Immunization Using Autologous Dendritic Cells Pulsed with the Melanoma-Associated Antigen gp100-Derived G280-9V Peptide Elicits CD8+ Immunity


Abstract

**Purpose:** To determine the toxicity, maximal tolerated dose, and clinical and immunologic response to autologous dendritic cells pulsed with melanoma-associated antigen gp100-derived G280-9V peptide.

**Patients and Methods:** Twelve HLA-A*0201+ patients with advanced melanoma were administered dendritic cells pulsed with G280-9V peptide. Cohorts of three patients were administered 5 × 10^6, 15 × 10^6, and 50 × 10^6 cells i.v. every 3 weeks according to a dose escalation scheme. Three additional patients were treated at the highest dose. No additional cytokines or therapies were coadministered. The immunogenicity of G280-9V-pulsed dendritic cells was measured by IFN-γ ELISPOT assay, tetramer assay, and ^51Cr release assay comparing prevaccination to postvaccination blood samples. Response to treatment was assessed by Response Evaluation Criteria in Solid Tumors.

**Results:** CD8+ immunity to the native G280 was observed in 8 (67%) patients as measured by ELISPOT and in 12 (100%) patients as measured by tetramer assay. Of the 9 patients tested, 9 (100%) had measurable high-avidity CTL activity as defined by lysis of allogeneic melanoma lines, which coexpress HLA-A*0201 and gp100. The median follow-up of the entire cohort is 43.8 months. Two (17%) partial responses were observed and 3 (25%) patients had stable disease. The median survival of the treated population was 37.6 months. At this time, three patients are alive, including one patient who continues to respond without additional treatment.

**Conclusion:** The high rate of immunization as measured by three independent assays and the occurrence of clinical regression support continued investigation of G280-9V peptide as a candidate epitope in melanoma vaccine formulations.

Melanoma incites an immune response by the host, and a variety of strategies have been pursued in an attempt to take therapeutic advantage of this observation (1). Some of these strategies involve nonspecific attempts to augment antitumor immunity, such as the cytokines interleukin (IL)-2 and IFN-α2b (2, 3). These are now accepted conventional therapies. However, most recent investigational work has been focused on attempts to induce specific antitumor immunity by engendering responses to defined single or multiple antigens expressed by tumor cells. The central line of this research has been the identification and isolation of the antigens present on melanomas recognized by T cells (4).

The isolation of melanoma-associated antigens has allowed investigators to design peptide vaccines that can be coadministered with a variety of adjuvants (4, 5). Dendritic cells serve a critical role in capturing and presenting antigen in the initiation of immunity against infectious pathogens and neoplasia (6). A variety of technical advances in isolating human dendritic cells as well as an improved understanding of dendritic cell biology have led investigators to propose using autologous dendritic cells as adjuvant for peptide vaccination in cancer (7, 8).

The initial clinical reports conclude that dendritic cell immunization in patients with advanced melanoma is safe and well tolerated; however, response rates are <10% in most studies (9 – 11). Laboratory correlative studies show that some patients develop T-cell immunity after dendritic cell vaccination with a variety of schedules and routes of administration.
Most in vitro studies conclude that immature dendritic cells are optimal at antigen capture and processing, whereas mature dendritic cells are best at presenting antigen to elicit immunity (15). Limited studies in human dendritic cell vaccine trials tend to support this conclusion, but additional controlled trials are needed to more carefully address this issue (16, 17).

We report the results of a phase I study of peptide-pulsed dendritic cells for patients with advanced melanoma. In the current study, monocyte-derived dendritic cells are loaded with soluble peptide antigen ex vivo and irradiated before reinfusion through an i.v. route every 21 days for six doses. We find immunization using G280-9V-pulsed dendritic cells elicited melanoma-reactive CD8+ T cells specific for the native (G280) epitope in all 12 patients as judged by ELISPOT assays, tetramer analysis, and cytolytic assays using peripheral blood obtained from longitudinal samples from each patient.

### Patients and Methods

#### Study design

Adult patients with histologically proven regional (unresectable stage III) or metastatic (unresectable stage IV) cutaneous melanoma were eligible. All subjects were HLA-A*0201+, had Eastern Cooperative Oncology Group performance status of 0 or 1, had gp100+ biopsy-proven melanoma metastases confirmed by immunohistochemistry using the monoclonal antibody HMB45, had no evidence of autoimmune disorder, and were >4 weeks from any therapy with corticosteroids, chemotherapy, radiotherapy, immunotherapy, or despite positive HBV serology, or were pregnant or nursing mothers. A negative pregnancy test was required from females of childbearing age.

All patients signed a valid informed consent for treatment on a phase I protocol reviewed and approved by the Dana-Farber Cancer Institute Human Protection Committee in accord with an assurance filed with the Department of Health and Human Services numbered as WA0001121. The Food and Drug Administration approved procedures for dendritic cell preparation and administration according to investigational new drug application IND 7847.

### Preparation of dendritic cell vaccine

Patients underwent leukapheresis according to standard procedures. For the first immunization, fresh peripheral blood mononuclear cells (PBMC) were initiated into culture for generation of dendritic cells. For subsequent use, the remaining cells were aliquoted for single use according to dose level and cryopreserved in RPMI 1640 with 15% autologous plasma and 10% DMSO. Cryopreserved cells were thawed in sterile PBS containing 1.25% human serum albumin (Bayer 684-20, Research Triangle Park, NC), 40 units/ml heparin (Elkins-Sinn, Saint Davids, PA), and 10 μg/ml DNase I grade II (Boehringer Mannheim, Germany). After washing, cell viability was determined by trypsin blue exclusion.

Dendritic cells were prepared as described (18). PBMC suspended in RPMI 1640 with 5% autologous plasma at 5 × 10⁶ cells/mL were dispersed into T75 culture flasks. After 1-hour culture at 37°C in 5% CO₂, nonadherent cells were removed by three washes with PBS. Adherent cells were then cultured in RPMI 1640 with 1% autologous xplasma/25 mmol/L HEPES/glutamine supplemented with granulocyte macrophage colony-stimulating factor at 100 ng/ml (Immunex, Seattle, WA) and IL-4 at 20 ng/mL. Fresh medium containing granulocyte macrophage colony-stimulating factor (GM-CSF) was added every 2–3 days. After 7 days, cells were harvested by centrifugation, washed, and cryopreserved.

#### Table 1. Details of subject characteristics, dose, and clinical responses

<table>
<thead>
<tr>
<th>ID</th>
<th>Age*</th>
<th>Sex</th>
<th>Stage</th>
<th>Disease sites</th>
<th>Prior therapy</th>
<th>Dose level</th>
<th>Total dendritic cell dose</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>72</td>
<td>F</td>
<td>IV</td>
<td>Tissues, mesentery</td>
<td>Surgery</td>
<td>1</td>
<td>30 × 10⁶</td>
<td>SD</td>
</tr>
<tr>
<td>102</td>
<td>49</td>
<td>F</td>
<td>IV</td>
<td>Distant lymph nodes</td>
<td>Surgery, IFN</td>
<td>1</td>
<td>30 × 10⁶</td>
<td>SD Now NED</td>
</tr>
<tr>
<td>103</td>
<td>55</td>
<td>M</td>
<td>IV</td>
<td>Soft tissue, lung, mesentery</td>
<td>Surgery, XRT, chemotherapy</td>
<td>1</td>
<td>30 × 10⁶</td>
<td>Twice³ PR; then PD</td>
</tr>
<tr>
<td>201</td>
<td>69</td>
<td>M</td>
<td>IV</td>
<td>Lymph node, liver</td>
<td>Surgery, XRT</td>
<td>2</td>
<td>90 × 10⁶</td>
<td>PD</td>
</tr>
<tr>
<td>202</td>
<td>51</td>
<td>F</td>
<td>IV</td>
<td>Skin, soft tissue, adrenal</td>
<td>Surgery, IFN</td>
<td>2</td>
<td>90 × 10⁶</td>
<td>PD</td>
</tr>
<tr>
<td>203</td>
<td>29</td>
<td>F</td>
<td>III</td>
<td>Skin</td>
<td>Surgery, IFN</td>
<td>2</td>
<td>90 × 10⁶</td>
<td>PD now NED</td>
</tr>
<tr>
<td>301</td>
<td>67</td>
<td>M</td>
<td>IV</td>
<td>Lung</td>
<td>Surgery, XRT</td>
<td>3</td>
<td>250 × 10⁶</td>
<td>PD</td>
</tr>
<tr>
<td>302</td>
<td>75</td>
<td>M</td>
<td>III</td>
<td>Skin</td>
<td>Surgery, IFN</td>
<td>3</td>
<td>300 × 10⁶</td>
<td>PR</td>
</tr>
<tr>
<td>303</td>
<td>59</td>
<td>M</td>
<td>IV</td>
<td>Skin, lung, liver, brain</td>
<td>Surgery, XRT</td>
<td>3</td>
<td>300 × 10⁶</td>
<td>PD</td>
</tr>
<tr>
<td>304</td>
<td>71</td>
<td>M</td>
<td>IV</td>
<td>Liver</td>
<td>None</td>
<td>3</td>
<td>300 × 10⁶, 500 × 10⁶</td>
<td>SD; then PR then PD</td>
</tr>
<tr>
<td>305</td>
<td>60</td>
<td>M</td>
<td>IV</td>
<td>Abdominal mass</td>
<td>Surgery, IFN</td>
<td>3</td>
<td>300 × 10⁶</td>
<td>PD</td>
</tr>
<tr>
<td>306</td>
<td>50</td>
<td>F</td>
<td>IV</td>
<td>Lung</td>
<td>Surgery, chemotherapy</td>
<td>3</td>
<td>200 × 10⁶</td>
<td>PD</td>
</tr>
</tbody>
</table>

Abbreviations: PD, progressive disease; SD, stable disease; PR, partial response; NED, no evidence of disease; AWD, alive with disease; XRT, radiation therapy.

*Median age, 59.5; range, 29–75.

†Five men; seven women.

‡Dose level 1: 10 × 10⁶; 2.5 × 10⁶; 3.5 × 10⁶.

§Subject 103 experienced a PR, underwent a second course of therapy, and then progressed.

‖Subject 304 showed stable disease after six treatments but went on to experience a partial response 4.5 months after the last treatment without additional therapy.
factor and IL-4 was added every 2 to 3 days. On day 5 of culture, an aliquot (2 \times 10^6) of cells were removed for phenotypic analysis and microbiological studies. On day 7, dendritic cells were harvested, washed twice in serum free medium, and resuspended to 2 \times 10^6 cells/mL. Generally, an average yield of 4% to 8% dendritic cells was achieved from PBMC. The dendritic cells were cultured for multiple dendritic cell markers and met lot release criteria as shown in Table 2. Phenotyping was done on aliquots of cultured dendritic cells before pulsing. They were CD86^+, CD80^low, CD83^low, and HLA-DR/CD11c double positive (see Results).

Dendritic cells were resuspended in X Vivo 15 medium (BioWhittaker, Walkersville, MD) supplemented with recombinant human granulocyte macrophage colony-stimulating factor (100 units/mL) and IL-4 (20 ng/mL) at 2 \times 10^6 cells/mL. Generally, an average yield of 4% to 8% dendritic cells was achieved from PBMC. The dendritic cells were cultured for multiple dendritic cell markers and met lot release criteria as shown in Table 2. Phenotyping was done on aliquots of cultured dendritic cells before pulsing. They were CD86^+, CD80^low, CD83^low, and HLA-DR/CD11c double positive (see Results).

**Peptides.** HLA-A*0201-associated gp100: 280-288 (G280) peptide (YLEPGPVTA) with modification at the terminal anchor residue (position 9) called G280-9V (YLEPGPVTV) was used in this study. GMP-grade lyophilographed peptide with >99% purity by high-performance liquid chromatography was purchased from Bachem AG (Bubendorf, Switzerland). The peptide for vaccination was dissolved in 5% DMSO in sterile water at 2 mg/mL followed by 0.2 μm filtration. The peptide was divided into aliquots, vialed for single use, and frozen at -80°C. The peptide was tested for sterility, biological activity by T2 target cell assay, and stability before pulsing dendritic cells (data not shown).

**Immunologic monitoring**

**ELISPOT assay.** ELISPOT was done against the immunogen G280-9V (the anchor modified), native G280 (the relevant target expressed on melanoma), and HIV-gag (SYLNTVATL) peptide (control background) in batch to minimize interassay variation. Briefly, 96-well plates (MAHAS4510, Millipore, Bedford, MA) were coated with 100 μl anti-IFN-γ antibody (10 μg/mL, 1-D1k, Mabtech, Mariemont, OH) overnight at 4°C. After six washings with PBS, the plates were blockaded with 150 μL/well RPMI 1640 with 5% human AB serum (Valley Biomedical, Inc., Winchester, VA) at 37°C for 2 hours. After removal of the blocking medium, thawed PBMCs were incubated at 2 \times 10^5 PBMCs per well in triplicate in a total of 100 μL RPMI 1640 with 5% human AB serum with each of the peptides G280 and G280-9V at 20 μg/mL for 24 hours. Plates were washed thrice with 0.01% Tween 20 in PBS. Next, biotinylated anti-IFN-γ antibody (100 μL/well, 1 μg/mL, 7-B6-1, Mabtech) in PBS with 0.5% bovine serum albumin (Fisher Biotech, Fair Lawn, NJ) was added. After incubation at room temperature for 2 hours, plates were washed thrice with each of 0.01% Tween 20 in PBS and PBS alone. Next, streptavidin-APL (100 μL/well; 1:1,000 dilution in PBS) was added and incubated at room temperature for 1 hour. After four washings with PBS, 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium substrate solution (Bio-Rad, Hercules, CA) was added. The plates were incubated at room temperature in the dark to develop. The colorimetric reaction was then stopped by washing with running water. Plates were allowed to dry, and the number of spots was read on an ELISPOT reader (Autoimmune Diagnostika GmbH, Strassberg, Germany). Values are corrected by subtracting background spots caused by PBMC incubation with control peptide HIV-gag. Phthalamagglutinin-positive controls were employed and in all cases yielded >300 spots per well.

To define an ELISPOT response, we determined the mean number of spots at baseline for patients and the between-patient SD. A positive result was designated as an elevation of 2 SDs above baseline. Data from our laboratory (data not shown) have shown that in patients, using the average of duplicate values, the mean and SD were 1.9 ± 1.6 for the G209 peptide in 17 patients and 2.6 ± 2.7 for the G209-2M peptide in 15 patients in an assay using the same conditions as reported here. For this study (see Results), the mean and SD were 6 ± 6.8 spots for G280 and 4.4 ± 3.4 spots for G280-9V. Thus, a positive response to G280 was an increment of 14 spots over baseline. For G280-9V, a positive result was an increment of 7 spots over baseline.

**Tetramer assay.** PBMCs (1 \times 10^6) were washed with 1% fetal bovine serum/PBS and followed by incubation with antigen-presenting cell–labeled G280:HLA-A*0201 tetrarmers and phycoerythrin-labeled anti-human CD8 antibody (Pharmingen, San Diego, CA) on ice for 30 minutes. In some experiments, G280-9V:HLA-A*0201 tetrarmers were used. After washing twice with 1% fetal bovine serum/PBS, cells were fixed by 2% paraformaldehyde in PBS and analyzed by a FACSCalibur with CellQuest (BD Biosciences, San Jose, CA). HIV-gag peptide:HLA-A*0201 tetramer (Beckman Coulter Immunomics, San Diego, CA) was used as background control. Data are presented as the percentage of CD8^+ cells positive for tetramer binding based on gating variables set with appropriate isotype control nonbinding antibodies. The optimal concentrations of CD8-phycoerythrin and antigen-presenting cell–labeled G280:HLA-A*0201 tetrarmers with temperature and time of reaction were determined in preliminary experiments.

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**Table 2. Dendritic cell phenotype**

<table>
<thead>
<tr>
<th>Mean percentage of positive cells by two-color fluorescence-activated cell sorting (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DR</td>
</tr>
<tr>
<td>CD11c</td>
</tr>
<tr>
<td>HLA-DR/CD11c</td>
</tr>
<tr>
<td>CD80</td>
</tr>
<tr>
<td>CD86</td>
</tr>
<tr>
<td>CD80/CD86</td>
</tr>
<tr>
<td>CD14</td>
</tr>
<tr>
<td>CD11c</td>
</tr>
<tr>
<td>CD14/CD11c</td>
</tr>
<tr>
<td>CD3</td>
</tr>
<tr>
<td>CD19</td>
</tr>
<tr>
<td>CD3/CD19</td>
</tr>
</tbody>
</table>

**NOTE:** Release criteria: HLA-DR, >75%; CD11c, >75%; CD86, >75%; CD14, <60%.
The mean ± SD staining at baseline was 0.1 ± 0.04%. As for ELISPOT, a positive result was designated at 2 SDs above baseline or results greater than 0.18%.

**Cytolytic assay.** Purified CD8+ T cells were obtained by negative selection (20). Autologous dendritic cells pulsed with 10 μg/mL peptide were added to purified CD8+ cells at a 1:20 ratio and cultured in 48-well trays (Costar 3548, Cambridge, MA) in RPMI 1640 containing 10% autologous plasma. IL-2 (50 units/mL, Chiron, Emeryville, CA) was added to each well beginning on day 1 and every 2 to 3 days thereafter. T cells were harvested on day 7 and assayed for lytic activity in triplicate using a standard 4-hour 51Cr release assay. T2 (TAP1-deficient, A*0201+ ) cells, DM6 (A*0201+ and gp100 ), DM13 (A*0201+ and gp100 ), and A375 (A*0201+ and gp100 ) cells were used as target cells in lysis assays as described.

**Statistical methods.** The Kaplan-Meier plot was used to present the survival curve of the 12 patients. The Kruskal-Wallis test was used to test differences in the three dose groups with respect to the three immunologic end points. The immunologic end points were defined as the difference between prevaccine and postvaccine readings. In addition, univariate analysis of the signed rank test on the three immunologic end points was done.

**Results**

**Patient characteristics.** From October 1998 to April 2001, we enrolled 12 patients evaluable for clinical and immunologic responses. Fourteen additional patients were deemed ineligible as a consequence of being typed HLA-A2+. Two patients enrolled had unsectable recurrent stage III melanoma and 10 patients had stage IV disease (see Table 1). Five patients had prior adjuvant IFN, whereas two patients had received prior chemotherapy for metastatic melanoma. Two patients had prior brain metastases controlled with radiation therapy and were deemed stable before study entry.

**Feasibility.** The production of dendritic cells for therapeutic administration was feasible and reproducible. The mean yield of dendritic cells from initial PBMC was 6.77% (range, 2.62-17.75%). No dendritic cell lot failed release criteria. Dendritic cell phenotypes as determined by two-color flow cytometry are listed in Table 2.

**Safety.** The overall toxicities of the G280-9V-pulsed dendritic cell vaccine were mild. Five patients reported rashes during the study. Two of these were grade 1 rashes possibly related to treatment. Two of them were grade 1 and related to contrast administration. One was grade 2 contact dermatitis and unrelated to treatment. Four patients reported upper respiratory or flu-like symptoms; all were grade 1. There were three reports of constitutional symptoms, nausea or vomiting. All were grade 1 in intensity. Generally, the vaccine was well tolerated, and no serious adverse events related to therapy were reported.

**Efficacy.** Using Response Evaluation Criteria in Solid Tumors, patients were evaluated by computed tomography imaging after three vaccinations and again after six vaccinations. Two patients (103 and 302) experienced a partial response after six vaccination cycles. One of these patients (103) had tumor in multiple soft-tissue sites and had already experienced a prior complete response to whole-brain radiation therapy as well as a partial response to prior chemotherapy, whereas the other patient (302) had disease confined to the skin of the scalp that responded after treatment. Three patients (101, 102, and 304) had stable disease after completion of the study. Patient 304 experienced stable disease of a solitary (biopsy-proven) liver metastasis after study but went on to show a near-complete tumor response with additional follow-up in the absence of further treatment and remains alive with disease. Patient 101 had stable disease but ultimately experienced disease progression of pulmonary nodules 11 months after completion of therapy. Patient 102 had stage IV disease with extensive i.p. adenopathy but is currently alive with disease after additional treatment with systemic biochemotherapy and i.p. chemotheray for mesenteric disease.

The remaining seven patients had documented disease progression. Interestingly, patient 203 had extensive in-transit stage III disease at entry and had a vigorous CTL response as measured by ELISPOT, tetramer, and lysis assays and progressed to stage IV disease involving distant skin. After further investigational therapy with a farnesyl transferase inhibitor (no response) followed by isolated limb perfusion (complete response), the patient is disease free. Remarkably, the latter procedure prompted regression of the regional metastases as well as a distant metastatic lesion on the contralateral (nonperfused) limb.

The median follow-up of the entire cohort is 43.8 months and the median survival of the entire population is 37.6 months. The median survival of the 10 stage IV patients is 21.1 months. Three patients are still alive; two of them having undergone additional treatments as described above. Figure 1 illustrates the overall survival in a Kaplan-Meier plot.

**Immunologic response.** The ELISPOT (IFN-γ) assay was used to measure the frequency of cells reactive to the native G280 peptide as well as the anchor-modified peptide G280-9V used for immunization. Baseline (preimmunization) values were calculated, and the mean and SD for the population were determined. Positive values were those >2 SDs above the pretreatment mean. As shown in Fig. 2A, 8 (67%) patients responded to G280 immunization with a significant increase in ELISPOT number. Figure 2B illustrates the response to the immunizing peptide, G280-9V; 8 (67%) patients responded to the modified peptide. Two patients (202 and 305) show no detectable immunity to either peptide. Two additional patients (103 and 302) mounted weak yet significant responses to G280-9V without significant responses to the native peptide.

To evaluate the frequency of cells specific for the G280:HLA-A*0201 complex, tetramer analysis was done on PBMC from

![Fig. 1. Kaplan–Meier plot of survival of study population.](image-url)
12 patients after three vaccinations and after the final (sixth) vaccination. Baseline values were determined as above. As shown in Fig. 2C, all 12 immunized patients showed significant increases in CD8+ cells specific for G280:HLA-A*0201 3 weeks after the third and sixth immunizations. Peripheral blood samples from patient 306 were tested at baseline and after the third immunization only.

Standard 51Cr release assays were done and repeated at least once to measure the lytic activity of CD8+ effector T cells from blood samples obtained at baseline and after study completion. Purified CD3+CD8+ cells from peripheral blood were cultured for 7 days with G280-9V-pulsed dendritic cells and tested using T2 target cells or allogeneic melanoma cell lines. All nine patients studied (304, 305, and 306 were not evaluated due to insufficient PBMC) had measurable immunity induced by dendritic cell immunization. In Fig. 3A, CD8+ T cells from each donor were tested from prevaccination and postvaccination blood samples using T2 cells pulsed with the G280-9V peptide and all nine patients had detectable response. In parallel cultures, all nine patients
had detectable immunity against G280 peptide-pulsed T2 cells elicited by dendritic cell immunization (Fig. 3B). Moreover, dendritic cell vaccination elicited CTL that lysed DM6 melanoma coexpressing gp100 and HLA*0201 (Fig. 3C). As a control, CD8\(^+\) T cells were cocultured with influenza A matrix (M1) peptide and tested against T2 cells pulsed with the M1 peptide. Both prevaccination and postvaccination blood samples contained influenza A–specific CTL, suggesting that all patients were immunocompetent at study enrollment and study conclusion based on reactivity to M1 peptide (Fig. 3D)

Cultured CD8\(^+\) T cells from all donors were tested for natural killer cell activity against K562 target cells and were nonreactive (data not shown).

Patients 103 and 203 were studied in greater detail to confirm the above findings and prove antigen specificity. In separate experiments, CD8\(^+\) T cells from both donors were stimulated in vitro for 7 days with G280-9V-pulsed dendritic cells and tested at various E:T ratios using either T2 target cells or additional melanoma cell lines. Postvaccination CTL from each donor lysed T2 target cells pulsed with the G280 native peptide as well as the G280-9V-pulsed targets (data not shown). In a second test of specificity, both donor CTL efficiently lysed DM6 and DM13 (gp100\(^+\), A*0201\(^+\)) melanomas; however, the A375 (gp100\(^+\), A*0201\(^+\)) melanoma target is not recognized by CTL supporting the requirement for naturally processed gp100 antigen. Postvaccination CTL from both donors can effectively kill G280-pulsed A375 melanoma cells showing reconstitution with exogenous peptide. As a control, prevaccination and postvaccination CD8\(^+\) T cells specific for influenza A M1 peptide are detected from each patient.

As a final test of antigen specificity, CD8\(^+\) T cells from 12-day in vitro cultures from postvaccination blood samples are evaluated by tetramer analysis with flow cytometry. At study completion, patient 103 had 0.65% tetramer/CD8\(^+\) double-positive PBMC, whereas 203 had 0.82% tetramer/CD8\(^+\) double-positive PBMC (data not shown). After 12 days of in vitro culture with G280-9V-pulsed dendritic cells, the expansion of peptide-specific CD8\(^+\) T cells was significant with detection of 64% tetramer/CD8\(^+\) cells seen in donor 103 (Fig. 4B) and 88% tetramer/CD8\(^+\) cells seen in donor 203 (Fig. 4D). CTL from these cultures efficiently killed DM6, DM13, and G280-pulsed A375 melanomas as confirmation of specificity as shown for both donors (Fig. 4A and C).

In an effort to evaluate antigen receptor avidity, T2 target cells were pulsed with 10-fold dilutions (range, 10 fmol/L-100 nmol/L) of G280, G280-9V, or M1 peptide before addition of effector CD8\(^+\) cells from postvaccination samples. We calculated the peptide concentration sufficient to render the target cells susceptible to lysis and express this value as 50% half-maximal lysis. A representative experiment is shown with donor 203 using effector T cells obtained by 12-day in vitro culture (Fig. 4E). The 50% half-maximal lysis was calculated as 12.53 pmol/L for G280-9V and 14.32 pmol/L for G280. The M1 peptide was not recognized at any concentration tested. A similar analysis was conducted using postvaccination blood from donor 103 and similar results were obtained which support the finding of high-avidity CTL after dendritic cell immunization. We conclude that dendritic cell vaccination of melanoma patients with G280-9V elicits CTL that fully cross-react and retain specificity for the native peptide sequence naturally presented by A*0201\(^+\) melanomas.

The Kruskal-Willis test did not show significant differences between the vaccine dose groups with respect to the three immunologic end points. Because the dose groups did not differ significantly, the effect of the vaccine dose on the end points was assumed to be homogeneous. Next, the signed rank test (one sided P) was used to evaluate the presence of an effect of the vaccine on the induction of immunity as measured by ELISPOT (P = 0.003), tetramer (P = 0.005), and lysis (P = 0.0005) assays. These data suggest that an increase in the magnitude of the response seen in each assay is due to the vaccine; however, the responses are not affected by the vaccine dose administered.

**Discussion**

We postulated that immunization with dendritic cells is effectively required to elicit antigen-reactive T cells capable of eradicating melanoma. A phase I dose escalation clinical trial was designed to test the hypothesis that immunization with G280-9V-pulsed dendritic cells can effectively elicit tumor-reactive CTL in patients with advanced melanoma. Results from this study allow us to conclude that peptide-specific CD8\(^+\) T cells can be elicited through repeated dendritic cell immunization with a modified peptide designed for improved immunogenicity. Single amino acid substitution of neutral
Immunotherapy of cancer is based on the premise that dendritic cells (DCs) can be used to present tumor-associated antigens to T cells, thus inducing an immune response. DCs are professional antigen-presenting cells that are responsible for processing and presenting antigens to T cells. They play a critical role in initiating and regulating the immune response. DCs can exist in two functional states: immature (or tolerogenic) and mature (or immunogenic). Immature DCs are effective at tolerizing T cells, whereas mature DCs are effective at immunizing against tumors.

Recent studies have shown that immunization with immature DCs can lead to the induction of immature dendritic cells, which are less effective at inducing an immune response. In contrast, mature DCs are more effective at stimulating T cells and inducing an immune response. This suggests that targeting the maturation process of DCs could be a strategy to enhance the immunogenicity of DCs and improve the efficacy of cancer vaccines.

One approach to improving the immunogenicity of DCs is to modify their phenotype. This can be achieved through the use of irradiation, which has been shown to increase the expression of costimulatory molecules on DCs and improve their immunogenicity. However, the optimal dose of irradiation is not well-defined and may vary depending on the DC population and the specific antigen being presented.

The use of appropriate DC populations and antigens is also critical for the success of DC-based vaccines. Dendritic cells derived from different sources, such as blood or skin, have different characteristics and may require different approaches for optimal immunogenicity. Similarly, the choice of antigen and its modification can affect the immunogenicity of DCs. For example, modified peptides can serve as potent immunogens when presented on DCs.

In conclusion, the use of dendritic cells in cancer immunotherapy is a promising approach. However, further research is needed to optimize the use of DCs, including the selection of appropriate DC populations, antigens, and irradiation doses, to improve the immunogenicity of DCs and enhance the efficacy of cancer vaccines.
showed significant responses to vaccination in all patients. Two patients at the highest dose level exhibited >1% circulating CD8+ T cells specific for the native G280. Finally, all nine patients tested had measurable high-avidity CTL that lysed allogeneic melanoma cell lines that coexpressed A*0201 and gp100.

Recently, Cerundolo et al. (35) reviewed progress in the dendritic cell therapy field. They chose as the standard for dendritic cell trials those using characterized peptides and ELISPOT or tetramer assays for measurements of T-cell frequencies in the blood. They compared these with trials done using peptide alone or recombinant viruses alone. There is a very wide variation in T-cell responses. Response to gp100 (209-217M) was 28% in the single trial they analyzed; other melanoma antigen dendritic cell peptide trials report responses ranging from 22% to 73%. The results reported here compare favorably with other studies.

We conclude that dendritic cell–based vaccination with modified class I restricted G280-9V peptide uniformly elicits antitumor immunity and offers the prospect of clinical benefit for some patients (35).

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