Phase I/II Study of Treatment with Matured Dendritic Cells With or Without Low Dose IL-2 in Patients with Disseminated Melanoma

1Hersey, P., 2Halliday, G.M., 1Farrelly, M.L., 3DeSilva, C., 3Lett, M., 3Menzies, S.W.

From the: 1Oncology and Immunology Unit, Room 443, David Maddison Clinical Sciences Building, Cnr. King & Watt Streets, Newcastle, NSW 2300, Australia;
2Department of Dermatology, University of Sydney, Sydney, NSW 2006, Australia.
Melanoma and Skin Cancer Institute, Sydney University, Sydney, NSW 2006, Australia;
3Sydney Melanoma Unit, Royal Prince Alfred Hospital, Level 3, Gloucester House, Camperdown, NSW 2050, Australia.

Corresponding Author: Dr. Peter Hersey, Oncology and Immunology Unit, Room 443, David Maddison Clinical Sciences Building, Cnr. King & Watt Streets, Newcastle, NSW 2300, Australia.
Email: Peter.Hersey@newcastle.edu.au
Abstract

In the present study we have examined whether treatment of patients with metastatic melanoma with matured dendritic cell (DC) vaccines with or without low dose IL-2 may improve treatment outcomes. Sixteen patients received DC vaccines (DCs) sensitized with autologous melanoma lysates and 18 patients received DCs sensitized with peptides from gp100, MART-1, tyrosinase, MAGE-3.A2, MAGE-A10 and NA17. IL-2 was given subcutaneously (sc) at 1 million units/m² on the second day after each injection for 5-14 days in half of each group. DCs were given by intranodal injection. There were 2 partial responses (PR) and 3 with stable disease (SD) in the 9 patients receiving DCs + peptides + IL-2, and 1 PR and 1 SD in 9 patients treated with DCs + peptides without IL-2. There were only 2 patients with SD in the group receiving DCs + autologous lysates and no IL-2. Median overall survival for all patients was very good at 18.5 months but this was most probably due to selection of a favourable group of patients for the study. There was no significant difference in survival between the groups by log rank analysis. Treatment was not associated with significant side effects. The quality and yield of the DCs in the preparations was generally good. We conclude that mature DC preparations may be superior to immature DC preparations for presentation of melanoma peptides and that IL-2 may increase clinical responses to the DCs plus peptides. However, in our view the low response rates do not justify the cost and complexity of this treatment approach.

Keywords: Melanoma, dendritic cells, vaccines, interleukin-2, melanoma peptides, clinical responses.
Introduction

Treatment of melanoma once it has spread beyond locoregional sites continues to be unsatisfactory. Immunological responses are believed to play a role in the natural history of the disease and can be demonstrated in immunohistological studies by a variety of assays carried out ex vivo on lymphocytes from patients. Evidence from these sources has prompted clinical trials with melanoma vaccines using whole cells or cell lysates or more purified antigens known to be recognized by the immune system. The results of these studies have generally been disappointing, as reviewed elsewhere [8, 34].

Particular interest continues to be focused on use of dendritic cell (DC) vaccines based on the idea that there is often a paucity of these antigen presenting cells in or around tumors and that their function may be compromised by the growing tumor. In previous studies we and others have shown limited success in treatment of patients with metastatic melanoma using DC vaccines prepared from culture of human monocytes in IL-4 and GM-CSF [9,10]. It was hypothesized that the DCs prepared in this way were immature and that this may have contributed to treatment failure by inducing tolerance to the antigens [30] or by inducing immunoregulatory T cells [15]. Mature DCs were shown to have migratory capacity when injected into skin or to other lymph nodes (LNs) when injected into LNs [4]. The function of mature DCs may also be superior to immature DCs in induction of immune responses. De Vries et al [3] reported that it was only mature DCs that induced immune responses to the helper protein, KLH. Jonuleit et al [13] found that mature DCs induced melanoma peptide specific response in 5/7 patients.
whereas only 1/7 responses were seen in patients treated with immature DCs. Induction of CTL was seen in patients treated with mature but not immature DCs. Moreover, Dhodapkar et al [5] found that injection of immature DCs inhibited CD8 T cell function. In view of this, we matured DCs in the present study with a cytokine mixture of IL1-β, TNF-α and IL-6, to which was added PGE-2 [14]. The latter increased the yield of DCs and has since been shown to enhance the migratory capacity of DCs [4] and upregulate CCR7 expression by a cyclic AMP dependent mechanism [24, 16].

The present study also examined whether treatment may be more effective if the DC vaccine injections were followed by administration of low dose IL-2 to maintain activated T cells and to reverse anergy of the T cells to melanoma [31], e.g. in mice treated with peptides from the tyrosine related protein (TRP-2) differentiation antigen, regression of melanoma was only seen if the vaccinations were followed by IL-2 at low doses for 5 days [18]. Similarly, DC vaccines were only effective against established sarcomas in mice if given with low dose IL-2 [26]. Low dose IL-2 (500,000iu/m² were shown to maintain high frequencies of adoptively transferred MART-1 specific T cells in a melanoma patient [35]. Furthermore, previous studies reported a high response rate when melanoma patients were treated with a combination of melanoma peptides and IL-2 [23].

The results described below provide some evidence that the use of mature DCs with low dose IL-2 may improve clinical responses to melanoma peptides.
Methods and Materials

Patients

Patients entered into the study had AJCC stage IV melanoma that was clinically or radiologically measurable. Inclusion criteria were Eastern Co-Operative Oncology Group (ECOG) Performance Status of 0 or 1, low volume disease, no other concomitant malignancy, no previous chemotherapy or immunotherapy in the past 4 weeks and no other serious illness. The study was approved by the Ethics Committee in Royal Prince Alfred Hospital and Hunter Area Health region. All patients gave written informed consent prior to inclusion in the study.

Study Design

Patients who were HLA-A2 +ve and without resectable melanoma were allocated on an alternating basis to receive DCs + peptides or DCs + Peptides + IL-2 (Group 1). Patients who were HLA-A2 +ve or –ve with resectable melanoma were allocated on an alternating basis to receive DCs + lysates or DCs + lysates + IL-2 (Group 2). This 2 x 2 design was to allow evaluation of differences in responses between patients receiving peptides and melanoma lysates and with or without IL-2.

DC vaccines were given by injection into lymph nodes under ultrasound control at weekly intervals for the first four injections then once 2 weeks later and twice at 4-week
intervals, as described elsewhere [20]. Delayed hypersensitivity tests were carried out pretreatment, at 4 weeks and at the end of the study. Tumor measurements were carried out prior to treatment and at 8 and 16 weeks. Autoantibody tests were carried out at similar intervals. Metastatic lesions and clinical responses were evaluated by response evaluation criteria in solid tumors (RECIST), as described elsewhere [32]. In patients with PR or stable disease (SD; no progression over 3 months), DC vaccines were continued at 4-week intervals and tumour measurements reevaluated every 14 weeks. Observation of progressive disease (PD) led to cessation of treatment.

Administration of IL-2

IL-2 (Proleukin) was purchased from Chiron B.V. (the Netherlands) in vials containing 18 x 10^6 IU and made up by the pharmacy at Royal Prince Alfred Hospital into syringes for self administration by SC injection at a dose of 1 million units (1MU)/m². IL-2 was commenced on the second day after each DC injection and continued for 5 days after the first 3 DC injections, for 12 days after the fourth DC injection and for 14 days after the final 3 vaccines.

Preparation of DC Vaccines

Blood samples were collected 7 days prior to each vaccine administration. Mononuclear cells were separated from 80-100mls of heparinized blood by centrifugation on Ficoll-Hypaque in the GLP facility, Department of Dermatology, University of Sydney, and
resuspended in RPMI + 2% autologous serum and allowed to adhere to plastic flasks. After 2 hours, nonadherent cells were removed and the adherent cells were cultured with GM-CSF (Schering Plough, Baulkham Hills, NSW) 800 µg/ml and IL-4 (Schering Plough, Baulkham Hills, NSW) 500 µg/ml for 5 days (complete media).

For patients receiving DCs + peptides, on day 5, DCs were harvested and resuspended in RPMI and 1% autologous plasma at 2-5 x 10^6/ml and pulsed with KLH at 50µg/ml for 4 hours at 37°C. DCs were then washed and resuspended in the complete media and the maturation cocktail of IL-1β 10ng/ml, TNF-α 10ng/ml, IL-6 800u/ml and PGE2 1µg/ml for 48hrs. The cells were washed and resuspended at 2-5 x 10^6/ml in RPMI and 1% autologous plasma and 1 x 10^6 DCs pulsed with each peptide at 50µg/ml for 2hrs at 37°C. Then all DCs were pooled together after washing in PBS + 1% autologous plasma and resuspended in 1ml of the same solution for intranodal injection. 0.5mls were injected under ultrasound control in two normal inguinal lymph nodes. DC phenotypes were assessed on each patient on at least 3 DC preparations for CD1a, CD11c, HLA-DR and CD80, CD86 and CD83 expression.

For patients receiving DCs + autologous lysates, on day 5, DCs were washed and resuspended at 10^6/ml in RPMI + 2% autologous serum and pulsed with lysate (melanoma cells frozen and thawed 3 times) 100µg/ml and KLH 50µg/ml for 4 hours, then washed and resuspended at 2-5 x 10^6/ml in RPMI + 2% autologous serum and the maturation cocktail described above for 48hrs. Cells were then harvested for vaccination as above for intranodal injection.
Samples were taken for standard microbiological assessment from each sample on day 5. The development of DCs was monitored by appearance under an inverted microscope of DC-like aggregates in the cultures and by flow cytometry on a sample of the final cell culture.

Peptides Used in the Studies

Peptides from MAGE-A3, gp100, and MART-1 were supplied by the University of Pittsburgh Peptide Facility according to the instructions of current ‘Good Manufacturing Practice’, part 21 of the Code of Federal Regulations, Food and Drug Administration, USA (cGMP 21 CFR, FDA), as described elsewhere [7]. The MART-1 peptide was the modified decamer described by Valmori et al [33] and the gp100 peptides were the modified gp209 2M and gp280 9V peptides described by Parkhurst et al [22]. Tyrosinase, MAGE-A10.A2 [12] and NA17-A.A2 [7] were supplied by Clinalfa in ready to use vials, as used in the Ludwig Cancer Institute Trials. The tyrosinase peptide was the posttranslationally modified variant with aspartic acid in place of asparagines [28]. The influenza matrix peptide used as a positive control was kindly supplied by Dr. Andrew Scott, Ludwig Cancer Institute, Heidelberg, Victoria, Australia. The sequences of the HLA-A2-restricted peptides are as follows: Melan A/Mart-1, 26-35-ELAGIGILTV; tyrosinase, YMDGTMSQV; MAGE-3.A2, FLWGPRALV; gp100 280-9V, YLEPGPVT; gp100 209-2M, IMDQVPFSV; influenza matrix, GLGFVFTL; MAGE-A10, GLYDGMEHL; NA17-A, VLPDVFIRCV.
Skin Tests

Skin tests with the peptides used for treatment and the control influenza peptide were carried out prior to and at 4 and 14 weeks after commencement of therapy, as described by Jaeger et al [12]. Peptides (100µg) were given in 100µl of PBS by intradermal injection on the volar aspect of the forearm. Autologous lysates 100µg/ml were the same as those added to the DCs. They were given in 100µl of PBS. DTH reactions were evaluated at 48hr after injection. Reactions were considered positive when palpable skin induration was 2 mm or greater in diameter or if erythematous response at 48hr was greater than or equal to 20mm in diameter.
Results

Patient Details and Clinical Responses

In total, 34 patients were entered into the study. Sixteen patients received DCs plus autologous lysates (group 1) and 18 patients DCs plus melanoma peptides (group 2). Details of the patients and clinical responses to treatment are summarized in Tables 1 and 2.

In group 1 there were 8 females and 8 males aged from 44 to 75. All but two had an ECOG status of 0. Patient 7 and 16 had only 4 and 3 vaccines because of PD. All others had a minimum of 5 vaccines. However, in total, 6 patients did not complete the full course of treatment because of PD. Three patients had received prior chemotherapy with DTIC. Side effects were predominantly flu-like symptoms in patients given IL-2. Best responses seen were 2 with stable disease (SD) (no progression over 3 months) in patients 8 and 10.

In group 2 there were 12 males and 6 females aged from 33 to 74 years. All were HLA-A*0201. ECOG status was 0 in 14 and ECOG 1 in 4 patients. One patient had received chemotherapy with DTIC. Patients 5 and 10 had less than 5 injections of the DC vaccines. Six patients did not complete the course due to PD. There were 2 PR and 3 with SD in the patients treated with DCs plus melanoma peptides plus IL-2 and 1 PR and 1 with SD in those receiving the DCs + peptides. The difference in clinical response rates between groups 1 and 2 taking SD as a response was close to significant by Fisher exact test ($p = .086$).
Survival of Patients in the Study

As shown in Figure 1(a), the median overall survival of the 34 patients in the study from time of first metastasis was 18.5 months, which is well above that expected for a population of unselected patients with AJCC Stage IV metastatic melanoma. There was a slight trend for patients treated with peptides to survive longer [Figure 1(b)] \((p = 0.32)\) but this was not significant by log rank analysis. Similarly, there was a trend for patients receiving IL-2 to survive longer but this was not significant [Figure 1(c)] \((p = 0.4)\). There was no significant difference in survival between any of the 4 groups shown in Figure 1(d).

Delayed Hypersensitivity Skin Test (DTH)

The results in Table 3 show the diameter of the responses in mm before and at any time during vaccine administration (best result shown). Five of 6 patients immunized with lysates plus IL-2 and 5 of 7 immunized with lysates alone responded to KLH. None of the patients responded to autologous melanoma lysates.

In the patients immunized with melanoma peptides and given IL-2, 6 of 8 patients responded to KLH. In those not given IL-2, 6 of 8 responded to KLH. In those given IL-2 there were 6 of 8 responding to MART-1, 4 of 8 responding to NA17 and 5 of 8 responding to influenza peptide. In those not receiving IL-2 there were 4 of 8 responding to MART-1, 0 of 8 responding to NA17 and 5 of 8 responding to influenza peptide. The main
difference appeared to be more frequent responses to NA17 in those given IL-2. There were no responses to the gp100 280-9V, Tyrosinase or MAGE A-10 peptides. There was one response against the gp100 209-2M and MAGE 3.A2 peptides. There was no obvious correlation with clinical responses.

Quality and Yield of DCs Used in the Study

An example of the quality of the DCs used in the study is shown in Figure 2 (patient 17, group 2). As reported elsewhere [13, 14], the phenotype of the DCs incubated in the cytokine cocktail was typical for mature differentiated DCs with high HLA-DR, CD80, CD86, CD83 and CD11c expression but low CD1a expression. The yield and phenotype of the DCs used to treat individual patients is shown in Table 4 and 5. As judged by CD83, expression of the DCs from patients 2, 6, 8 and 10 in group 1 may have been less mature than the preparations from other patients. There was no correlation with clinical responses.
Discussion

The objectives of this clinical trial were to examine whether maturation of DCs ex vivo and administration of low dose IL-2 after each injection would improve the clinical response to DC vaccine administration. When compared to our previous studies on 33 patients treated with immature DC vaccines there were no substantial differences in the overall response rates [9]. In the present study on 34 patients there were 3 PR and 6 with SD compared to 3 PR, 1 MR and 9 with SD in the previous study on 33 patients. The main difference between the two studies was that all the responses in the previous study were in patients receiving DCs plus autologous lysates, whereas in the present study all the responses were seen in patients receiving DCs plus peptides. These differences are likely to be stochastical but may indicate that immature DCs are better at processing lysates for presentation to the immune system whereas mature DCs may be more effective in presenting preformed peptide epitopes.

Low dose IL-2 was reported in studies on animal tumor models to increase the potency of DC vaccines primed with autologous lysates and enhance cytotoxic T cell activity in the spleens of mice [26]. It was also shown that IL-2 given after exposure of T cells to antigen could prevent antigen induced non-responsiveness [31] and result in prolonged expansion of the T cell response [27]. Human studies showed that culture of T cells from LNs of melanoma patients in IL-2 promoted their proliferation and differentiation into CCR7-perforin +ve CTL [1]. Daily doses of IL-2 given s.c. to cancer patients at doses up to 1.25 million units were also shown to increase NK activity [17]. Given this
background, we examined whether this approach would improve clinical responses in patients treated by DC vaccines. Responses in group 1 treated with DCs + lysates were too low to allow any evaluation but in group 2 treated with DCs + peptides there was a trend for increased responses in those receiving IL-2. There was also a small increase in DTH responses to the peptides, particularly that from NA17. Reports by others using low dose IL-2 have reported low response rates, e.g. Nagayama et al. [19] reported 1SD and 2MR in 10 patients treated with mature DCs + IL-2 given s.c. three times a week. No responses were seen in patients with renal cell carcinoma treated with immature DCs plus IL-2 given s.c. for 5 days after each DC vaccine given two weekly [21]. Slingluff et al [29] reported 1PR and 1SD in 15 melanoma patients treated with DCs plus peptides given weekly for 6 weeks and 3 million units of IL-2 given s.c. daily for 6 weeks.

The results from the present studies are therefore not dissimilar to a number of other small studies using DC vaccines with or without IL-2 in patients with metastatic melanoma. Nevertheless, the low response rates called into question whether the DC preparations were satisfactory. The yields ranged from 4.3 million to 29 million and their phenotype was consistent with that of matured (differentiated) DCs with HLA-DR CD80, CD83, CD86 and CD11c expression, known to be effective in induction of T cell responses [3]. CD1a expression was generally low, as expected for differentiated DCs. There was no obvious correlation of clinical responses with the level of the phenotypic markers on the DCs or the numbers of DCs injected. Another question is whether the intranodal route of administration of the DCs may have been a factor in the low response rate. We followed the route of administration reported by Nestle et al [20], which was
associated with good response rates. Studies by de Vries et al [4] showed that DCs injected intranodally could migrate to other LNs in the chain of LNs. The patients in this study had a good performance status and only four patients had prior chemotherapy with DTIC.

Assessments of response rates in immunotherapy trials may not be the best outcome measure and may not correlate with overall survival. In view of this we examined the Kaplan-Meier estimates of overall survival in the patients treated with peptides or lysates or with and without IL-2. This showed that the median OS for all the patients was 18 months. This is significantly better than that recorded in several recent trials [2, 25] but it is likely this may be due to selection of patients with small volume disease. No significant differences were found in survival between the groups in the study. It was also noticeable that there were some long term survivors with deaths occurring after 4 and 5 years or alive with or without disease >3 years after first metastasis. In the absence of a control group, no undue significance can be attributed to this.

In conclusion, IL-2 given after intranodal injections of matured DC vaccines primed with peptides in melanoma patients tended to be associated with enhanced clinical response. The results however using DC vaccines prepared as described in this study, with or without low dose IL-2, do not appear compelling and in our opinion do not justify the technical difficulty and expense involved in this form of immunotherapy.
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References


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Legends to Figures

Figure 1. Survival of patients treated with dendritic cells + autologous melanoma cell lysates ± IL-2 compared to dendritic cells + melanoma peptides ± IL-2.

Figure 2. Typical phenotype of DCs used for treatment of patients (Patient 17, Group 2).

[Figures not available]