Mannan-MUC1–Pulsed Dendritic Cell Immunotherapy: A Phase I Trial in Patients with Adenocarcinoma

Bruce E. Loveland, Anne Zhao, Shane White, Hui Gan, Kate Hamilton, Pei-Xiang Xing, Geoffrey A. Pietersz, Vasso Apostolopoulos, Hilary Vaughan, Vaios Karanikas, Peter Kyriakou, Ian F.C. McKenzie, and Paul L. R. Mitchell

Abstract

Purpose: Tumor antigen-loaded dendritic cells show promise for cancer immunotherapy. This phase I study evaluated immunization with autologous dendritic cells pulsed with mannan-MUC1 fusion protein (MFP) to treat patients with advanced malignancy.

Experimental Design: Eligible patients had adenocarcinoma expressing MUC1, were of performance status 0 to 1, with no autoimmune disease. Patients underwent leukapheresis to generate dendritic cells by culture ex vivo with granulocyte macrophage colony-stimulating factor and interleukin 4 for 5 days. Dendritic cells were then pulsed overnight with MFP and harvested for reinjection. Patients underwent three cycles of leukapheresis and reinjection at monthly intervals. Patients with clinical benefit were able to continue with dendritic cell-MFP immunotherapy.

Results: Ten patients with a range of tumor types were enrolled, with median age of 60 years (range, 33-70 years); eight patients were of performance status 0 and two of performance status 1. Dendritic cell-MFP therapy led to strong T-cell IFN-γ Elispot responses to the vaccine and delayed-type hypersensitivity responses at injection sites in nine patients who completed treatments. Immune responses were sustained at 1 year in monitored patients. Antibody responses were seen in three patients only and were of low titer. Side effects were grade 1 only. Two patients with clearly progressive disease (ovarian and renal carcinoma) at entry were stable after initial therapy and went on to further leukapheresis and dendritic cell-MFP immunotherapy. These two patients have now each completed over 3 years of treatment.

Conclusions: Immunization produced T-cell responses in all patients with evidence of tumor stabilization in 2 of the 10 advanced cancer patients treated. These data support further clinical evaluation of this dendritic cell-MFP immunotherapy.

Dendritic cells as vectors for antigen delivery are a major focus of cancer immunotherapy. Early clinical studies have used diverse strategies, including the ex vivo loading of autologous monocyte-derived dendritic cells with antigens (synthetic peptides and recombinant proteins in melanoma, prostate, gastrointestinal, lung and breast cancer; refs. 1–6), with tumor cell lysates (in kidney and prostate cancer; refs. 7, 8), with DNA (9) or RNA (10), and with dendritic cell/tumor cell coinjection (11) or fusions (12). Patient benefit in these studies has been infrequent and inconsistent (reviewed in ref. 13), indicating the challenges to be surmounted if cellular therapy is to become clinically relevant.

We now report clinical and laboratory outcomes of a phase I clinical trial using monocyte-derived dendritic cells loaded with a mucin 1 (MUC1) VNTR recombinant protein conjugated to oxidized mannan [mannan-MUC1 fusion protein (MFP)]. The MUC1 antigen was chosen as it is highly and abnormally expressed with aberrant glycosylation by many epithelial tumors including breast, colon, kidney, lung, esophageal, stomach, and ovarian cancer. On normal tissues, low level expression of heavily glycosylated MUC1 is restricted to the apical cell surface facing the lumen of ducts and glands (14). Preclinical studies of the recombinant (not glycosylated) MUC1 antigen in mice indicated a particular advantage of conjugation to oxidized mannan, with enhanced stimulation of cellular immune responses (15). Unique features of mannan-conjugated antigen include targeting the complex to the mannose receptor of dendritic cells. Oxidized mannan rapidly traverses the endosome to deliver conjugated proteins (e.g., MUC1 VNTR) to the class I presentation pathway (16). However, in prior phase I clinical trials when MFP was injected (i.m., s.c., or i.p.) into >100 patients with advanced metastatic MUC1+ adenocarcinoma, while no toxicity was noted, there was a predominance of antibody-mediated, rather than cell-mediated, immunity and no clinical responses were observed...
Mice and humans differ markedly in their immune responses to MUC1. We had shown that the naturally occurring human anti-Galα1,3Gal antibodies in cancer patients cross-reacted with MUC1 peptides (20) and considered it likely that anti-Gal antibodies formed immune complexes with injected MFP, bound Fc receptors, and stimulated antibody production rather than cell-mediated immunity (21). This was clearly seen in Gal−/− mice (which in this regard resemble humans) where, in contrast to normal (Gal+/+) mice, the responses were predominantly antibody. The trend to antibody production in Gal−/− mice was reversed by the ex vivo loading of antigen-presenting cells with MFP (21). These studies led to the current clinical trial using autologous dendritic cells as vaccine carriers, aiming to maximize cellular immunity while minimizing the induction of antibody, which seemed to have no clinical benefit in the advanced disease setting (17–19).

This phase I study in patients with MUC1+ adenocarcinoma has shown that MFP loaded ex vivo into autologous monocyte-derived dendritic cells was without significant side effects, reliably led to T-cell immune responses, and was correlated with stabilization of disease in some patients.

Materials and Methods

Study design

The primary objective of the study was to assess toxicity with secondary objectives of assessing antitumor efficacy, immune responses, and procedure feasibility.

Eligible patients underwent leukapheresis at 4-weekly intervals on three occasions to obtain dendritic cell precursors. Autologous dendritic cells were cultured ex vivo and injected into patients at intradermal and s.c. sites. When it became evident that some patients were deriving clinical benefit, a protocol amendment allowed further dendritic cell-MFP immunotherapy.

Patients

Eligibility criteria were adenocarcinoma with no available curative therapy (i.e., either stage IV or with relapsed disease); no central nervous system involvement; no chemotherapy, radiotherapy, immunotherapy, or experimental treatment within the previous 4 weeks; life expectancy of >12 weeks; Eastern Cooperative Oncology Group performance status of 0 to 1; no other malignancy within 5 years except basal cell carcinoma of skin or noninvasive cervical cancer; no concurrent systemic corticosteroid therapy; adequate hematologic (Hb, >10 g/dL; WBC, >3 × 10^3/L; platelets, >10^5/L), renal (creatinine, <0.14 mmol/L), and hepatic function (bilirubin, <60 mmol/L) and no autoimmune disease apart from autoimmune thyroid disease. All patients were required to have strongly expressed MUC1 (VNTR) and the VNTR-containing sequences (103 amino acids; 12 kDa), was recombinant MFP, consisting of glutathione-S-transferase (26 kDa) and the VNTR-containing sequences (103 amino acids; 12 kDa), was produced in E. coli, solubilized by sonication, and purified by elution from glutathione-agarose columns (Sigma, St. Louis, MO). Mannan (Sigma) was oxidized with sodium periodate and conjugated to fusion protein to make MFP (23, 24). For in vitro assays of MUC1 vaccine–specific immune responses in delayed-type hypersensitivity (DTH), Elispot, and ELISA assays, an alternative source of recombinant MUC1 antigen (pVNTR), not containing glutathione-S-transferase, was prepared using the pFRHIsB vector (Invitrogen) and purified on a Nickel-NTA column (Qiagen, Melbourne, Australia). MFP and pVNTR were tested for sterility and for endotoxin content by the LAL assay to be <30 endotoxin units/250 μg protein.

Generation of dendritic cells in ex vivo cell culture, antigen loading, and injection

Patients underwent three leukaphereses at 4-weekly intervals to generate dendritic cells for three sets of injections within the 12-week trial period. Peripheral blood mononuclear cells (PBMC) were collected without priming over 2.5 to 3.5 hours using a Haemocytes MCS plus machine, were enriched for dendritic cells using standard monocyte adherence methods (reviewed in ref. 26), and cultured with recombinant human granulocyte macrophage colony-stimulating factor (500 units/mL; Schering-Plough Ltd., Sydney, Australia) and recombinant human interleukin 4 (500 units/mL; Peprotech, Inc., Rocky Hill, NJ) in AIM-V medium (Life Technologies, Gaithersburg, MD) and 2% v/v autologous serum. The MFP vaccine (10 μg/mL) was added to cell cultures on day 5 before recovering nonadherent dendritic cells on day 6, which were released for injection based on culture morphology (>50% dendritic cell-like) and Gram stain (negative). The cells were resuspended to 4 × 10^7/mL in BP saline for injection (Astra Pharmaceuticals, Sydney, Australia) with 2% v/v autologous serum, and injected into two intradermal sites (10^7 viable cells/site) with the remainder divided between two to four s.c. sites in the upper arms and/or legs. A sample was analyzed for phenotype by flow cytometry as described below.

Assessment of immune responses

PBMC and serum samples were cryopreserved at six time points: at each leukapheresis (weeks 1, 5, and 9) and 3 weeks after each injection (weeks 4, 8, and 12). Where possible, samples were again taken at 6 and 12 months. The intradermal injection sites were assessed at 48 hours for erythema and edema. In five patients (A01-A05), a protocol amendment allowed two skin biopsies of injection sites to be taken at week 10, with one formalin fixed for histology and the other cryopreserved and sectioned for immunohistochemistry (staining with anti-CD4, anti-CD8, and anti-CD86 monoclonal antibodies) to assess DTH. In five patients (A01-A05), skin tests at week 12 were used to assess general cellular immunity using MCI Multitest Kits (Pasteur Merieux, Lyon, France) for Tetrats, Diphtheria, Streptococcus (group C), Tuberculin, Candida (albicans), and HLA typing. Hepatitis B and C and HIV serology were required to be negative for patients to undergo leukapheresis. Baseline imaging of disease sites was done within 3 weeks of study entry. Following each injection of cell product, patients were monitored for 3 hours. Patients were reviewed thrice at monthly intervals during the 12-week study period, including blood tests and repeat imaging of disease sites at 12 weeks. Patients who received ongoing therapy were reviewed with repeat imaging 3-monthly and monthly tumor markers.

Preparation of vaccine and test antigens

MFP vaccine was prepared as previously described (23, 24) The cDNA insert (pDP9.3; ref. 25) encoded 103 amino acids of the human MUC1 protein, including three copies of the VNTR motif (PDTRPAPG-STAPAHGVTSA) flanked by natural variants of this sequence. Recombinant MFP, consisting of glutathione-S-transferase (26 kDa) and the VNTR-containing sequences (103 amino acids; 12 kDa), was produced in E. coli, solubilized by sonication, and purified by elution from glutathione-agarose columns (Sigma, St. Louis, MO). Mannan (Sigma) was oxidized with sodium periodate and conjugated to fusion protein to make MFP (23, 24). For in vitro assays of MUC1 vaccine–specific immune responses in delayed-type hypersensitivity (DTH), Elispot, and ELISA assays, an alternative source of recombinant MUC1 antigen (pVNTR), not containing glutathione-S-transferase, was prepared using the pFRHIsB vector (Invitrogen) and purified on a Nickel-NTA column (Qiagen, Melbourne, Australia). MFP and pVNTR were tested for sterility and for endotoxin content by the LAL assay to be <30 endotoxin units/250 μg protein.

Results

Patients. The characteristics of the 10 patients entered on study are detailed in Table 1. Median age was 60 years (range, 33-70 years) and performance status was 0 in eight patients and 1 in two patients. Tumor types (MUC1\textsuperscript{+}) were renal carcinoma in three patients, breast carcinoma in two, ovarian/fallopian tube in two, and one patient each with non–small-cell lung, colon, and esophageal carcinoma. Eight patients had stage IV disease whereas two patients (A03 and A09) had recurrent, incurable stage III disease. All patients had received prior therapy in addition to surgery, which included chemotherapy in five patients, radiotherapy in four, IFN in two, and hormonal therapy in one patient.

Treatment. Nine of 10 patients completed all three rounds of leukapheresis, cell culture, and immunization over the 12-week study period, with the third leukapheresis of A01 abandoned due to poor vascular access. From these 29 leukaphereses, the mean PBMC yield was 2.8 ± 1.4 (SE) \times 10\textsuperscript{8} cells and after 6 days of culture with granulocyte macrophage colony-stimulating factor and interleukin 4, the mean viable cell recovery ("dendritic cells") was 8.8 ± 4.9 \times 10\textsuperscript{7} cells. However, within this broad range of cell recoveries, there were reproducible "patient-specific" features; i.e., some individuals had consistent low PBMC yields but high dendritic cells recoveries (e.g., A11: 1.9 ± 0.4 \times 10\textsuperscript{7} PBMC; and 15.3 ± 3.4 \times 10\textsuperscript{7} dendritic cells, being 7.9% of the starting number) or the converse (e.g., A09: 4.9 ± 0.6 \times 10\textsuperscript{7} PBMC; and 5.8 ± 1.3 \times 10\textsuperscript{7} dendritic cells, being 1.2% of the starting number). The content of dendritic cells in the harvested cultures varied considerably (estimated to be 60-85% based on flow cytometry as CD86\textsuperscript+CD14\textsuperscript-CD3\textsuperscript+ as detailed below) and the residual nondendritic cells (largely T cells by CD3 staining) were not expected to affect the immunogenicity of the injected cells. All recovered MFP-loaded cultured cells were injected into the patients: 10 million cells into each of two intradermal sites in the upper arm, with the remainder divided equally between two to four (for higher cell recoveries) s.c. sites.

Toxicity. The toxicity of leukapheresis and cell injections was mild. Two patients had anxiety and lower back pain related to leukapheresis. There was no clinical toxicity of the vaccine. One patient developed elevation of antithyroid antibodies up to a maximal titer of 1:400 with no evidence of clinical autoimmune disease, and another had anti–smooth muscle antibodies at 1:80.

Antitumor efficacy. In four patients (A02, A08, A09, and A10), disease remained stable during the 12-week study period (Table 1). This included two patients, with breast and colon carcinoma, respectively, who had stable or slowly progressing disease at study entry, which was not considered to have been altered by study therapy. The other two patients, with ovarian (A09) and renal carcinoma (A08), had documented progressive disease at study entry and their clinical courses are presented here in detail.

Patient A09, a 64-year-old woman, presented with stage III ovarian carcinoma 26 months before study entry, and underwent debulking surgery followed by six cycles of carboplatin and paclitaxel chemotherapy. Incurable recurrent disease, diagnosed by elevated serum CA125, occurred 20 months from presentation and she was treated with four cycles of carboplatin, with normalization of the CA125 level. Study therapy commenced 3 months following completion of chemotherapy, at which time serum CA125 remained normal at 14 (normal <35) with no evaluable disease on computed tomography scanning (as typical of many ovarian cancer cases). After 6 weeks on study, serum CA125 rose to 47 IU/ml (normal <35), then to 64 at 2 months from study entry, indicating definite progressive disease during the early stage of trial therapy. Following the second dendritic cell-MFP injection, CA125 stabilized and remained in the range of 57 to 70 for 4 months, after which the level again began to increase, increasing from 64 to 165 over 4 months (indicated as periods ii and iii in Fig. 1 using extrapolated trendlines).
In view of the observed 4-month stabilization, then increasing CA125 levels, approval for a protocol amendment was obtained from the Institutional Human Research Ethics Committee to allow for further therapy. This resulted in a delay of 7 months before restarting intradermal dendritic cell-MFP immunotherapy, first at monthly intervals (three injections), then 3-monthly (using cryopreserved MFP-loaded dendritic cells). Serum CA125 on recommencing study therapy was 165, increasing to 191 during the next month. After the second of the additional injections at 11 months, the patient had largely stable disease for a period of 18 months, although with very slowly increasing CA125 levels which remained in the range of 182 to 258 IU/mL (Fig. 1, period iv). From ~27 months, there has been an inexorable increase in CA125 (most recently to 1,256 IU/mL 34 months from recommencing therapy and 43 months since study entry), interspersed with brief intervals where dendritic cell-MFP injections are associated with a transient reduction in serum CA125 (e.g., at 31, 35, and 40 months; Fig. 1). Reversion to monthly injections at 35 months seemed to slow the CA125 increase for 5 months only. Through >3 years, there has been no evaluable disease on computed tomography scanning.

The second patient, A08, a 69-year-old man, underwent right nephrectomy for a clear cell carcinoma of the kidney 22 months before study entry. The 7-cm tumor was adherent to the liver and extended into perinephric fat. The patient received adjuvant IFN for 6 months but stage IV disease with metastases to mediastinal nodes and liver was noted at 14 months from diagnosis. Computed tomography scanning at study entry showed progressive disease in mediastinal nodes (Fig. 2A, C, and E). The disease sites remained stable through the 12-week initial study period. After an interval of 8 months, during which the patient received no therapy and disease remained stable, the patient recommenced leukopheresis with three dendritic cell-MFP injections at monthly intervals followed by ongoing 3-monthly injections. Computed tomography scanning at 14 months showed the earlier disease sites to be stable or reduced in size (Fig. 2B, D, and F). The patient remained clinically well on dendritic cell-MFP therapy, with overall stable disease 36 months from commencing study therapy. At 20 months, a newly enlarged 2.0-cm aorto-pulmonary window lymph node was noted, which later increased to 3.5 cm in diameter but resolved by 36 months.

**Dendritic cell yield and phenotype.** In preliminary culture experiments to establish procedural details, the numbers and proportion of monocyte-derived dendritic cells (CD86<sup>+</sup>CD14<sup>−</sup>CD3<sup>−</sup>) increased markedly from day 4 to day 6 but little more to day 8 (data not shown). Because the cell yield was considered important, a 6-day culture period was used. Addition of the MFP antigen was on day 5, with rapid uptake into dendritic cells as monitored by surface and intracellular staining with anti-VNTR monoclonal antibodies after 0.5, 2, 6, and 24 hours (data not shown). Although no specific steps to mature the dendritic cells were undertaken, CD40, CD83, CD86, and CMRF44 expression on MFP-loaded dendritic cells tended to be greater than on untreated

---

**Table 1. Patient characteristics, immunologic responses, and antitumor efficacy**

<table>
<thead>
<tr>
<th>ID</th>
<th>Age/sex</th>
<th>HLA</th>
<th>Primary adenocarcinoma diagnosis</th>
<th>Best response</th>
<th>DTH*</th>
<th>Elispot</th>
<th>ELISA&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A01</td>
<td>58/F</td>
<td>DR2,4</td>
<td>B38,62 C6</td>
<td>Progression</td>
<td>None</td>
<td>+</td>
<td>Not detected</td>
</tr>
<tr>
<td>A02</td>
<td>51/M</td>
<td>DR2,3</td>
<td>A2,24 B7,18 C5,7</td>
<td>Colon</td>
<td>Stable 7 mo&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Inj 3</td>
<td>+</td>
</tr>
<tr>
<td>A03</td>
<td>62/M</td>
<td>DR2,4</td>
<td>A2,24 B7,60 C3,7</td>
<td>Lung</td>
<td>Progression</td>
<td>Inj 3</td>
<td>+</td>
</tr>
<tr>
<td>A04</td>
<td>62/F</td>
<td>DR2,4</td>
<td>A24,29 B45,75 C4,6</td>
<td>Oesophagus</td>
<td>Progression</td>
<td>Inj 3</td>
<td>+</td>
</tr>
<tr>
<td>A05</td>
<td>56/M</td>
<td>DR1,2</td>
<td>A1,24 B7,35 C4,6</td>
<td>Renal cell</td>
<td>Progression</td>
<td>Inj 2,3</td>
<td>+</td>
</tr>
<tr>
<td>A07</td>
<td>51/F</td>
<td>DR1,3</td>
<td>A1,11 B8,35 C4,6</td>
<td>Breast</td>
<td>Progression</td>
<td>Inj 1,3</td>
<td>+</td>
</tr>
<tr>
<td>A08&lt;sup&gt;†&lt;/sup&gt;</td>
<td>69/M</td>
<td>DR2,7</td>
<td>A2 B7,50 C6,7</td>
<td>Renal cell</td>
<td>Stable 44 mo&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Inj 2,3</td>
<td>+</td>
</tr>
<tr>
<td>A09&lt;sup&gt;†&lt;/sup&gt;</td>
<td>64/F</td>
<td>DR1,3</td>
<td>A1,26 B8,27 C1,7</td>
<td>Ovary</td>
<td>Stable 43 mo&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Inj 2,3</td>
<td>+</td>
</tr>
<tr>
<td>A10</td>
<td>33/F</td>
<td>DR11 A1,25</td>
<td>B44,57 C5,6</td>
<td>Breast</td>
<td>Stable 8 mo&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Inj 2,3</td>
<td>+</td>
</tr>
<tr>
<td>A11</td>
<td>70/M</td>
<td>DR2,11</td>
<td>A24,30 B51 C15</td>
<td>Renal cell</td>
<td>Progression</td>
<td>Inj 2,3</td>
<td>+</td>
</tr>
</tbody>
</table>

* DTH reactions at the dendritic cells intradermal injection sites are indicated following the specified injection times.
† Low titer (1/100) anti-VNTR serum antibody reactions or none detectable, measured within 6 months of treatment.
‡ Stable or slow progression at study entry and hence not considered to have clinical benefit from dendritic cell-MFP therapy.
§ Received ongoing dendritic cell-MFP treatment. Overall slow progression (A09) or stable with transient episode of resolving progression (A08).

---

![Fig. 1. Serum CA125 levels in patient A09, monitored from pre-trial to 42 months, with the 23 MFP/dendritic cell injections (arrows) below the X axis. Injections were given as two triple injection series every month with a 6 month interval, then every 3 months to 33 months, then monthly. The dashed linear trendlines (Microsoft Excel), for periods of relatively systematic CA125 levels, indicate marked reductions in the rate of CA125 increase following the early injection series (sections ii and iv, marked by vertical lines) and acceleration from 28 months (section v).](image-url)
dendritic cells (Fig. 3) whereas other phenotypic markers did not change (data not shown).

Dendritic cell–like cells were largely CD3−CD86+ and contained minimal CD3+ and CD14+, high expression of MHC class I and class II, and high CD86, whereas CD83 expression was low to moderate and CMRF44 expression was moderate to strong. The mannose receptor, detected by the binding of FITC-conjugated mannan, was usually expressed on a majority of, but not all, dendritic cell–like cells (data not shown). There were no notably variant cultures generated from the 29 leukaphereses of these 10 patients.

**Multitest CMI Kit and anti-VNTR DTH responses.** Five patients were tested for skin DTH responses to seven common antigens using a Multitest CMI kit and to a local i.d. injection of the MUC1-VNTR vaccine antigen. All five had skin DTH reaction responses to tetanus and four had weak DTH responses to the low challenge dose of the VNTR peptide, with variable responses to the other antigens (data not shown). These results showed the participants to be immunocompetent and the DTH responses to MUC1-VNTR injection correlated with the *in vitro* T-cell assays.

**Elispot assays of IFNγ secretion.** The *ex vivo* 18-hour Elispot assay did not require cell expansion as it detected memory effector cells (both CD4+ and CD8+ cytokine-producing). It is a sensitive method that has been used to monitor cellular immune responses in patients receiving immunotherapy (30, 31). Purified protein derivative was used as a positive control antigen to validate the stored PBMC samples, and to which all patients were expected to respond.

IFNγ-secreting T cells were generated promptly in all 10 patients immunized with dendritic cells pulsed with oxidized mannan-MUC1, were specific for MUC1 pVNTR antigen (with no glutathione-S-transferase), and detected as early as 2 weeks after the first injection (Fig. 4A). The relative responses to the purified protein derivative and pVNTR antigens by a cell sample, rather than absolute values, were considered the most appropriate measure of immune response because of interassay variation. The patients developed specific VNTR IFNγ-secreting T cells either 2 weeks after the first injection (A02, A04, and A11), 4 weeks after the first injection (A01, A03, A05, A07, A08, and A09), or 4 weeks after the second injection (A10). Follow-up tests after 6 to 12 months for A02 to A11 as available, and 2 to 3 years for A08 and A09 during extended treatments, showed a long-term sustained immune response to the vaccine antigen (Fig. 4A). In addition, when PBMC from patients A04 to A11 were depleted of CD4+ or CD8+ T cells and analyzed for VNTR-induced IFNγ secretion, it was clear that both CD4+ and CD8+ T cells specific for MUC1 VNTR were present in all patents (A04 and A06 shown in Fig. 4B). Concurrent assays of intracellular IFNγ production by flow cytometry showed that the responding cells were either CD4+ or CD8+ T cells and not other cells, excluding a significant natural killer cell response (data not shown).

Thus, all patients made vaccine-specific IFNγ responses within the 12-week trial period after the three dendritic cell-MFP injections and these responses, in the nine patients who were further monitored, were shown to be sustained long-term.

**Measurement of antibodies by ELISA assays.** Low titer (1/40) immunoglobulin M (IgM) anti-VNTR serum antibodies were induced in 3 of 10 participants (A04, A08, and A10) and seroconverted from the second injection to low titer IgG in two of the patients (data not shown). A09 hadunchanging low titre IgG and no IgM throughout the treatment period. There was no significant increase in antibody titers when assayed 6 to 12 months after the immunizations.

**DTH responses.** In previous phase I trials of MFP injection (see Karanikas et al. refs. 17, 19), we had assiduously measured DTH responses: most were weak and the biopsies showed few infiltrating cells. In this study, however, all but one patient developed DTH-type responses of 1 to 2 cm at intradermal injection sites 24 to 48 hours after the second or third injection of cell product. Patients A08 and A09, who then received ongoing dendritic cell-MFP therapy, continued to show DTH responses at injection sites. The exception was patient A01 who had no DTH after the second injection (although an *in vitro* cellular immune response; Fig. 4A) and had no further therapy due both to poor venous access precluding apheresis and to rapidly progressive disease. Typically, there was intense erythema up to 2 cm in diameter, with or without edema. These responses lasted 48 to 72 hours. Biopsy at 48 hours in 4 patients showed an infiltration of CD4+ T cells in substantial numbers whereas rarely could CD8+ cells be detected (data not shown).

**Discussion**

Targeting dendritic cells to load a mannan-conjugated recombinant MUC1 VNTR (MFP) antigen for immunotherapy was well tolerated and was associated with prolonged essentially stable disease in 2 of 10 patients treated who had advanced ovarian and renal carcinoma, respectively. The patient with ovarian carcinoma was in second relapse at the
time of study entry, indicating tumor escape from the effects of chemotherapy ceased 3 months earlier. The patient with renal cancer had cancer metastatic to liver and lymph nodes. These two patients, with progressive disease at study entry, continued on study therapy for over 3 years.

The trial was intended to establish reliable and practical procedures to generate and use MFP-pulsed autologous monocyte-derived dendritic cells, to assess vaccine toxicity, and, by immunologic monitoring, to provide a measure of vaccine immunogenicity. The culture process minimized the use of components that are subject to batch variations and regulatory controls (e.g., minimal reagents to enrich monocytes or deplete other cells and no additional cytokines or chemicals to mature the dendritic cells) and was based on standard methods to generate dendritic cells from adherent monocytes with granulocyte macrophage colony-stimulating factor, interleukin 4, and autologous serum.

This immunotherapy produced cellular responses to the MUC1 immunogen in all patients treated, a result which had not previously been observed after direct injection of the MFP antigen (17–19), emphasizing the important adjuvant effect of the autologous dendritic cells to stimulate a much stronger immune response. Peripheral blood T cells from all 10 patients made IFNγ in Elispot assays (frequency ~ 1/1,000) after only two or three dendritic cell-MFP injections at 4-week intervals. There was an extensive cellular infiltrate as a DTH response at the sites of boosting injections and little production of anti-MUC1 serum antibody. The maintenance of the DTH responses was consistent with the detection of IFNγ Elispot results in vitro. We suspect that the rapid development of DTH responses, well established within 24 hours after the third or subsequent boosting injections, was a result of antigen-loaded dendritic cells being injected and no additional time was required for antigen to be taken up locally, processed, and presented (which usually takes several hours).

The low frequency of a serologic response was consistent with the ex vivo MFP vaccine uptake and internalization into dendritic cells with transient MUC1 detectable on the cell surface. Thus, it was likely that only low quantities of MUC1 protein were available to induce anti-vaccine serum antibodies whereas internalized protein was available for processing and presentation to stimulate T-cell responses.

Dendritic cells as antigen carriers are now receiving considerable attention, particularly in tumor immunotherapy where it has been difficult to obtain either appropriate immune responses or to produce tumor regression (13, 32). Apart from dendritic cells being the optimal antigen-presenting cell, there are additional advantages in targeting them ex vivo: removing them from an immunosuppressive cytokine milieu (e.g., interleukin 10) which does not encourage immune responses; ensuring the target antigen has ample opportunity to be taken up and processed by the dendritic cells; and, after injection intradermally, providing an opportunity for effector T-cell recruitment and activation to occur in draining lymph nodes distant from sites of metastatic disease. Although leukapheresis and cell culture in a GMP facility have logistical difficulties, there was no significant clinical toxicity and the quality of the immune responses generated, along with the clinical effects, justifies the additional procedures. In this phase I trial, each patient received all cells generated (though i.d. doses were standardized to 20 million cells), which did not allow assessment of cell dosage effects. A current follow-up phase II trial of this dendritic cell/MFP therapy is using standardized doses of $4 \times 10^6$ cells per treatment.

The T-cell responses to VNTR test antigen were striking, with all patients developing vaccine-specific IFNγ-secreting T cells, both CD4+ and CD8+, which was not unexpected. The particular recombinant VNTR antigen contains 103 amino acids from MUC1 protein (three copies of the conserved amino acid sequence: Thr-Pro-Thr).

Fig. 3. Phenotypic changes to monocyte-derived dendritic cells following pulsing with MFP: representative results from two patients (A10 and A11). Dendritic cells were cultured for 5 days in vitro and then pulsed for an additional 18 hours with 10 µg/mL MFP (——) or without MFP (——). Harvested cells were stained with the panel of antibodies and flow cytometry data for CD40, CD83, CD86, and CMRF44 are shown here. Large mononuclear cells as determined by the wide and forward angle light scatter profile. Appropriate isotype controls (matching the isotype of the specific antibody) were included, with MFP (——) and no MFP (———).
20-mer VNTR motif plus two flanking variants), providing considerable epitope diversity. Thus, both CD4 “helper” and CD8 “cytotoxic” responder cells were generated in the HLA-diverse patient group (Table 1), as proven by Elispot (IFNγ) and Intracellular Cytokine assays of cell subsets. Two questions arise: why were the dendritic cell-MFP so effective in stimulating responses, and why was the response not eradicating tumors? Mannan-antigen targeted dendritic cells are likely
to be potent because the mannose receptor enables rapid antigen uptake and its cross-linking initiates some of the progressive dendritic cell maturation processes (Fig. 3), which are likely to provide a direct immunogenic benefit after injection.

As to why clinical efficacy was less striking than T-cell responses, there are a number of potential factors whereby dendritic cell-MFP therapy was not delivered to the right patients or in an optimal fashion. First, it is increasingly being recognized that the mere detection of T-cell responses may not necessarily correlate with the observation of clinical response may have the best opportunity of a benefit. We note that in patient A09, recent dendritic cell-MFP injections were associated with a drop in CA125 levels 1 to 3 months later, which then rebounded, although perhaps affected by the immunosuppressive environment of the tumor (36). Thus, protocols where the frequency of dendritic cell-based immunotherapy injections can be tailored to a patient with early stage (minimal residual) disease by careful stimulation and epitope mapping studies. Second, whereas dendritic cells used in a number of other studies induced good T-cell responses, the 3-monthly interval between injections may have been too long to achieve a greater antitumor effect. The rate of CA125 change is an accepted surrogate marker of ovarian cancer progression (35), and initially in patient A09 there seemed to be a good correlation between treatment episodes and response; however, this deteriorated in the 3rd year of treatment even after reverting to monthly injections (Fig. 1). It is possible that effector T cells which are (newly) recruited by dendritic cell-MFP injection have only a limited period to destroy targeted cancer cells, and might not have the potential to renew, to a patient with early stage (minimal residual) disease by observation of clinical response may have the best opportunity of a benefit. We note that in patient A09, recent dendritic cell-MFP injections were associated with a drop in CA125 levels 1 to 3 months later, which then rebounded, although perhaps affected by the immunosuppressive environment of the tumor (36). Thus, protocols where the frequency of dendritic cell-based immunotherapy injections can be tailored to a patient with early stage (minimal residual) disease by observation of clinical response may have the best opportunity of a benefit. We note that in patient A09, recent dendritic cell-MFP injections were associated with a drop in CA125 levels 1 to 3 months later, which then rebounded, although perhaps affected by the immunosuppressive environment of the tumor (36).

## Acknowledgments

We thank the following people for their valuable help with different aspects of the clinical trial: Belinda Bardsway, Sharon France, Vanessa Waddell, and Dr. John Bates (PRIMA BioMed Ltd.); Dr. Jayesh Desai and Wayne Saunders (Austin Hospital); Lesley Barber, Dr. Dominic Wall, and Kerrie Stokes (Centre for Blood Cell Therapies, Peter MacCallum Cancer Centre); Carla Olsinski, Harry Aletreas, Dr. Li Wenjun, and Violeta Bogdanoska (The Austin Research Institute); Dr. Carole Smith, Tamla Tait, and staff of the Apheresis Service (Austin Hospital); and Louise Keelan and Sharon Wilson (Department of Histopathology, Austin Hospital). Brendan Toohey prepared the pTRC-VNTR antigen. Thanks also to Dr. Pat Mottram for reviewing the manuscript.

## References


Mannan-MUC1–Pulsed Dendritic Cell Immunotherapy: A Phase I Trial in Patients with Adenocarcinoma

Bruce E. Loveland, Anne Zhao, Shane White, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/12/3/869

Cited articles
This article cites 35 articles, 10 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/12/3/869.full#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/12/3/869.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.