate was 5%, the probability of observing 3 patients or more experiencing DLT events of 9 patients was 0.008. Interim analyses were conducted for immune response and clinical outcome, as detailed in Supplementary Text.

**Statistical analysis**

Immune response rate, objective clinical response rate, DCR, and toxicity incidence were compared between treatment arms using the Fisher exact test (27). Distributions of OS and PFS were estimated using the Kaplan–Meier method (28), with 95% confidence intervals (CI) calculated using Greenwood formula. Treatment effect on survival was tested using log-rank tests. Associations between immune response and clinical response were explored using Fisher exact test and logistic regression. Logistic regression models for rare events were also fit for testing association between immune response and clinical response as sensitivity analysis (29). Multivariable Cox proportional-hazards models (30) were built to explore associations between immune response and OS using the landmark method (31), with 2 months from randomization as a landmark time point. In the landmark analysis, OS was defined from landmark to the event (death); patients with OS events before the landmark were excluded from that analysis. All reported P values were for 2-sided tests. Significance level was set at 0.05. Given the exploratory nature of this study, no adjustments were made for multiple comparisons.

**Results**

**Patients**

One hundred seventy-five participants were enrolled from March 2005 to January 2009 (Table 2, Consort Diagram Fig. 1). At interim analysis, Arm C met protocol criteria for stopping based on clinical outcome, after accruing 37 patients. On eligibility review, 21 patients were ineligible (19 treated). Reasons for ineligibility are provided in Supplementary Text. Among the 154 eligible participants, 6 never started assigned therapy; thus, 148 eligible and treated patients were used for clinical outcome analysis. Toxicity analysis was based on the 167 participants who received protocol therapy and had toxicity reported, regardless of eligibility. Immune response analyses were based on 140 eligible participants with HTL data, and 128 with HTL data. Forty-five patients (30%) received booster vaccines (16, 8, 7, and 14, on arms A–D, respectively); 7 completed all 6 cycles. Study arms were well matched for known prognostic factors (Supplementary Table S1), but prior immunotherapy was less frequent for arm B than others (P = 0.01). Overall, serum LDH was elevated in 28%; 80% had 2 or more metastatic sites; M-stage was 7% M1a, 22% M1b, and 72% M1c.

**Immune response**

**CTL response rate.** CTL response rates to 12 melanoma peptides for Arms A to D were 43%, 47%, 28%, and 5%, respectively (Fig. 2A, Supplementary Table S2), and differed among treatment arms (P < 0.001) and for arms A to C versus D (P < 0.05), but not for A versus B or B versus C. In multivariable logistic analysis, the odds of CTL response was lower for patients on arm D, patients with more metastases, and increased age (Table 1). Responses to each peptide and responses to booster vaccines are summarized in Supplementary Text and Supplementary Table S3.

**HTL responses.** HTL response rates to 6 MHP for Arms A to D, respectively, were 3%, 0%, 40%, and 41% (P < 0.001); for tetanus peptide, they were 11%, 59%, 4%, and 0%, respectively (P < 0.001, Fig. 2A, Supplementary Table S2).

**Cytokine and chemokine production.** Antigen-specific cytokine and chemokine production by PBMC was evaluated in the 47 analyzable patients with HTL responses to 6 MHP (26), tet (20), or both (1). Among arm B patients, there were treatment-related increases in tetanus-specific production of IL-2 (d2, P = 0.0383), IL-5 (d2, P = 0.0149; d5, P = 0.0088; IFN-γ (d2, P = 0.006; d5, P = 0.0118), GM-CSF (d5, P = 0.0377), CXCL9 (d2, P = 0.0144;
Among 29 arm C+D patients, there were significant treatment-related increases in 6MHP–specific production of IL-2 (d2, \( P = 0.021 \)), IL-5 (d2, \( P = 0.0212 \); d5, \( P = 0.0072 \)), IL-12p70 (d5, \( P = 0.0359 \)), CXCL9 (d2, \( P = 0.0176 \); d5, \( P = 0.0434 \)), and CXCL10 (d5, \( P = 0.0076 \)). These data for IL-2, IL-5, CXCL9, and CXCL10 are shown in Fig. 3. Within groups C+D, for patients with partial response or SD (\( n = 9 \)), after stimulation with 6MHP, there were treatment-related increases in CCL3 (d2, \( P = 0.0212 \)) and CCL4 (d2, \( P = 0.0125 \)); for patients with PD (\( n = 19 \)), there were increases in IL-2 (d2, \( P = 0.0413 \)), IL-5 (d2, \( P = 0.0292 \); d5, \( P = 0.0096 \)), GM-CSF (d5, \( P = 0.0238 \)), and CXCL10 (d5, \( P = 0.0151 \), data not shown).

**Clinical outcome**

Seven patients had PR (4.7%, 95% CI: 1.9–9.5), and 27% had SD at 8 weeks (Table 2). There were no CRs. ORRs (CR+PR, ORR) did not differ significantly across study arms (\( P = 0.74 \)). The DCR (CR+PR+SD, DCR) was 32% (47/148; 95% CI: 24–40) overall. For arms A to D, PR rates were 2, 3, 6, and 7%, respectively. DCRs were 46%, 15%, 25%, and 36%, respectively, differing among arms (\( P = 0.026 \)), with A > B (\( P = 0.006 \)). SD persisted to 6 months in 5, 2, 3, and 2 patients on arms A to D, respectively; thus, PR or durable SD were observed in 15%, 9%, 16%, and 12%, respectively.

Of the 148 analyzable patients, median OS was 11.8 (95% CI: 10.2–14.0) months. For arms A to D, median OS and 95% CI were 14.9 (10.1–18.6), 10.2 (6.7–12.2), 12.4 (4.8–16.8), and 11.1 (8.8–14.2) months, respectively. The respective 1-year OS probabilities were 59% (42–72), 38% (21–54), 53% (35–69), and 49% (33–63) [50% (42–58) overall], and were assessed in the context of prior outcomes in multicenter cooperative group trials (32): correcting for cited variables (gender, visceral disease, performance status), the observed 1-year OS rate exceeds prediction by at least 2-fold, for each study arm (Supplementary Tables S4 and S5) and the observed values fall above the 95th percentile curves for arms A, C, D, and overall (Fig. 4D). PFS was not a designated endpoint of the study and did not differ significantly among study arms (Supplementary Text).

**Association between immune response and clinical outcomes**

For eligible and analyzable patients with and without CTL responses to 12 MP, ORRs were 9.8% and 3.0% (\( P = 0.194 \)), and DCRs were 37% and 32% (\( P = 0.695 \)), respectively (Fig. 2B). Among patients vaccinated with 12MP (groups A–C), ORRs were 10.3% and 0% (\( P = 0.022 \)) and DCRs 36% and 30% (\( P = 0.661 \)), respectively. Landmark analysis showed no significant association between OS and CTL response (\( P = 0.253 \), Fig. 4B). In multivariable regression analyses for groups A to D, CTL response was not associated with clinical response (OR 5.9; 95% CI: 0.7–48.6; \( P = 0.102 \)) or OS (HR 0.87; \( P = 0.557 \); Table 3, Model 1). The landmark analysis data are comparable if eligible and ineligible patients were assessed (\( P = 0.312 \) for 12 MP; data not shown).

Among 128 analyzable patients with or without HTL response to 6MHP, ORRs were 15% (4/27) and 3% (3/101; \( P = 0.036 \)), respectively (Fig. 2B). Evaluating only the 64 patients on arms C and D, ORRs were similar: 15.4% and

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**Figure 1. CONSORT diagram for E1602 trial.**

<table>
<thead>
<tr>
<th>Arm A (12 MP)</th>
<th>Arm B (12 MP/Tet)</th>
<th>Arm C (6 MP/MHP)</th>
<th>Arm D (6 MHP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allocated</td>
<td>Allocated</td>
<td>Allocated</td>
<td>Allocated</td>
</tr>
<tr>
<td>(n = 47)</td>
<td>(n = 43)</td>
<td>(n = 37)</td>
<td>(n = 48)</td>
</tr>
<tr>
<td>Ineligible &amp; no therapy (n = 0)</td>
<td>Ineligible &amp; no therapy (n = 2)</td>
<td>Ineligible &amp; no therapy (n = 0)</td>
<td>Ineligible &amp; no therapy (n = 0)</td>
</tr>
<tr>
<td>Eligible &amp; no therapy (n = 2)</td>
<td>Eligible &amp; no therapy (n = 3)</td>
<td>Eligible &amp; no therapy (n = 1)</td>
<td>Eligible &amp; no therapy (n = 0)</td>
</tr>
<tr>
<td>Received protocol therapy (n = 45)</td>
<td>Received protocol therapy (n = 38)</td>
<td>Received protocol therapy (n = 36)</td>
<td>Received protocol therapy (n = 48)</td>
</tr>
</tbody>
</table>

**Discontinued arm A:**
- Treatment completed (n = 1)
- Disease progression (n = 37)
- Toxicity (n = 4)
- Died (n = 0)
- Withdrawal (n = 3)
- Other (n = 0)

**Discontinued arm B:**
- Treatment completed (n = 2)
- Disease progression (n = 26)
- Toxicity (n = 4)
- Died (n = 0)
- Withdrawal (n = 1)
- Other (n = 2)

**Discontinued arm C:**
- Treatment completed (n = 4)
- Disease progression (n = 30)
- Toxicity (n = 3)
- Died (n = 1)
- Withdrawal (n = 0)
- Other (n = 2)

**Discontinued arm D:**
- Treatment completed (n = 3)
- Disease progression (n = 43)
- Toxicity (n = 1)
- Died (n = 1)
- Withdrawal (n = 0)
- Other (n = 0)

**Number of patients included:**
- Efficacy analysis (n = 41)
- Safety analysis (n = 45)
- CTL response analysis (n = 40)
- HTL response analysis (n = 37)
- Efficacy analysis (n = 33)
- Safety analysis (n = 38)
- CTL response analysis (n = 30)
- HTL response analysis (n = 27)
- Efficacy analysis (n = 42)
- Safety analysis (n = 48)
- CTL response analysis (n = 41)
- HTL response analysis (n = 39)
analyses showed that OS, for 64 patients on arms C and

response rate is shown for patients based on the presence or absence of

respectively), and 128 eligible participants had HTL response data (40, 30, 29, and 41 on arms A–D, respectively). B, the objective clinical response rate is shown for patients based on the presence or absence of CTL or HTL response to peptides in the vaccines (n = 140 for response to 12MP, and n = 128 for response to 6MHP or tetanus peptide; patients in each category are listed under the x-axis); C, median survival and 1-year survival based on 2-month Landmark analyses, by CTL response to 12MP, HTL response to 6MHP or tetanus, and considering just patients vaccinated with the respective peptides.

2.6%, respectively (P = 0.149 due to the smaller sample size). In contrast, response to tetanus peptide was not associated with clinical response (P = 1.00). Landmark analyses showed that OS, for 64 patients on arms C and

Table 1. Multivariable logistic regression for CTL response to 12MP (N = 140)

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Levels</th>
<th>OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arm A vs. Arm D</td>
<td>11.9</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Arm B vs. Arm D</td>
<td>33.1</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Arm C vs. Arm D</td>
<td>30.2</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;65 vs. &lt;65</td>
<td>0.2</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female vs. Male</td>
<td>1.3</td>
<td>0.657</td>
<td></td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 vs. 0</td>
<td>0.6</td>
<td>0.289</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Bold highlights P < 0.05.
Abbreviations: ECOG, Eastern Cooperative Oncology Group; LDH, serum lactate dehydrogenase.

D, was strongly and significantly associated with HTL response to 6MHP (P = 0.0045), with 1-year survival rate of 65% and 24%, and median OS of 14.7 and 7.9 months, respectively, for patients with or without HTL response (Figs. 2C and 4C). This association remained significant in multivariable Cox landmark analysis for arms C+D (HR 0.50; 95% CI: 0.26–0.96; P = 0.038; Table 3, Model 2). In contrast, of 27 analyzable patients on arm B, landmark analysis showed no significant association of survival with response to tetanus peptide (P = 0.153, Fig. 2C). The landmark analysis data are comparable if eligible and ineligible patients were assessed (P = 0.0183 for 6MHP; data not shown); and the effect is in the same direction when groups C and D are assessed individually, though not significant for arm D given the smaller sample size (Arm C: P = 0.009, Arm D: P = 0.127, Supplementary Fig. S2).

Of the 128 analyzable patients with data on both CTL response and HTL response, patients without response to 12MP or 6MHP had an ORR of 1.5%; corresponding rates were 6.9% with response to either, and 50% for response to both (P = 0.005, Fig. 2B).

Toxicity

Treatment-related toxicities are summarized in Supplementary Table S6. Rates of grade 3 or higher toxicity were 13%, 24%, 19%, and 8% on arms A to D, respectively. There was no significant difference in toxicity rates among
treatment arms (P > 0.05). There were 6 grade 4 toxicities in 4 patients (neutrophils, troponin elevation, lung infection, lymphopenia, CNS ischemia, and AST elevation) and no treatment-related mortality. The most common toxicities of grade 3 or higher were fatigue and injection site reaction.

Discussion

The cytotoxic T-lymphocyte response rates in arms A and B met primary study objectives by exceeding 40%; however, the study failed to show increased immunogenicity of a 12 MP melanoma vaccine by addition of melanoma-specific helper epitopes (6MHP), as only 28% had cytotoxic T-lymphocyte responses in arm C. This negative result is consistent with a parallel study conducted in the adjuvant setting (22). Possible explanations may include expansion of antigen-specific regulatory T cells, suboptimal cytokine induction, homing of T cells to tumor deposits, or sequestration of T cells at vaccine sites (33). Prior experience with 6 MHP vaccines argues against a Th12-dominant cytokine response (Dillon and colleagues, manuscript in preparation) and against expansion of total regulatory T cells (23). However, effects on melanoma-specific regulatory T cells and T-cell trafficking warrant further study.

Data from the HIV literature show that induction of HTLs can restore function in CTLs (34). However, combining epitopes for HTLs and CTLs into one colinear peptide may be preferable to using them separately in vaccines (35–37). Short peptides (9–11 residues) can bind directly to class I MHC molecules of cells in the vaccine-site microenvironment (VSME; ref. 38). On the other hand, longer peptides require uptake and intracellular processing before presentation on nascent MHC molecules in professional APC, which migrate from the VSME to draining lymph nodes after vaccination (38). It is possible that the intermediate length helper peptides in 6MHP (14–23 amino acids) may be presented primarily by professional APC in the vaccine-draining lymph node (VDLN). If peptides for CTLs and HTLs are presented in different compartments (VSME and VDLN), intended synergy may not be achieved.

Despite a body of literature that supports the use of GM-CSF as a vaccine adjuvant (4, 39, 40), 2 prospective randomized phase II trials have shown negative effects of GM-CSF in combination with other vaccine adjuvants (21, 41). The overall CTL response rates (43 and 47% in arms A and B) are based on ELISPOT assays conducted after in vitro stimulation; which has been required for other studies when GM-CSF was used in the adjuvant (4, 20, 21); we expect that the magnitude of these responses would be greater using incomplete Freund’s adjuvant without GM-CSF. It is also notable that the CTL response rates of 43% to 47% fall short of the circulating CTL response rate of 83% to the same 12 class I MHC-restricted peptides in a prior study (20); this may be due to effects of shipping blood from many centers or to cryopreservation methods (manuscript in preparation), or to the more advanced stage of the patients in the present study. However, this CTL response rate is at least as high as observed with a prior ECOG multipeptide vaccine trial (42).

Although the MHPs did not increase circulating CTL responses in this study, the HTL response rates to 6MHP in arm C (40%) and D (41%) were similar and were comparable with prior studies (22, 23). Thus, HTL responses to 6 MHP are not impacted negatively by addition of 12 melanoma peptides. The response to

### Table 2. Clinical response by treatment arm

<table>
<thead>
<tr>
<th>Best overall response</th>
<th>Arm A n (%)</th>
<th>Arm B n (%)</th>
<th>Arm C n (%)</th>
<th>Arm D n (%)</th>
<th>Total n (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyzable (eligible and treated) patients n = 41</td>
<td>n = 33</td>
<td>n = 32</td>
<td>n = 42</td>
<td>n = 148</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>PR</td>
<td>1 (2.4)</td>
<td>1 (3.0)</td>
<td>2 (6.3)</td>
<td>3 (7.1)</td>
<td>7 (4.7)</td>
<td></td>
</tr>
<tr>
<td>SD (at 8 weeks)</td>
<td>18 (43.9)</td>
<td>4 (12.1)</td>
<td>6 (18.8)</td>
<td>12 (28.6)</td>
<td>40 (27.0)</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>22 (53.7)</td>
<td>27 (81.8)</td>
<td>23 (71.9)</td>
<td>27 (64.3)</td>
<td>99 (66.9)</td>
<td></td>
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<tr>
<td>Un-evaluable</td>
<td>0</td>
<td>1 (3.0)</td>
<td>1 (3.1)</td>
<td>0</td>
<td>2 (1.4)</td>
<td></td>
</tr>
<tr>
<td>ORR (CR+PR)</td>
<td>1 (2.4)</td>
<td>1 (3.0)</td>
<td>2 (6.3)</td>
<td>3 (7.1)</td>
<td>7 (4.7)</td>
<td>0.741</td>
</tr>
<tr>
<td>DCR (CR+PR+SD)</td>
<td>19 (46.3)</td>
<td>5 (15.1)</td>
<td>8 (25.1)</td>
<td>15 (35.7)</td>
<td>47 (31.7)</td>
<td>0.026</td>
</tr>
</tbody>
</table>

| All patients n = 47 | n = 43 | n = 37 | n = 48 | n = 175 | | |
| CR | 0 | 0 | 0 | 0 | 0 | |
| PR | 1 (2.1) | 1 (2.3) | 2 (5.4) | 3 (6.2) | 7 (4.0) | |
| SD (at 8 weeks) | 19 (40.4) | 4 (9.3) | 5 (13.5) | 7 (18.9) | 13 (27.1) | 43 (24.6) |
| PD | 24 (51.1) | 1 (2.3) | 26 (70.3) | 29 (60.4) | 110 (62.9) | |
| Un-evaluable | 3 (6.4) | 7 (16.3) | 2 (5.4) | 3 (6.2) | 15 (8.6) | |
| ORR (CR+PR) | 1 (2.1) | 1 (2.3) | 2 (5.4) | 3 (6.2) | 7 (4.0) | 0.662 |
| DCR (CR+PR+SD) | 20 (42.5) | 5 (11.6) | 9 (24.3) | 16 (33.3) | 50 (28.6) | 0.008 |

**NOTE:** P-values were from Fisher’s exact test. Among analyzable patients, SD at 3 months was observed in 10, 4, 5, and 5 patients on arms A to D, respectively; SD at 6 months was observed in 5, 2, 3, and 2 patients on arms A to D, respectively. Abbreviations: PD, progressive disease; ORR, objective response rate; DCR, disease control rate.
Figure 3. Cytokine/chemokine produced by HTLs. From 47 patients with proliferative responses to tetanus peptide or 6MHP, 2-day (A, B) and 5-day (C, D) supernatants of PBMC from patients on arm B (A, C) or arms C+D (B, D) stimulated in vitro with 6MHP (6 MHP-0, 6 MHP+) or tetanus peptide (Tet-0 or Tet+) were assayed by Luminex for 10 cytokines and 5 chemokines, with increases in IL-2, IL-5, CXCL9, and CXCL10, shown here. Each line represents data from PBMC taken pretreatment (6 MHP-0 or Tet-0) or after 3 to 6 vaccines (6MHP + or Tet +). The median concentrations induced by PHA for IL-2, IL-5, CXCL9, and CXCL10 were 4,600, 198, 2,248, and 760 pg/mL, respectively.
tetanus peptide and to 6MHP includes specific induction of low levels of T\(_{H1}\)-associated cytokine IL-2, but also significant production of the TH2 cytokine IL-5. In prior studies, HTL responses to tetanus peptide were primarily TH1 dominant (24), as were responses to 6MHP (Dillon P and colleagues, manuscript in preparation). Regardless, it is anticipated that improved vaccine adjuvants are needed to induce T cells with stronger TH1 bias. Also, TH1-associated chemokines, CXCL9–10, implicated in T-cell homing to tumor (43, 44), were produced (Fig. 3); so if these vaccine-induced CD4\(^+\)T cells infiltrate melanoma metastases, they may be helpful in recruiting melanoma-reactive CD8\(^+\)T cells to the tumor. Notably, there was a strong association between immune response to the 6MHP and clinical response and patient survival, unlike tetanus peptide. These findings suggest a more important clinical role of HTL responses to melanoma antigens than previously appreciated.

The overall OR rate of 4.7% is consistent with prior experience with peptide vaccines (45); however, 1-year survival for all study groups exceeded prior experience for advanced melanoma in cooperative group trials (32), even after correcting for multiple risk factors. The encouraging 1-year survival data may be impacted by patient selection, by the fact that 6 untreated patients were not included, and by capping LDH in the eligibility; however, 72% of analyzable patients had M1c disease, 28% had elevated LDH, 80% had 2 or more metastatic sites, 43% had PS 1, and prior systemic therapy included immunotherapy in 44% and chemotherapy in 49%; these features may be compared with patients treated with carboplatin and taxol on the recent E2603 trial, for which 57% had M1c disease, 39% had elevated LDH, 39% had PS1, and 58% were treatment-naïve; overall median survival with carboplatin and taxol was 11.1 months (46); overall median survival for the present trial E1602 was 11.8
months. Despite modestly encouraging early survival data with these multipeptide vaccines, most patients still died of their melanoma within 2 years. Thus, continued investigation of multipeptide vaccines in patients with advanced melanoma should be in combination with other active agents. In particular, BRAF inhibitors may transiently improve antigen expression by melanoma cells and T-cell infiltration into melanoma metastases (47–49). So, combination with vaccines may be considered as an approach to improve clinical activity of both agents.

Disclosure of Potential Conflicts of Interest

C.L. Slingluff has a commercial research grant from Glaxo Smith Kline, has ownership interest (including patents) in UVA Licensing and Ventures Group (patents on peptides, through UVA), and is a consultant/advisory board member of Immatics and Polynoma. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.L. Slingluff, L.H Butterfield, T.L. Whiteside, P.D. Leming, J.M. Kirkwood
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.L. Slingluff, S. Lee, F. Zhao, L.H Butterfield
Writing, review, and/or revision of the manuscript: C.L. Slingluff, S. Lee, F. Zhao, K.A. Chianese-Bullock, W.C. Olson, L.H Butterfield, P.D. Leming, J.M. Kirkwood

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.L. Slingluff, F. Zhao, W.C. Olson, J.M. Kirkwood
Study supervision: C.L. Slingluff, J.M. Kirkwood
Providing peptides for use in the trial, from our research program at UVA: C.L. Slingluff
Development of the clinical trial protocol: K.A. Chianese-Bullock

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Table 3. Multivariable Cox landmark regression analysis for OS by immune response and clinical variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model 1: OS by CTL response to 12MP, n = 139, Arms A-D</th>
<th>Model 2: OS by helper T-cell response to 6MHP, n = 64, Arms C, D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>P value</td>
</tr>
<tr>
<td>CTL response</td>
<td>0.87</td>
<td>0.557</td>
</tr>
<tr>
<td>Helper T-cell response to 6 MHP</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Treatment</td>
<td>1.57</td>
<td>0.124</td>
</tr>
<tr>
<td>Treatment</td>
<td>1.05</td>
<td>0.863</td>
</tr>
<tr>
<td>Treatment</td>
<td>1.42</td>
<td>0.198</td>
</tr>
<tr>
<td>Treatment</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Age</td>
<td>1.02</td>
<td>0.041</td>
</tr>
<tr>
<td>Sex</td>
<td>0.92</td>
<td>0.684</td>
</tr>
<tr>
<td>ECOG performance status</td>
<td>0.95</td>
<td>0.810</td>
</tr>
<tr>
<td>Number of sites</td>
<td>1.74</td>
<td>0.033</td>
</tr>
<tr>
<td>Uterine level</td>
<td>2.66</td>
<td>0.003</td>
</tr>
<tr>
<td>Ulceration</td>
<td>0.81</td>
<td>0.390</td>
</tr>
<tr>
<td>Ulceration</td>
<td>0.75</td>
<td>0.310</td>
</tr>
<tr>
<td>Clark level</td>
<td>0.035</td>
<td>0.877</td>
</tr>
<tr>
<td>Serum LDH</td>
<td>1.06</td>
<td>0.859</td>
</tr>
<tr>
<td>Serum LDH</td>
<td>1.45</td>
<td>0.107</td>
</tr>
</tbody>
</table>

NOTE: Two models for OS are summarized in the table: Model 1 includes all patients and includes the measures of CTL response; Model 2 includes patients vaccinated with 6MHP (arms C and D) and includes measures of the immune response to 6MHP.

One patient died within 2 months and was not included in the landmark analysis for OS. Bold highlights P < 0.05.
References


A Randomized Phase II Trial of Multiepitope Vaccination with Melanoma Peptides for Cytotoxic T Cells and Helper T Cells for Patients with Metastatic Melanoma (E1602)

Craig L. Slingluff, Jr, Sandra Lee, Fengmin Zhao, et al.

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