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Phase Ib trial assessing autologous, tumor-pulsed dendritic cells as a vaccine administered with or without IL-2 in patients with metastatic melanoma

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Abstract

Twenty four subjects with metastatic melanoma were treated on a randomized Phase Ib trial evaluating an autologous tumor lysate-pulsed dendritic cell (DC) vaccine with or without IL-2. The vaccine consisted of autologous DCs obtained from peripheral blood mononuclear cells (PBMC) cultured in GM-CSF and IL-4 then pulsed with autologous tumor cell lysate and KLH. The primary endpoints of the trial were safety and immune response to vaccine. Subjects were randomized to vaccine administered every other week times 3, vaccine x 3 followed by low dose IL-2, or vaccine x 3 followed by high dose IL-2. Immune response was monitored pretreatment and at 2 and 4 weeks after the third vaccine administration. Disease evaluation was performed at 4 weeks after the third vaccination. Therapy was well tolerated with no local vaccine toxicity greater than Grade 1 in any arm. IL-2 toxicity was as expected without additional toxicity from the addition of IL-2 to vaccine. Immune response defined as DTH, PBMC interferon gamma ELISPOT, and PBMC proliferation, to both autologous tumor and KLH were detected in all arms. Interferon gamma ELISPOT response to KLH (7 of 10 patients) and autologous tumor (4 of 10 patients) were also detected in subjects with available vaccine draining lymph node cells. There were no differences in immune response between treatment arms. No clinical responses were seen. Autologous tumor lysate-pulsed DC vaccine with or without IL-2 was well tolerated and immunogenic but failed to induce clinical response in patients with advanced melanoma.

Keywords

dendritic cell; vaccine; melanoma; immunity

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INTRODUCTION

Dendritic cells (DC) are antigen presenting cells (APCs) that are important in regulating cellular and humoral immune responses. Dendritic cells are unique APC in that they can stimulate secondary as well as primary T and B cell responses to antigen (1). We and others have shown that DC can be generated ex-vivo under various culture conditions from human peripheral blood mononuclear cells (PBMC) (2-4). We had previously reported on a Phase I trial in which ex-vivo generated DC that were pulsed with autologous tumor lysate were capable of inducing an immunologic response to tumor antigens when utilized as a cancer vaccine (4). Antitumor activity (a partial response) was also seen in one patient with metastatic melanoma. As in other trials of DC based vaccination strategies immunologic responses occur at a much greater frequency than actual clinical responses.

Research continues on methods to enhance the clinical response to DC based vaccines in cancer therapy. Interleukin-2 (IL-2) is a cytokine that has clinical activity in advanced melanoma and can augment the activity of both cytotoxic T lymphocytes (CTL) and natural killer cells (NK). We showed previously in a pre-clinical murine model that the addition of systemically administered IL-2 to a tumor lysate pulsed DC vaccine could enhance antitumor immunity and tumor regression (5). In this pre-clinical evaluation the dose of IL-2 utilized was lower than that required for the successful treatment of established murine tumors when used as a single agent. Based on our pre-clinical studies we have compared in a Phase Ib trial the addition low dose vs. high dose IL-2 to an autologous tumor lysate-pulsed DC vaccine in patients with advanced melanoma.

MATERIALS AND METHODS

Patient Selection

Patients older than 18 years old with previously treated or untreated metastatic melanoma with a performance status of equal to or greater than 70% were eligible. Patients may have received prior IL-2 based therapy. Because of the requirement for autologous melanoma cells patients needed to have a tumor site that was easily harvested usually involving cutaneous, subcutaneous, or peripheral lymph nodes. Patients who were undergoing palliative resection of metastatic disease were also eligible. Patients were required to have evaluable or measurable disease in addition to the disease that was to be resected for manufacture of the vaccine. Adequate pre-study hematologic and organ function was required. This included; platelet count $\geq 100,000/\text{mm}^3$, total white blood cell count $\geq 3,000/\text{mm}^3$, absolute granulocyte count $\geq 1,500/\text{mm}^3$, absolute lymphocyte count $\geq 500/\text{mm}^3$, creatinine $\leq 1.5 \text{ mg/dl}$, total bilirubin $\leq 1.5 \text{ mg/dl}$, aspartate aminotransferase ≤ 2.5 times the upper limit of normal, prothrombin time < 14 seconds, and partial thromboplastin time < 40 seconds. Patients may not have received any antineoplastic therapy within 4 weeks of entry into study. All patients had to have a negative MRI of the brain within 4 weeks of study entry. In addition all patients over the age of 50 or with any history of cardiac disease had to have a negative cardiac stress test within 6 months of study entry. Patients were ineligible if they; had received any prior antitumor vaccine, had an autoimmune disease, were serologically positive for HIV or hepatitis B surface antigen, pregnant, required ongoing or within the 4 weeks prior to study entry any corticosteroids, any ongoing serious medical condition, or required therapeutic anticoagulation.

Study Design

The primary endpoint of this trial was to characterize the immune response to autologous tumor lysate pulsed DC alone and with a low dose or high dose IL-2 regimen. Eligible patients were randomized to DC alone, DC followed by low dose IL-2, or DC followed by high dose IL-2. The secondary endpoint was antitumor response. Each patient underwent a pretreatment

leukapheresis to obtain PBMC for DC vaccine preparation and also to obtain pretreatment lymphocytes for immunologic monitoring. Pretreatment each patient received DTH testing with irradiated autologous melanoma cells. Each cohort of patients received for each vaccination 10^7 DC pulsed with KLH and autologous melanoma lysate by i.d. injection near an inguinal or axillary nodal region felt to be free of disease. A total of 3 vaccinations administered at the same site at 2 week intervals were planned (week 0, 2 and 4). Vaccination preceded IL-2 administration in those subjects receiving IL-2. For those patients randomized to low dose IL-2 the IL-2 was administered at a fixed dose of 3 million IU subcutaneously once a day for 4 days starting the day of the vaccination. For those patients randomized to high dose IL-2 the IL-2 was administered at 360,000 IU/kg by 15 minute IV infusion every 8 hours beginning the day of vaccination for a planned maximum of 9 doses of IL-2 after each vaccination. A scheduled dose of IL-2 was omitted for toxicity rather than dose reduced or delayed. Reasons for omitting IL-2 doses were; systolic blood pressure < 90 mmHg refractory to fluid boluses or requiring doses of dopamine > 5 mcg/kg/hour, respiratory distress requiring supplemental oxygen, mental confusion, tachydysrhythmia or cardiac ischemia (patients with these events received no further IL-2 during the study), or any other serious toxicity that in the judgment of the investigator (B.G.R.) warranted omitting IL-2 doses. At week 7 patients had a lymph node removed draining the vaccine site under conscious sedation. At week 9 patients were assessed for tumor response and underwent a repeat leukapheresis or large volume peripheral blood draw (100 ml) to obtain lymphocytes for immunologic monitoring. Also at week 9 patients underwent repeat DTH testing with irradiated autologous melanoma cells as well as DTH testing to KLH. Tumor response was determined by RECIST criteria. Patients who had a tumor response (at least a PR) were eligible for retreatment at week 11. The protocol was approved by the University of Michigan Institutional Review Board and University of Michigan Comprehensive Cancer Center. Each patient provided written informed consent. The study was conducted under United States Food and Drug Administration IND BB6958.

Vaccine Toxicity Grading System

For systemic toxicities, the National Cancer Institute Common Toxicity scale was used. For local vaccine toxicities, the following scale was used: grade I, erythema and induration <20 mm; grade II, erythema and induration $\geq$ 20 mm without ulceration; grade III, ulceration or painful adenopathy; and grade IV, permanent dysfunction related to local toxicity.

Tumor Cell Harvest and Cryopreservation

Tumors were harvested surgically and cryopreserved. Tumors were kept sterile on ice and transported from the operating room to the laboratory. A single cell suspension was made by a combination of mechanical and enzyme dispersion techniques as described previously (6). The tumor cells were cryopreserved in 90% human AB serum plus 10% DMSO in a liquid nitrogen freezer. Each freezing vial contained $2-4 \times 10^7$ tumor cells.

Leukapheresis and Cryopreservation of PBMCs

In accordance with the University of Michigan Blood Bank guidelines, protocol patients underwent a 4-h leukapheresis on a COBE spectrum apheresis system to ensure adequate numbers of PBMCs for DC culture and for immune monitoring. PBMCs were obtained by taking the apheresis product, diluting it 4-fold in DPBS and overlaying it on Ficoll-Hypaque gradients. The cells were then centrifuged at $900 \times g$ for 30 min at room temperature. The interface representing the PBMCs were then collected and washed in DPBS twice to reduce platelets. Aliquots of PBMCs were then cryopreserved in 70% human AB serum 20% X-VIVO 15 and 10% DMSO for future use in cryopreservation bags (Baxter Corp., Deerfield, IL) or cryovials.

Vaccination Preparation

DC cultures and antigen pulsing were performed in the Human Applications Laboratory of the General Clinical Research Center, which is a facility that operates under Good Manufacturing Procedures. Vaccines were prepared from cryopreserved PBMCs obtained from the pretreatment leukapheresis. PBMCs were resuspended in serum-free X-VIVO 15 medium (BioWhittaker, Walkerville, MD) at 1×10^7 cells/ml for a total volume of 30 ml in 225-cm² flasks. The cells were allowed to adhere for 2 h at 37°C in 5% CO₂, and the nonadherent cells were removed after gentle rocking of the flasks and aspiration of the medium. Immediate replacement of 30 ml of X-VIVO 15 medium containing GM-CSF (100 µg/ml; Schering-Plough, Kenilworth, NJ) and IL-4 (50 µg/ml, Schering-Plough) was completed, and the cells were incubated for 6 days at 37°C, 5% CO₂ before pulsing with tumor lysate and KLH.

The adherent DCs were harvested from the flasks using 10 ml of EDTA (3 mM) for each flask and allowed to incubate for 10 min. The detached DCs were harvested, washed, and resuspended at 1×10^6 cells/ml in fresh X-VIVO 15 medium containing GM-CSF and IL-4. Ten ml of the cell suspension were placed in 75-cm² flasks (10^7 DCs/flask) for pulsing with tumor lysate and KLH. Single cell suspensions of tumor were snap freeze-thawed three times in rapid succession, irradiated at 10,000 cGy, and stored at -80° C for later use. Tumor lysate suspension was added to DCs at 1:1 cell equivalent ratio. Specifically, a volume of tumor lysate equal to 10^7 tumor cells was added to the flask and incubated for 18h at 37°C, 5% CO₂. A volume of 300 µl of KLH stock solution diluted in PBS (50 µg/ml; Calbiochem, San Diego, CA) was added to the flask and incubated for 18h.

After incubation, the tumor lysate-pulsed and KLH-pulsed DCs were harvested and counted. The DC suspension was adjusted to a total volume of 0.5 ml of PBS at 10^7 DC for injection.

Flow Cytometric Analysis of DCs

An aliquot of the vaccine preparation was retained to examine the expression of cellular markers. FITC-conjugated CD83 was purchased from Caltag Laboratories (Burlingame, CA).. PerCP-conjugated HLA-DR, PE-conjugated HLA-Class I, CD14, mouse IgG1, mouse IgG2a, CD11c, CD58 and FITC-conjugated CD80, CD86, mouse IgG1, and mouse IgG2b were purchased from PharMingen (San Diego, CA). Vaccine cells were washed with FACS buffer (PBS + 2.5 % bovine serum albumin, and counted. One µg of each labeled antibody was added to culture tubes containing one million cells in 100 µl of FACS buffer. Cells were incubated on ice for 30 min, washed two times with FACS buffer, and then suspended in PBS + 1% formaldehyde and stored at 4°C before FACS analysis.

Release Criteria for DC Vaccine

During DC vaccine manufacturing, in process samples were obtained for routine microbial culture. In addition a day 8 final cell suspension sample was obtained for immediate gram stain and microbial culture. Release criteria for vaccine required all in process cultures to be negative with a negative gram stain on day 8. The day 8 microbial culture was for quality control. DC also had to be $\geq 70\%$ viable by trypan blue exclusion.

Immune Monitoring Using PBMCs

PBMCs were harvested pretreatment at the time of leukapheresis for DC generation and 1 month after the third vaccination when tumor response assessment was determined. All of the assays were done in batch on cryopreserved PBMCs. Cells were used soon after thawing. Cell viabilities ranged from 67 to 98% between patients, but for a given patient, viabilities between pre- and post treatment samples were within 15%. PBMC were thawed, washed with sterile PBS, and suspended in complete medium: X-VIVO-15 supplemented with 2% HEPES, 100

units/ml penicillin, 10 mg/ml streptomycin, 2 mM glutamine, 50 μ M 2-mercaptoethanol, and 3% AB serum. Counts and viability were determined with trypan blue. All incubations were conducted at 37°C, 5% CO₂. Antigens for assays were KLH (40 μ g/ml, Calbiochem, San Diego, CA), tumor lysate (cell equivalence), *C. albicans* (1/100 dilution of cellular lysate, (Allermed, San Diego, CA). The following assays were performed by the Immunologic Monitoring Core of the University of Michigan Comprehensive Cancer Center.

Proliferation Assay—Cryopreserved PBMCs were thawed, washed, and suspended in complete medium. Viability was assessed by trypan blue exclusion, and cell concentrations were adjusted to 5×10^6 /ml. Cells were added to 96-well, round-bottomed plates (Falcon-BD, Franklin Lakes, NJ) in 100 μ l volumes and incubated in a final volume of 200 μ l with either medium alone, KLH (40 μ g/ml), or tumor lysate (prepared to deliver lysate at tumor cell equivalence) for a total of 6 days at 37°C, 5% CO₂. Phytohemagglutinin (10 μ g/ml; Sigma Chemical Co.) was added to some of the wells as a positive control on day 3. The cultures were pulsed with 1 μ Ci-well of [³H]thymidine (ICN, Costa Mesa, CA) on day 5 and incubated overnight before harvest onto glass fiber filter plates (Millipore, Bedford, MA). Data were collected on a TopCount NXT scintillation counter (Meriden, CT). A stimulation index (SI) was calculated:

$$SI = \frac{\text{Avg. cpm of antigen-stimulated culture}}{\text{Avg. cpm of unstimulated culture}}$$

ELISPOT Assay—One day prior to assay, ELISPOT plates (Millipore, Bedford, Ma) were pre-wet with 70% ethanol, immediately washed with sterile PBS, then incubated overnight at 4°C with 75 μ l/well of anti-IFN- γ coating Ab (Pierce, Rockford, IL) suspended at 4 μ g/ml in sterile 0.1 M carbonate buffer. The day of assay, the plates were washed with sterile PBS (Mediatech, Herndon, VA) and then blocked for 1 hour with complete medium. PBMCs were prepared as above and adjusted to 1×10^7 /ml. One hundred μ l of PBMCs were added to each well and incubated with antigen as above. Negative controls for the assay were unstimulated PBMCs. Background counts for these samples were quite low and were subtracted from the counts generated from stimulated cultures. Positive controls were stimulated with phorbol myristate + ionomycin. Cultures were incubated undisturbed at 37°C, 5% CO₂ for 24 h.

After 24h, cells were removed, and the plates were washed two times with PBS and then two times with wash buffer (tris-buffered saline + 0.05% Tween-20). Biotinylated secondary Ab suspended in assay buffer (TBS + 0.2% casein) at 2 μ g/ml was added and incubated for 2 hours. Plates were washed 5 times and incubated for 1 hour with streptavidin-alkaline phosphatase (Sigma). After 6 washes, plates were developed with NBT-BCIP substrate (Bio/FX, Owings Mills, MD) for 20-40 minutes, and stopped in running water. Plates were allowed to dry at least 24 hours before analysis using an ImmunoSpot Series 1 analyzer (Cellular Technologies Ltd, Cleveland, OH). Background counts were generally low and subtracted from the responses in stimulated cultures for presentation. We defined a positive ELISPOT as a 3X increase over the pretreatment result or if the pretreatment was 0 spots then the post-vaccine result had to be > 10 spots.

Lymph Node Assays—Harvested lymph nodes were teased apart and cells were washed and cryopreserved prior to assay. ELISPOT and proliferation assays were performed as above using pre-vaccine PBMC as antigen presenting cells. For both assays, LN cells and PBMC were added at 10^5 /well. The proliferation assay was performed in 96 well plates and developed using a dye-conversion assay (Dojindo, Gaithersburg, MD).

DTH Testing

In addition to *in vitro* immune monitoring, we assessed patients for *in vivo* immune reactivity to KLH and autologous tumor by DTH testing. For KLH reactivity, patients were given intradermal injections of 2, 20, and 100 µg of KLH in 0.2-ml volumes of PBS. Induration was measured 48 h later in two perpendicular diameters. For autologous tumor reactivity, patients were assessed before treatment and 1 month after treatment with irradiated (6,000 cGy) autologous tumor cells at 10⁴, 10⁵, and 10⁶ doses i.d. Induration was measured in a similar fashion as KLH. Positive DTH reactions were scored if the average perpendicular measurements exceeded 5 mm.

Statistics

Differences between pre and post-vaccine immune responses were assessed with a Wilcoxon Signed Rank Test. A $p \leq 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

Patient demographics are shown in Table 1. A total of 24 subjects were registered and randomized. Overall the patients were relatively young (median age 44 years old) and the majority had not received any systemic therapy for Stage IV disease. Only 3 subjects had a diagnosis of non-cutaneous primary melanoma (1 ocular, 2 mucosal). Twenty two subjects received at least one vaccine. Two subjects were not treated due to problems with vaccine production. Eighteen subjects received all 3 vaccines with 3 receiving 2 and 1 receiving 1 vaccine. Of the 3 subjects who received 2 vaccines, 2 had symptomatic progression of disease and 1 had vaccine production problems. The subject receiving 1 vaccine was due to production difficulties. All vaccines were prepared in antibiotic free medium as required at that time by the FDA. Of the 18 subjects who received all 3 vaccines, 14 had post treatment PBL harvest and 13 had post treatment lymph node biopsy. The 14 subjects for which there was post treatment PBL were randomized to; 5 no IL-2, 4 low dose IL-2 and 5 high dose IL-2.

Vaccine Product

A total of 61 vaccines were administered to subjects. The viability for the 61 vaccines administered was 91 % $\pm$ 6.8 (mean $\pm$ SD). The DC phenotype of the final vaccine product (day 8) was obtained for 23 of the vaccines. The phenotype was (mean $\pm$ SD) CD86, 74.2 % $\pm$ 16.7; HLA-DR, 83.1 % $\pm$ 13.5; and CD14, 7.76 % $\pm$ 12.5; representing a DC population. Additional in process phenotyping of the product for 15 vaccines from 15 separate subjects was also performed. In process phenotype was obtained on Day 1 from the PBMC seeded, Day 7 prior to pulsing with KLH and melanoma cell lysate and Day 8 prior to administration to subjects (Table 2). The phenotype on Day 1 was monocyte predominant with Day 7 and 8 showing a shift towards DC. The final phenotype was of an immature DC population with a low percentage of CD83+ cells. A maturation effect of pulsing with tumor lysate was not observed.

Toxicity

The vaccine was well tolerated by all subjects with no subject experiencing greater than Grade 1 toxicity at the vaccine site. Local vaccine toxicity was not enhanced by the administration of IL-2. The toxicity from IL-2 administration was as expected. Those subjects receiving low dose IL-2 had fever and malaise of Grade 1 or less. High dose IL-2 administration toxicity was greater with each cycle with the mean number of IL-2 doses administered in the first and third cycle of 8 and 7 respectively. One subject (patient #14) experienced an episode of atrial

fibrillation with rapid ventricular response at the end of the third cycle, which spontaneously converted to normal sinus rhythm.

DTH Response

Fourteen subjects received all 3 vaccines and had pre and post DTH responses assessed. No subject had a pretreatment DTH response to autologous tumor. Three subjects converted to positive DTH response to autologous tumor, one in each of the treatment arms. Nine subjects had a post treatment DTH response to KLH (2/4 high dose IL-2, 3/4 low dose IL-2, 4/5 vaccine alone). All 3 subjects with response to autologous tumor also had a response to KLH.

Analysis of PBMC

Pre and post treatment PBMC were available from 14 subjects. Interferon-gamma ELISPOT to KLH and autologous tumor was determined (Figures 1). Across all treatment arms the post treatment response to KLH was significantly increased compared to pretreatment ($p = 0.005$). A similar significant increase was seen between pre and post treatment interferon gamma response to autologous tumor ($p = 0.011$). A similar pattern was seen (Figures 2) with respect to the proliferative responses to KLH and autologous tumor across all treatment arms ($p < 0.001$, $p = 0.005$, respectively). The three treatment arms were not significantly different from one another in respect to interferon-gamma ELISPOT or proliferation. Table 3 summarizes the DTH and ELISPOT responses by patient.

Analysis of Vaccine Draining Lymph Nodes

Vaccine draining lymph nodes were harvested approximately 10 to 14 days after the third vaccination. Ten subjects had vaccine draining lymph nodes retrieved and were analyzed for reactivity to KLH and autologous tumor lysate by ELISPOT and proliferative assays (Figures 3 and 4). By IFN γ ELISPOT assay, 9 of 10 subjects demonstrated reactivity to KLH whereas 4 of 10 had responses to autologous tumor lysate. The greater reactivity to KLH compared to tumor lysate was borne out in the proliferation assay as well. A ratio of proliferation was calculated for each subject (net absorbance of presenters + KLH to presenters) yielding ratios >1 . The mean ratio for KLH was 1.61 and for tumor lysate was 1.28; this difference was statistically significant ($p=0.03$ by paired t-test). These data indicate that KLH immune reactivity was reliably elicited in draining lymph nodes; and was significantly more prevalent than reactivity to autologous tumor lysate. Due to the small number of subjects, no differences between the randomized groups could be observed.

Clinical Response

There was no tumor response as defined by RECIST criteria in any subject. There were 2 minor responses (patients 32 and 33) both in subjects who received high dose IL-2 and vaccine. These patients had reduction in size of their metastatic lesions but not enough to meet RECIST criteria for a partial response. One of these minor responses occurred in a subject who had progressed on high dose IL-2 prior to participation in the DC vaccine trial.

DISCUSSION

DC play a central role in tumor immunology (for review see 7). These cells are an integral part at the interface between innate and adaptive immune responses to various pathogens as well as potentially of the immune response to malignant cells. DC have been evaluated as a component of various vaccine strategies in cancer therapy. We have previously reported on the use of autologous tumor lysate-pulsed DC in patients with advanced malignancies (4). Other sources of antigens and methods of loading DC for vaccination purposes have included

using allogeneic tumor, recombinant proteins, viral vectors, plasmid DNA and RNA transfection (reviewed in 8).

Based on our previous preclinical and clinical DC investigations we performed a Phase Ib trial evaluating the safety and immunologic response of autologous tumor lysate-pulsed DC with or without two different dose levels of IL-2. Therapy was well tolerated and no additional toxicities were seen other than those associated with IL-2. IL-2 administration did not result in any additional vaccine site changes than those that were seen in our prior autologous tumor-lysate DC vaccine trial. We did not see any evidence of clinical autoimmunity as has been reported by others when IL-2 treatment accompanies an antitumor vaccine (9). However we used IL-2 only for 4 days after vaccination where as the previous report used low dose IL-2 daily for 42 days.

Autologous tumor lysate-pulsed DC vaccination with or without IL-2 administration did result in enhanced immune response against autologous tumor. Enhanced immune response was seen both in interferon-gamma release by ELISPOT and in a proliferation assay when unselected PBL were used as responders. We did not detect a significant difference in immune responses between the randomized groups for those patients receiving no IL-2, low dose IL-2 or high dose IL-2. Another group has also shown the immune responses seen with a melanoma DC vaccine were not enhanced by the addition of low dose IL-2 (10). In addition in our study there was no decrease in immune responsiveness in PBMC from patients receiving high dose IL-2 as has been reported previously (11).

Despite the immunologic responses to DC vaccination in this trial there were no clinical anti-tumor responses. Similar results of detectable immune responses with little or no clinical response has been reported for most DC based vaccination strategies in subjects with metastatic cancer. There are numerous reasons that have been proposed for this discordance. These range from the subtype of DC utilized (plasmacytoid and/or immature DC result in immune tolerance), to the mode of DC administration (intravenous, intradermal, or intranodal). However, when the different subtypes of DC and different methods of administration are taken into account there remains little, if any reported evidence of consistent clinical activity in DC-based vaccines in advanced cancer patients.

Autologous tumor lysate-pulsed DC vaccination can consistently result in an antitumor immune response in patients with advanced melanoma. However, the lack of clinical anti-tumor response remains a major hurdle of this strategy. Although continued efforts of enhancing of DC-based seem warranted, it will be important to also address potential host factors such as immunosuppressive networks that may prevent a circulating immune response from achieving clinically-meaningful anti-tumor responses in advanced cancer patients (12). In this regard, recent studies suggest that the use anti-CTLA-4 (13), anti-PD-1 (14), and selective removal of T regulatory cells (15) may be worthy of further considerations. Strategies that incorporate methodologies to inhibit the immunosuppressive network during and after vaccination may have the potential to improve clinical antitumor responses.

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a

b**Figure 1. IFN- γ ELISPOT to KLH and autologous tumor lysate**

PBMC response to KLH (Figure 1a) and autologous tumor lysate (Figure 1b) was examined pretreatment and post treatment 4 weeks after last vaccination, as described in Materials and Methods.

a

b**Figure 2. Proliferation to KLH and autologous tumor lysate**

PBMC response to KLH (Figure 2a) and autologous tumor lysate (Figure 2b) was measured pretreatment and post treatment 4 weeks after last vaccination, as described in Materials and Methods.

a

b**Figure 3. IFN- γ ELISPOT of vaccine draining lymph node cells**

Vaccine draining lymph node cells response was examined to KLH (Figure 3a) and autologous tumor lysate (Figure 3b), as described in Materials and Methods.

a

b**Figure 4. Proliferation of vaccine draining lymph node cells**

Vaccine draining lymph node cells response was measured to KLH (Figure 4a) and autologous tumor lysate (Figure 4b), as described in Materials and Methods.

Table 1**Patient Characteristics**

Characteristic		N	%
Sex			
	Male	12	50
	Female	12	50
Age (years)			
	Median	44	
	Range	22-75	
Primary			
	Cutaneous	21	88
	Mucosal	2	8
	Ocular	1	4
Prior Treatment for Stage IV			
	None	16	67
	IL-2	5	21
	Chemo/Bio	3	12
Karnofsky Performance Status			
	100	10	42
	90	10	42
	80	4	16

Table 2**In-Process DC Culture Phenotype**

Marker	Day 1 [*]	Day 7	Day 8
CD14	76+/-16	24+/-23	11+/-14
CD58	97+/-6	98+/-5	98+/-17
HLA-Class I	98+/-2	82+/-15	91+/-5
HLA-DR	76+/-17	84+/-14	83+/-13
CD86	36+/-30	65+/-24	74+/-17
CD11c	ND	99+/-0.1	99+/-3
CD80	ND	3+/-1	4+/-3
CD83	ND	11+/-9	10+/-13

ND = not done

*
mean %+/-SD

Table 3**DTH and ELISPOT Response**

	Post Tx DTH		Post Tx ELISPOT	
	KLH	Tumor	KLH	Tumor
Patient				
11	-	-	+	+
12	+	ND	-	-
13	+	-	+	+
14	+	-	-	-
15	+	+	-	-
16	+	-	+	+
21	+	+	+	+
22	+	-	+	+
23	-	-	+	+
24	+	-	+	+
31	-	-	+	-
32	+	+	-	-
33	-	-	+	+
34	-	-	+	+

ND- Not Done