Abstract

Purpose: A melanoma vaccine incorporating six peptides designed to induce helper T-cell responses to melanoma antigens has induced Th1-dominant CD4+ and/or CD8+ T-cell responses in most patients, and induced durable clinical responses or stable disease in 24% of evaluable patients. The present study tested whether this vaccine also induced antibody (Ab) responses to each peptide, and whether Ab responses were associated with T-cell responses and with clinical outcome.

Experimental Design: Serum samples were studied from 35 patients with stage III-IV melanomas vaccinated with 6 melanoma helper peptides (6MHP). IgG Ab responses were measured by ELISA. Associations with immune response and overall survival were assessed by log-rank test and $\chi^2$ analysis of Kaplan–Meier data.

Results: Ab responses to 6MHP were detected by week 7 in 77% of patients, and increased to peak 6 weeks after the last vaccine and persisted to 6 months. Ab responses were induced most frequently to longer peptides. Of those with T-cell responses, 82% had early Ab responses. Survival was improved for patients with early Ab response ($P = 0.0011$) or with early T-cell response ($P < 0.006$), and was best for those with both Ab and T-cell responses ($P = 0.0002$).

Conclusions: Vaccination with helper peptides induced both Ab responses and T-cell responses, associated with favorable clinical outcome. Such immune responses may predict favorable clinical outcome to guide combination immunotherapy. Further studies are warranted to understand mechanisms of interaction of these Abs, T-cell responses, and tumor control.

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Introduction

A primary goal of cancer vaccines is to elicit immune responses to cancer antigens, and thus to mediate lysis of malignant cells. Many cancer vaccines use peptide antigens, and are primarily designed to elicit CD8+ and/or CD4+ T-cell responses (1, 2). Few studies of peptide vaccines have addressed whether cancer vaccines also elicit humoral responses and whether this affects clinical outcome. A melanoma vaccine incorporating six peptides designed to induce helper T-cell responses to melanoma antigens has induced Th1-dominant CD4+ T-cell responses in 81% of patients, and induced durable clinical responses or stable disease (SD) in 24% of evaluable patients (3, 4). We hypothesized that this 6MHP vaccine may induce Ab responses to peptides in the vaccine.

Spontaneous autoantibodies are present in patients with a variety of malignancies (5); however, whether such Ab support or inhibit immune-mediated tumor control is debated (6–8). Spontaneous Ab to the cancer testis antigen NY-ESO-1 (9) has been detected in about 50% of patients with NY-ESO-1–expressing melanomas while undetectable in patients with NY-ESO-1–negative tumors and in healthy adults (10) and has been associated with tumor progression (11). Several studies have reported clinical benefits in patients with an integrated humoral (Ab) and cellular response to cancer vaccines or to CTLA4 blockade (12–14), whereas others have not identified clinical impact of Abs induced by cancer vaccines (15, 16). Vaccination of melanoma patients with the MAGE-A3 or NY-ESO-1 recombinant protein or peptides induced T-cell responses and Ab responses but clinical impact was not reported (17–19), while another study suggested overall clinical benefit in patients vaccinated with NY-ESO-1 who developed Ab responses, but control groups were not available for comparison (20). Despite the reported humoral responses in these studies, cancer vaccine research remains focused on T-cell responses, while the effects of Ab responses remain unclear.

The capacity to produce Ab to specific antigen is the primary purpose of B cells and plasma cells, and Ab may either enhance the immune response to tumor (21, 22) or promote tumor growth (7, 23). The presence of Ab to TAA may also serve as prognostic (7, 24) or diagnostic (25) biomarkers. The role of B cells in the tumor microenvironment has received new attention as active participants in the host response to TAA (26) but they also can have regulatory function (27) in the tumor microenvironment. Changes in the B-cell compartment of tumor-involved nodes have also been reported in patients with malignant disease (28, 29) indicating a role for B cells in the response to tumor. Indeed, increases in the both T and B lineage cells.
Translational Relevance

We have examined the circulating IgG antibody (Ab) response to vaccination with a mixture of 6 melanoma “helper” peptides (6MHP). Prior studies have shown that the 6MHP vaccine has clinical activity in a subset of patients. In the present study, high Ab titers were detected in most patients. Detection of Ab to one or more peptides by week 7 (end of the vaccine regimen) was associated with significantly improved patient survival. The Ab response was also associated with a helper T-cell response, and the best survival was for patients with both Ab and T-cell responses. The favorable clinical outcome associated with the Ab responses, and especially the combination of Ab and T-cell responses, suggests that the Ab responses may participate in the clinical benefit of these helper peptide vaccines.

Patients and Methods

Patients and immunization protocol

Details of clinical trial (NCT00089219) design and patients have been previously described (3, 4). Briefly, 37 eligible patients with stage IIIB to IV melanoma were administered six immunizations of a vaccine containing 6 melanoma helper peptides (6MHP) at increasing doses of 200 mcg (Arm A, 12 patients), 400 mcg (Arm B, 12 patients), or 800 mcg (Arm C, 13 patients) per vaccine. Peptides were administered in emulsions with incomplete Freund’s adjuvant (IFA, Seppic Inc) and GM-CSF (Berlex) over a 7-week period. Seventeen of these patients had measurable disease. Blood, for lymphocytes and serum, was collected prevaccine (week 0), one week after vaccines 1, 3, and 6 (weeks 1, 3, 5, and 7) and in follow-up at weeks 12 and 18 with individual peptides, where that serum was obtained from a patient on a different trial with the 6MHP vaccine and reacted with that peptide. The FORECAST function in Microsoft Excel was used to calculate the Ab titer of patients’ sera (32). The titer is defined as the reciprocal of the serum dilution that yields a fluorescence intensity ten times greater than the cutoff value. The cutoff value is defined as the average fluorescence obtained from the first four dilutions of serially diluted normal donor serum (negative control). Antibody titers less than 100 were considered negative.

ELISA method

Patient sera were evaluated by ELISA (32) for IgG antibody to each peptide in the 6MHP vaccine, or to the pool of all 6 peptides, before (pre, week 0), 1 week after 4 to 6 injections (weeks 5–7), and (for 30 of the patients) at later time points (weeks 11 or later) as well. Briefly, 96-well half-area cluster plates (Corning Costar) were coated with 30 mcL of 6MHPs (individually or pooled) diluted in carbonate/bicarbonate buffer (pH 9.4, Sigma-Aldrich) at 1.67 mcg/mL of each peptide. For quantitation of specific serum levels of anti-peptide antibody, purified IgG immunoglobulin (Fitzgerald Industries International) was prepared in coating buffer at 1 mcg/mL, serially diluted 4-fold to 0.25 ng/mL, and 30 mcL of each dilution added to duplicate wells. After incubation overnight at 4°C, plates were washed with PBS with 0.1% Tween 20 (TPBS), then blocked for 1 hour with 5% nonfat dry milk in TPBS (blocking buffer). Beginning at 1:100, 4-fold serial dilutions of patient and control sera were prepared in blocking buffer and added to individual wells. After 2 hours of incubation at room temperature (RT) and subsequent washing, secondary antibody (Goat anti-human IgG AP conjugate, Southern Biotech) was added to all wells, incubated 1 hour at RT then washed. Attophos substrate (Sigma) was added to each well for 30 minutes. After incubation, 3N NaOH was added to stop the reaction, and fluorescence recorded on a Molecular Devices SPECTRAmax Gemini EM Fluorescent plate reader, excitation 450 nm, emission 580 nm. A positive control serum was used in all assays with individual peptides, where that serum was obtained from a patient on a different trial with the 6MHP vaccine and reacted against 5 of the 6 peptides by this ELISA assay (all except MAGE-A3 and MAGE-A5). The Ab response also was associated with a helper T-cell response, and the best survival was for patients with both Ab and T-cell responses. The favorable clinical outcome associated with the Ab responses, and especially the combination of Ab and T-cell responses, suggests that the Ab responses may participate in the clinical benefit of these helper peptide vaccines.

Table 1. Antibody and T-cell responses to each peptide in the 6MHP vaccine

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Sequence</th>
<th>Number of aa</th>
<th>Ab responsea</th>
<th>T-cell responseb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosinase</td>
<td>FLLIHAFVDSIFEGWLR</td>
<td>21</td>
<td>78%</td>
<td>32%</td>
</tr>
<tr>
<td>Melan-A/MART1</td>
<td>RNGYRALMDKSLHVGTCA</td>
<td>23</td>
<td>66%</td>
<td>24%</td>
</tr>
<tr>
<td>gp100</td>
<td>WNRQLPYETWAGRL</td>
<td>16</td>
<td>41%</td>
<td>5%</td>
</tr>
<tr>
<td>Tyrosinase</td>
<td>AGNLLSNAPLGPQF</td>
<td>15</td>
<td>6%</td>
<td>5%</td>
</tr>
<tr>
<td>MAGE-A5</td>
<td>TSVYAVLMHMKSG</td>
<td>15</td>
<td>0%</td>
<td>40%</td>
</tr>
<tr>
<td>MAGE-A1,2,3</td>
<td>LLKYRARFPVKAE</td>
<td>14</td>
<td>0%</td>
<td>22%</td>
</tr>
</tbody>
</table>

aPercentage of patients with Ab response to each peptide, n = 32.

bPercentage of patients with T-cell response to each peptide, as previously reported, n = 37 (31).
negative. A standard curve of IgG concentration and fluorescent intensity was generated from data averaged across 18 plates from 5 separate assays. Upper and lower limits were established based on the lowest and highest fluorescence of IgG standard concentrations bracketing the values used to produce a polynomial curve with a correlation coefficient greater than 0.99. Anti-peptide IgG serum concentrations were extrapolated according to the polynomial expression derived from this curve.

Reactivity to individual peptides. Patients identified as having reactivity to 6MHP were evaluated further to define reactivity to each of the 6 peptides. Sera from early (≤7 weeks after first vaccine) and at the time of peak reactivity (>10 weeks) were assayed at one dilution for reactivity to each of those peptides. Wells were coated with peptide at 30 mcL at 1 mcg/mL. The ELISA assay was performed as described above, using 1:200 dilution of patient serum. Positive responses were defined as fluorescence intensity 10-fold than the mean normal donor response to the peptides.

CD4+ T-cell responses
T-cell responses have been measured by 5-day proliferation assay, as described (4).

Data analysis
Kaplan–Meier survival curves were generated with MedCalc software and utilized updated patient clinical follow-up data in the Cancer Center clinical trials office database, and previously published data on the CD4+ T-cell responses to 6MHP vaccine (4); survival curves were compared with log-rank test and $\chi^2$. Differences in Ab titer between study arms were compared using a two-tailed Student t test with equal variance. Early and late serum titers were compared using the Student t test for paired samples.

Results
Vaccination with 6MHP induces IgG antibody responses
Ab responses to the 6MHP vaccine were assessed in 30 patients both early (weeks 5–7) and late (>10 weeks). There was a 4.2-fold (mean) increase in Ab titer between early and late time points ($P = 0.0001$) in 26 of the 30 patients (Fig. 1A). Of these patients, 77% had positive Ab titers (>100) after 4 to 6 vaccines (weeks 5–7), and 87% had positive Ab titers at the later time points, more than 5 weeks after the sixth vaccine (Table 2). Ab titers increased significantly from 5 to 7 weeks into the vaccine schedule to more than 10 weeks (>5 weeks after the last vaccine) at each dose level (Fig. 1B, $P < 0.04$, paired Student t test). However, Ab titers did not differ among vaccine doses at either time point ($P > 0.9$ at weeks 5–7; $P > 0.15$ at >10 weeks; Student t test with equal variance).

Association of T-cell responses and Ab responses, within 7 weeks
The CD4+ T-cell proliferative response (stimulation index) has been reported previously (4). Here, we report also the timing of that response, as it relates to the Ab response. The overall T-cell response rate in PBMC was 57% (21/37; ref. 4). Eighty-four percent of those responses were evident by week 7 (18/37, 49%). In that study, we also assessed immune response in the vaccine-draining lymph node (sentinel immunized node, SIN) in 36 patients, which was collected at week 3. T-cell responses were detected in the SIN in 28 of 36 patients (78%), including all 3 of the patients who developed T-cell responses in the blood after week 7, plus 9 patients who did not have T-cell responses detected in PBMC. Thus, 30 of 37 patients (81%) had T-cell responses evident by week 7 in PBMC or SIN (data not shown). Of the 34 patients with Ab and T-cell data available by week 7, 28 (82%) had T-cell responses in PBMC or SIN by week 7, and 25 (74%) had Ab responses by week 5 to 7. This includes 23 with Ab and T-cell responses, 4 with neither, 2 with Ab only, and 5 with T cell only. Thus, there was substantial concordance of Ab and T-cell responses: 82% of those with T-cell responses also had Ab responses by week 7, and, conversely, 92% of those with Ab responses by week 7 also had T-cell response. This association approached significance ($P = 0.051$, $\chi^2$, MedCalc).

Quantification of serum Ab to peptide, over time
Ab titer was closely correlated with calculated concentration of anti-peptide Ab ($R^2 > 0.92$, Fig. 1C). Ab concentration increased from weeks 5 to 7 to peak at weeks 11 to 13 and was maintained at a relatively consistent level through week 25 or later (Fig. 1D). With one exception (3%), all prevaccine sera had titers less than 100 (<0.7 mcg/mL). One patient had a prevaccine titer of 141 (0.8 mcg/mL) that increased to >5,000 after 10 weeks. Similar to what was found with titer, peak peptide-specific IgG levels in serum were similar among dosage arms ($P$ values 0.14, 0.83, and 0.14 for A vs. B, B vs. C, and A vs. C, respectively; Student t test with equal variance; Table 3).

Longest peptides induced Ab responses at highest frequency
Ab responses were most frequent to the longer helper peptides in the vaccine (Table 1). The two peptides with more than 20 amino acids (FLL: tyrosinase386-406; RNG: MART-1/Melan-A51-73) induced IgG responses in 78% and 66% of the patients, respectively (Fig. 1E). The third longest peptide (WNR: gp10044-59, 16 amino acids) induced IgG responses in 41%, whereas slightly shorter peptides were much less immunogenic (0%–6%). Peptide length and Ab response rate were closely associated ($R^2 \sim 0.82$, Fig. 1E). Ab responses to individual peptides were assessed in paired serum from 23 patients at two time points (Supplementary Fig. S2), and show increases for 3 of the 4 peptides (Tyrosinase386-406 [$P < 0.0001$], MelanA/MART151-73 [$P < 0.0001$], and gp10044-59 [$P = 0.012$). Only 2 patients responded to the Tyrosinase56-70 with one showing an increase and the other patient not showing an increase over time.

Patient survival is improved in patients with both Ab and CD4+ T-cell responses to 6MHP vaccination
The study population included a range of patient presentations (stage III–IV, with or without measurable disease). Not surprisingly, patients without measurable disease had better survival than those with measurable disease ($P = 0.003$, Fig. 2A). However, patient survival was not associated with stage ($P = 0.21$, Fig. 2B), age ($P = 0.16$, Fig. 2C), or gender ($P = 0.18$, not shown). The survival curve for patients in Arm C (highest dose) is lower than that for Arms A and B, but this was not significant ($P = 0.10$, Fig. 2D). Arm C differed from Arms A+B by having more stage IV patients (77% vs. 67%), more measurable disease (54% vs. 42%), and fewer patients with performance status 0 (54% vs. 71%; ref. 4). Although Arm C may have had a lower survival curve than Arms A and B, the response rates for Ab production by week 7 were 70%, 73%, and 77% for Arms A, B
Figure 1.
Antibody response to the 6MHP vaccine mixture. A, the Ab response to 6MHP peptides, as the serum Ab titer prevaccine (week 0), early after vaccination (week 5–7), or at maximal titer time point (more than 10 weeks) plotted on a square root scale. B, Ab responses to 6MHP (plotted on a log to the base 4 scale) early versus late by study arm (Arm A $P = 0.03$, Arm B $P = 0.01$, Arm C $P = 0.003$). C, Ab responses were defined by titer and by serum concentration, and these measures were closely correlated ($R^2 = 0.92$). D, serum Ab concentrations measured through week 25, plotted on a square root scale, with box plots (each box 25th to 75th percentiles; vertical lines define maximum and minimum; horizontal lines represent median values). E, % of patients with detectable Ab to each peptide (graphed by peptide amino acid length).
and C, respectively. On the other hand, immune responses to peptides in the vaccine were associated with better survival: this was true for early (by week 5–7) Ab response ($P = 0.0011$, Fig. 3A, median survival 6.6 vs. 1.2 years) and for T-cell response [ref. (4); $P < 0.006$, Fig. 3B, median survival 5.0 vs. 1.2 years]. Survival was best for those with both Ab and T-cell responses by week 7 ($n = 23$, median 6.6 years), less for those with only Ab or T-cell responses ($n = 7$, median 1.3 years), and least for those with neither ($n = 4$, median 0.8 years; $P = 0.001$ across the 3 groups, Fig. 3C). Similarly, survival was better for those with both Ab and T-cell responses compared with all others ($P = 0.0002$, Fig. 3D).

Even when accounting for disease status, the association of survival with combined Ab and T-cell immune response was supported. The associations of immune responses and survival could not be ascribed to the immune responses to any single peptide (data not shown) but were associated with responses to the 6MHP mixture. Early Ab responses were detected in 15 of 17 patients without evidence of melanoma (88%) and in 10 of 16 patients with measurable disease (63%); this difference is not significant ($P = 0.19$, $\chi^2$). Among the subset of patients with measurable disease, there was better survival with early Ab response ($P = 0.03$). Among the subset without measurable

Table 2. Antibody responses as function of study arm and time on study

<table>
<thead>
<tr>
<th>Arm</th>
<th>A</th>
<th>B</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide vaccine dose</td>
<td>200 mcg</td>
<td>400 mcg</td>
<td>800 mcg</td>
</tr>
<tr>
<td>Evaluated patients</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Weeks after 1st vaccine</td>
<td>5–7</td>
<td>&gt;10</td>
<td>5–7</td>
</tr>
<tr>
<td>Number of responders</td>
<td>7</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Response rate</td>
<td>70%</td>
<td>90%</td>
<td>70%</td>
</tr>
</tbody>
</table>

Figure 2.

Associations of clinical factors with patient survival. Kaplan–Meier curves represent overall survival of patients with clinical features (A) disease status: measurable disease versus no evidence of disease (NED, $P = 0.003$); B, AJCC stage (III vs. IV, $P = 0.21$); C, age (<60 vs. ≥60, $P = 0.16$); and (D) study arm ($P = 0.10$).
Figure 3. Associations of immune response with patient survival. Kaplan–Meier curves represent overall survival of patients with immune response findings: A, antibody response by week 7 (P = 0.0011); B, T-cell response in PBMC or SIN by week 7 (P < 0.006); C and D, combined Ab and/or T-cell responses (P = 0.001 and P = 0.0002, respectively); E, combined Ab plus T-cell response for patients with measurable disease (P = 0.033); F, combined Ab plus T-cell response for patients with no evidence of disease (NED, P = 0.015).
disease, only 2 patients failed to generate early Ab responses and they had poor outcomes, but there were too few patients in that group for a meaningful statistical comparison. However, a combined Ab + T-cell immune response by week 7 was associated with improved survival for those with measurable disease \((P = 0.033, \text{Fig. 3E, median 3.0 vs. 6.0 years})\), and for those with no evidence of disease, \((P = 0.015, \text{Fig. 3F, median 7.1 vs. 1.3 years})\).

Among the patients on this trial with measurable disease, two (12%) experienced objective partial responses (PR), and two others (12%) experienced durable SD; all 4 of these PRs and SD were durable, for 1 to 7 years (4). Ab responses were detected by week 7 in all 4 of those patients, and CD4+ T-cell responses were also observed in all 4 of these patients. Thus, combined Ab and T-cell responses were associated both with improved survival and with objective clinical responses.

**Discussion**

Peptide vaccines have been designed to stimulate T-cell responses to defined cancer antigens, either using short peptides restricted by Class I MHC molecules to stimulate CD8+ T-cells, or longer peptides restricted by Class II MHC molecules to stimulate CD4+ T-cells. Both approaches have induced T-cell responses, but optimal vaccine regimens remain to be defined. Most peptides used in cancer vaccines, including those in the present study, are from intracellular proteins; so Ab to the peptides are not expected to bind to viable tumor cells and thus have not been the focus of immune monitoring of cancer vaccines. However, Ab induced by vaccines could have implications for vaccine immunogenicity or for tumor control. On one hand, Ab could neutralize peptides and reduce T-cell responses on repeat immunization. On the other hand, Ab could opsonize peptides in immune complexes, which could increase uptake by dendritic cells, limit peptide degradation, and enhance T-cell responses. Also, Ab may opsonize intracellular melanocytic proteins after tumor cell death, to support cross-presentation of proteins released by dying tumor cells. Thus, in the present study, we have assessed IgG Ab responses to each of the 6 peptides in this vaccine, and have evaluated associations with T-cell responses and patient survival.

We have found that a vaccine incorporating six HLA-DR restricted peptides derived from melanocytic differentiation proteins (MDP) and cancer-testis antigens (CTA) induced IgG humoral immune responses in melanoma patients in addition to CD4+ T-cell responses. Anti-peptide IgG Ab were detected 5 to 7 weeks after the first vaccine (in 77% of patients), peaked about 6 weeks after the last vaccine to a maximum Ab response rate of 87%, and were longlasting, persisting to 6 months. The Ab responses were of high magnitude (median 56 mcg/mL, mean 121 mcg/mL; Table 3). There was no significant difference in Ab response rate or magnitude by vaccine dose; however, there were marked differences in immunogenicity by peptide. Tyros\textsubscript{386-406} was immunogenic in 76% of patients.

The next most immunogenic was MelanA/MART-1\textsubscript{151-173}. Overall, Ab responses were greatest for the longest peptides (>20 amino acids), and also for peptides of melanocytic proteins. Other studies have shown that long (25–30 amino acids) peptides from the cancer-testis antigen NY-ESO-1 induce strong Ab responses (19, 33). Also a shorter (14 amino acid) NY-ESO-1 peptide converted 2 seronegative patients to seropositive after 5 to 9 months, but Ab was not detected at early time points. We conclude that early immunogenicity may depend in part on peptide length, but that peptides from both melanocytic antigens and cancer-testis antigens can induce Ab responses.

Ab was detected as early as 5 weeks in some patients, but was not detected at 3 weeks \((n = 9)\). In prior analyses of this trial (4), CD4+ T-cell responses were detected at 3 weeks in the sentinel immunized lymph node in 78% of patients evaluated. The Ab responses persisted without apparent change to 6 months or later (Fig. 1D). T-cell responses peaked at week 7 then declined slightly but were still detected at 9 months (4). Thus, Ab responses have appeared later than the T-cell responses but both commonly persisted, and overlapped temporally. Interestingly, the most immunodominant peptides for CD4+ T-cell responses were MAGE-A\textsubscript{3281-295} and Tyros\textsubscript{386-406} with responses in 49% and 32% of patients, respectively, and there was no association with peptide length (31). The Tyros\textsubscript{386-406} peptide is highly immunogenic for Ab as well as for T-cells, whereas the MAGE-A\textsubscript{3281-295} peptide is immunogenic only for T-cells (Table 1). MART-1/MelanA\textsubscript{51-73} was also immunogenic for T-cells and for Ab. These data reveal that Ab and T-cells may respond to the same or to different peptides. Because Ab responses occurred to two peptides that were also highly immunogenic for CD4+ T-cells, it appears unlikely that the Ab interferes with induction or persistence of CD4+ T-cell responses.

Early Ab response was associated with improved patient survival, as was CD4+ T-cell response to the peptides. The best survival overall is for those who had both early Ab response and T-cell response, with median survival of 6.6 years. Even among those with advanced measurable disease at study entry, median survival with both Ab and T-cell responses exceeded 3 years. There was an association between Ab responses and CD4+ T-cell responses, but some patients had only Ab responses, and others had only T-cell responses. As shown in Fig. 3C, the 7 patients with only Ab, or only T-cell response had less favorable survival than those with both. Other studies have shown that vaccination with NY-ESO-1, MAGE-A3 peptides, or protein induces integraded Ab and T-cell responses (12, 15–20). In two of those studies, there was a suggestion of clinical activity of vaccines that induce Ab and T-cell responses (12, 20). Also, Ab response to surface or secreted protein vaccines (Her2 or β-hCG) in epithelial cancer patients has been associated with prolonged survival (34). CTLA4 blockade has increased Ab responses to intracellular cancer antigens, with some evidence for association of Ab response and clinical activity (13, 14). Interestingly, Ab responses also arise to cancer antigens spontaneously, with data suggesting that they may support anti-tumor immunity or may interfere with it (5, 7, 24). In another study with the 6MHP vaccine, we have found that CD4+ T-cell responses are associated with improved survival (35). The present work is the first to show statistically better survival for patients with Ab responses, and with combined Ab and CD4+ T-cell responses after vaccination with peptides from intracellular proteins.

| Table 3. Peak concentrations of IgG-Ab to 6MHP vaccine (mcg/mL) |
|------------------|------------------|--------|----------|
| Patients evaluated | Arm A | Arm B | Arm C | Overall |
| Mean (mcg/mL)     | 10    | 10    | 10    | 50      |
| Minimum-maximum (mcg/mL) | 0.9-678 | 0.3-200 | 0.6-209 | 0.3-678 |
| Median (mcg/mL)   | 54    | 15    | 60    | 56      |

Melanoma Helper Peptides Vaccine Induces Antibody Responses

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These novel findings suggest that Ab responses to a peptide vaccine may have significant prognostic value, especially when combined with T-cell response data. Thus, it may be possible to identify patients early for whom benefit of the vaccine approach is unlikely, and for whom alternate therapy may be considered. The association of Ab with improved patient survival raises questions about potential mechanisms for that finding. Melanoma antigens represented in the vaccine are intracellular proteins; thus, antibodies to the peptides would not likely be involved in direct killing of tumor cells by antibody-dependent cellular cytotoxicity or complement-mediated cytotoxicity. However, the Abs we have detected may form immune complexes with the peptides, supporting their uptake by dendritic cells. The vaccines may also bind the source proteins released from dying melanoma cells. As proteins are released into this environment and Ab is present, immune complexes (ICs) may form and be taken up by dendritic cells (DC, reviewed in ref. 36, 37). ICs augment cellular immune responses by activating DC, even in the absence of CD4+ T-cells (38), and cross-presentation is facilitated through Fc receptors and signaling pathways (39, 40). Opsonization of antigen can also lead to increases in DC migration from peripheral tissues to the draining lymph nodes (41). Alternatively, Ab may neutralize peptide, or excess IC can interfere with antigen uptake and presentation (42). However, in light of the positive association with clinical outcome, we hypothesize that in this setting, the Abs are supporting antitumor immunity. Future studies are planned to test this hypothesis more directly, to characterize the IgG subtypes of Ab induced by peptides, and to identify vaccine adjuvants that are most effective at inducing Ab that support antitumor immunity.

Disclosure of Potential Conflicts of Interest

C.L. Slingluff reports receiving a commercial research grant from GlaxoSmithKline and is a consultant/advisory board member for Immatics and Polynoma. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: C.L. Slingluff
Development of methodology: N.D. Cresce, C.L. Slingluff, W.C. Olson
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.M. Reed, N.D. Cresce, C.L. Slingluff, W.C. Olson
Writing, review, and/or revision of the manuscript: C.M. Reed, N.D. Cresce, I.S. Mauldin, C.L. Slingluff, W.C. Olson
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.M. Reed, N.D. Cresce, I.S. Mauldin, C.L. Slingluff, W.C. Olson
Study supervision: C.L. Slingluff, W.C. Olson

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References

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immune cells are associated with clinical outcome in metastatic melano-

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Vaccination with Melanoma Helper Peptides Induces Antibody Responses Associated with Improved Overall Survival

Caroline M. Reed, Nicole D. Cresce, Ileana S. Mauldin, et al.


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