Advances in Brief

Priming Tissue-specific Cellular Immunity in a Phase I Trial of Autologous Dendritic Cells for Prostate Cancer\textsuperscript{1}


Abstract

We attempted to induce therapeutic immunity against prostate-derived tissues in patients suffering from progressive hormone-refractory metastatic prostate carcinoma. Thirteen patients were treated with two infusions, 1 month apart, of autologous dendritic cells (APC8015) preexposed \textit{ex vivo} to PA2024, a fusion protein consisting of human granulocyte/macrophage-colony stimulating factor (GM-CSF) and human prostatic acid phosphatase (PAP). The infusions were followed by three s.c. monthly doses of PA2024 without cells. Three groups of patients each received PA2024 at 0.3, 0.6, or 1.0 mg/injection. All Ps were two-sided. Treatment was well tolerated. After infusions of APC8015, patients experienced only mild (grade 1–2) short-lived fever and/or chills, myalgia, pain, and fatigue. One patient developed grade 3 fatigue. Four patients developed mild local reactions to s.c. PA2024. Twelve patients were evaluable for response to treatment. Circulating prostate-specific antigen levels dropped in three patients. T cells, drawn from patients after infusions of APC8015, but not before, could be stimulated \textit{in vitro} by GM-CSF ($P = 0.0004$) and PAP ($P = 0.0001$), demonstrating broken immune tolerance against these two normal proteins. Injections of PA2024 did not influence the reactivity of T cells against PAP and GM-CSF. However, antibodies to GM-CSF and, to a much lesser extent, to PAP reached maximum titers only after two or even three injections of PA2024, showing that directly injected PA2024 was involved in stimulation of humoral immunity. Dendritic cells exposed to antigen \textit{ex vivo} can induce antigen-specific cellular immunity in prostate cancer patients, warranting further studies of this mode of immunotherapy.

Introduction

Adenocarcinoma of the prostate is the most common malignancy in American men and the second most frequent cause of their death from cancer; nearly 40,000 men are projected to die from this disease in 1999 (1). For patients with advanced disease, standard care includes hormone ablation by bilateral orchiectomy or by agonists of luteinizing hormone-releasing hormone, with or without antiandrogens (2). Initially, most patients respond to this treatment, but in more than one half the disease becomes refractory to hormone therapy in \textless{}2 years (2). The treatment of hormone-refractory disease is less than satisfactory. Treatment options include supportive care only: second-line hormonal therapy (e.g., adding or withdrawing antiandrogens, corticosteroids, and others); chemotherapy; and/or experimental treatments (3). Nonetheless, the median survival of patients suffering from hormone-refractory disease is generally \textless{}1 year (4). Clearly, the lack of effective treatment for advanced hormone-refractory prostate cancer necessitates development of new therapies.

Recent advances in immunology, particularly in isolation and characterization of dendritic cells, have raised hopes that immunotherapy might provide such an additional therapeutic modality (5). Dendritic cells are the only antigen-presenting cells that can prime naive T cells and initiate an immune response (6). Isolated dendritic cells can be exposed to tissue- or disease-associated antigens \textit{in vitro} and reinfused to stimulate immunity to those antigens (5, 7, 8). Indeed, such \textit{ex vivo}-processed cells have been used successfully in experimental models (9, 10) and clinical trials (5, 8, 11–14). These trials used dendritic cells exposed to disease-associated antigens (i.e., idiotypic monoclonal protein, tumor cell lysate, and HIV-derived antigens). In this Phase I study of treatment safety, our secondary goal was to investigate if \textit{ex vivo}-processed autologous dendritic cells can prime cellular immunity against a normal tissue antigen such as PAP,\textsuperscript{3} characteristic both of normal prostate (15, 16) and prostate carcinoma (15, 16).

Studies in rats \textit{in vivo} indicate that rat dendritic cells exposed to rat PAP induce anti-PAP cellular immunity but do not induce antibodies to PAP (17). However, when the same

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\textsuperscript{3} The abbreviations used are: PAP, prostatic acid phosphatase; GM-CSF, granulocyte/macrophage-colony stimulating factor; PBMC, peripheral blood mononuclear cell; PSA, prostate-specific antigen; HLA, human leukocyte antigen.
antigen was injected s.c., it raised a strong antibody response (17). Accordingly, in this Phase I trial we administered two doses of dendritic cells “pulsed” ex vivo with antigen, followed by three injections of soluble antigen to men suffering from advanced hormone-refractory prostate cancer and monitored treatment safety and effects on cellular and humoral immunity.

Patients and Methods

Patients. Patients were enrolled into the trial after having signed the informed consent form approved by the Mayo Clinic Institutional Review Board. Men were eligible for the study if they suffered from histologically proven adenocarcinoma of the prostate that progressed despite the primary hormonal therapy (orchiectomy or administration of a LHRH agonist with or without an antiandrogen). Progression of disease was ascertained by the increase in the levels of circulating PSA and by radiography (bone scan and/or computed tomography). For enrollment into the study, any antiandrogens were withdrawn, and continued progression of disease had to be documented. Other eligibility requirements were age >18; Eastern Cooperative Oncology Group performance status 0 or 1; PSA ≤ 5.0 ng/ml; PAP equal to or above twice the upper limits of normal; negative serological tests for HIV, human T-cell lymphotrophic virus type I, hepatitis B, and hepatitis C; adequate hematological parameters (WBC ≥ 2,000/mm³; absolute neutrophil count ≥ 1,000/mm³; platelets ≥ 100,000/mm³; and hemoglobin > 9.0 g/dl); creatinine ≤ 2.0 mg/dl; total bilirubin equal to or less than twice the upper limit of normal; and aspartate aminotransferase and alanine aminotransferase equal to or less than five times the upper limit of normal.

Treatment and Assessment. The clinical trial included i.v. infusion of two doses of PAP antigen-loaded autologous dendritic cells [APC8015, autologous dendritic cells exposed ex vivo to PA2024 (see below); Dendreon] 1 month apart and followed by three monthly s.c. doses of the PAP antigen (PA2024, a fusion protein consisting of human GM-CSF and PAP; Dendreon; Fig. 1). Two days before each treatment, the patients underwent leukopheresis (1.5–2.0 blood volumes) to collect mononuclear cells, the fraction containing dendritic cell precursors. Patients received no chemotherapy or growth factors before leukopheresis. The APC8015 dose contained all dendritic cells prepared from one leukopheresis product and was infused i.v. over 30 min in the outpatient setting. The patients were observed for acute side effects for 1 h after infusion and then discharged. The dose of APC8015 applied in this study had already been shown to be safe in a Phase I trial (18). We tested three dose levels of s.c.-injected soluble antigen PA2024: 0.3, 0.6, and 1.0 mg. Cohorts of three patients were entered at each level as long as dose-limiting grade 3 toxicity (NIH Common Toxicity Criteria) was not observed in more than one patient/level. Six patients were to be treated at the final dose level determined either by grade 3 toxicity or by the attainment of the highest planned dose. PA2024 was injected s.c. in the thigh (one site for the first two dose levels and two sites for the third dose level). Patients were observed for acute side effects and then followed as outpatients. All adverse events were evaluated for the relationship to treatment with APC8015 or PA2024, and the severity was scored according to the NIH Common Toxicity Criteria.

Patients were monitored by history, physical examination, blood counts, serum chemistry, and measurements of PSA and PAP every 4 weeks during treatment (weeks 0–16). Tumor burden was evaluated by radiography at week 0 (baseline), week 20, and then every 8 weeks until progression. Unless accompanied by clinical progression, generally verified by repeated radiographic assessment, increasing levels of PSA or PAP before completion of the treatment were not taken as reasons for discontinuation of treatment.

Preparation of Antigen-loaded Dendritic Cells (APC8015). PA2024 antigen is a recombinant protein consisting of human PAP fused through its COOH terminus to the NH₂ terminus of GM-CSF by a Gly-Ser linker. The GM-CSF portion targets the fusion protein to dendritic cells (19). The PA2024 gene was cloned into the pBacPak8 vector (Clontech, Palo Alto, CA) and transfected into SF21 insect cells by the use of a commercially available kit (Clontech). Recombinant baculovirus was then cloned by plaque purification and propagated in SF21 cells adapted to grow in protein-free SPI00–2 medium (Life Technologies, Inc., Grand Island, NY) supplemented with hydrolyzed yeast extract (Life Technologies, Inc.). The PA2024 protein was released into the culture supernatant and purified for this trial by three sequential column chromatography steps to >90% purity.

Antigen-loaded Dendritic Cells (APC8015). PAP-loaded autologous dendritic cells were prepared at the Mayo Clinic Cell Processing Center. The Cell Processing Center comprises environmental control, staffing, and process controls that comply with the current Good Manufacturing Practices for somatic cell therapy. It contains a Class 10,000 clean room with Class 100 biosafety cabinets and other equipment for cell isolation and culture.

APC8015 was prepared by a modification of the method described by Hsu et al. (5). The leukopheresis product was collected at the adjacent Mayo Blood Bank and transferred to the Cell Processing Center. PBMCs were isolated by centrifuging over a buoyant density solution of 1.0770 g/ml, 320 mosM, and washed twice to remove platelets. The cells were then centrifuged over the second buoyant density solution of 1.0650...
g/ml, 320 mosM, to deplete monocytes. The high-density cells in the pellet containing dendritic cell precursors were suspended in AIM-V medium (Life Technologies, Inc.) at 1.0 × 10^7/ml and incubated with PA2024 (10 μg/ml) in the absence of exogenous serum or cytokines in a humidified 5% (v/v) CO_2 in air at 37°C. After 40-h of culturing, the cells were washed, suspended in lactated Ringer’s solution, and transported to the outpatient Mayo Infusion Therapy Center for infusion. Quality control for each cell lot included cell number and viability, Gram’s stain, cell surface marker phenotype, and tests of sterility, Mycoplasma, and endotoxin. The results of tests for sterility, Mycoplasma, and endotoxin were available only after APC8015 had been infused. Twenty-five lots of APC8015 were prepared for 13 patients. APC8015 contained 18.6 ± 9.4% (median, 18.0%; range, 6.0–39.0%) dendritic cells, defined as CD54bright, 65.1 ± 12.0% T cells (CD3^+), 16.6 ± 7.8% monocytes (CD14^+), and 5.0 ± 2.4% B cells (CD19^+). In APC8015 prepared for another prostate cancer immunotherapy study (18), we found that the CD54bright cells were also bright in CD40, CD86, HLA-A,B,C, and HLA-DR (data not shown); in APC8015 prepared from the blood of normal healthy donors, >90% of CD54bright cells were also HLA-DR^bright (data not shown).

Assessment of Immune Function. To assess the immune response to therapy, 20–40 ml of venous blood was collected in heparinized tubes at week 0 (baseline values before dendritic cell infusion) and in weeks 4, 8, 12, 16, and 20. Immediately after drawing, blood was transported at ambient temperature overnight from the Mayo Clinic to Dendreon (Mountain View, CA). There, serum was separated and frozen. Mononuclear cells containing lymphocytes were isolated by centrifuging over a buoyant density solution of 1.0770 g/ml and incubated with PA2024 (10 μg/ml) in the absence of exogenous serum or cytokines in a humidified 5% (v/v) CO_2 in air at 37°C. After 40-h of culturing, the cells were washed, suspended in lactated Ringer’s solution, and transported to the outpatient Mayo Infusion Therapy Center for infusion. Quality control for each cell lot included cell number and viability, Gram’s stain, cell surface marker phenotype, and tests of sterility, Mycoplasma, and endotoxin. The results of tests for sterility, Mycoplasma, and endotoxin were available only after APC8015 had been infused. Twenty-five lots of APC8015 were prepared for 13 patients. APC8015 contained 18.6 ± 9.4% (median, 18.0%; range, 6.0–39.0%) dendritic cells, defined as CD54bright, 65.1 ± 12.0% T cells (CD3^+), 16.6 ± 7.8% monocytes (CD14^+), and 5.0 ± 2.4% B cells (CD19^+). In APC8015 prepared for another prostate cancer immunotherapy study (18), we found that the CD54bright cells were also bright in CD40, CD86, HLA-A,B,C, and HLA-DR (data not shown); in APC8015 prepared from the blood of normal healthy donors, >90% of CD54bright cells were also HLA-DR^bright (data not shown).

Antigen-dependent T-cell proliferation was tested against PA2024, human seminal fluid PAP (Biodesign International, Kennebunk, ME), and GM-CSF (Leukine; Immunex, Seattle, WA). One hundred thousand PBMCs were plated per well of round-bottomed, 96-well microtiter plates. Antigens were dissolved in AIM-V medium containing 5.0% human AB serum and added to triplicate wells at the final concentration of 0.4, 2.0, 10, or 50 mg/ml in the total volume of 200 μl. Control wells contained no antigen. The cells were incubated at 37°C in 5% CO_2 for 5 days. Tritiated thymidine (Amersham, Piscataway, NJ), 1.0 μCi/well, was added for the last 16 h of incubation, after which the cells were harvested with a Tomtec harvester (Wallac, Gaithersburg, MD). The radioactivity of incorporated thymidine was measured as cpm in a Wallac-LKB Betaplate counter (Wallac). T-cell proliferation response was considered significant when the mean radioactivity of the wells containing antigen was at least twice above the mean radioactivity of the control wells.

Proliferation is expressed as proliferation index calculated from the formula.

\[
\text{logPI} = \frac{\text{average(logcpm}_{\text{exp}})) - \text{average(logcpm}_{\text{control}})\]

where PI is proliferation index, cpm_{exp} denotes cpm of radioactivity incorporated by the cells in the presence of antigen, and cpm_{control} is the radioactivity incorporated by the analogously treated cells devoid of antigen. The logarithmic transformation obviates the effects of the nonnormal distribution of cpm determinations representing the radioactivity incorporated by cells. Antibodies specific for PA2024, GM-CSF, and PAP in the serum were quantified by an ELISA that was comprised of immobilized antigens and goat antibodies specific for human IgG and IgM (Jackson ImmunoResearch, Westgrove, PA) conjugated to horseradish peroxidase. Multiswell plates (Dynex Technologies, Chantilly, VA) were coated with antigens dissolved at 1–2 μg/ml in Dulbecco’s PBS (D-PBS) by overnight incubation at 4°C, washed with D-PBS (pH 7.4), containing 0.05% Tween 20 (Sigma Chemical Co., St. Louis, MO), blocked with 5% powdered milk dissolved in Tris-buffered saline, and washed in D-PBS. Serial dilutions of the test serum were dispensed onto the plate, which included a negative control (pooled normal human serum) and a positive control serum. The plates were incubated for 1 h and washed. Then human immunoglobulin bound to the plate was detected by horseradish peroxidase-conjugated goat antihuman IgG/IgM. Color was developed from 3,3',5,5'-tetramethylbenzidene dihydrochloride and read on a plate reader at 492 nm. The coefficient of variability of controls for these measurements was routinely <20%. The absorbance measured for each tested serum was compared with the curve for a negative control serum obtained from a subject who did not suffer from prostate cancer. The measured values two times above the background were statistically significant. The titer of the tested antibody was determined as the highest dilution that resulted in absorbance two times above the background.

Statistics. Changes in antigen-specific cellular immunity were analyzed by comparing proliferation of T cells drawn at successive times before and during treatment at 4-week intervals. By the use of the PROC MIXED program package (SAS Institute, Cary, NC), the relationship was examined by comparing radioactivity (cpm) incorporated by cells from each of nine patients stimulated by four concentrations of each antigen. Each time/antigen combination was compared separately, yielding the respective P. The significance of differences between mean values was assessed by the two-tailed Student’s t test for unequal variances (see Table 2). The difference between median levels of antibodies to PAP was determined by the two-tailed Mann-Whitney test. The significance level for all comparisons was set at P < 0.05.

Results

Patients. Thirteen patients were enrolled between December 15, 1997, and July 7, 1998. They were all eligible and provided written informed consent before enrollment. The patients were evaluated if they received at least one dose of PA2024. One patient (no. 7) was not evaluated because the disease progressed after one dose of APC8015. Demographic information, which included age, weight, height, and disease progression after one dose of APC8015, was available for the 13 patients. The median age was 67 years (range: 52–83), the median weight was 75 kg (range: 59–110), and the median height was 180 cm (range: 165–195). The median disease progression after one dose of APC8015 was 3 months (range: 2–12).

Dendritic Cells in Prostate Cancer

PSA levels were reduced by more than one-half in the course of evaluated for the response to treatment. In patients 5, 10, and 11, primary therapy included venous access.

Leukopheresis, and all procedures were performed using peripheral venous access.

After injection of PA2024: patient 2 upon injections on weeks 12 and 16, patient 3 on week 8, patient 4 on weeks 12 and 16, and patient 7 on week 20.

The patients received the full course of PA2024 at week 12. We evaluated also the antibodies to GM-CSF (Table 3). After treatment, all patients developed antibodies to PA2024 (Table 3). After treatment, all patients developed antibodies to PA2024 because of early disease progression. Patients 5 and 8 received only one dose of PA2024, and patients 9 and 12 received only two doses.

Treatment Received. Table 2 shows the number of dendritic cells and the dose of PA2024 administered to each patient group. All patients received two doses of APC8015 except patient 7. The patients received the full course of PA2024 at each dose level except patients 5 and 8, who received only one injection, and patients 9 and 12, who received two injections. The achieved numbers of dendritic cells in the first and second doses of APC8015 were similar, demonstrating that cell processing was consistent. Variation in the total number of nucleated cells was slightly larger and ranged among patients between $1.1 \times 10^9$ and $5.4 \times 10^9$.

Adverse Events. Generally, the treatment was well tolerated. Five patients developed mild fever (grade 1–2) and/or chills within 1 h after infusion of APC8015; these symptoms lasted less than 24 h. Five patients developed mild (grade 1–2) myalgia or pain, usually 1–2 days after treatment with APC8015; these symptoms were fully resolved within 1 week.

Six patients reported transient grade 1–2 fatigue, and one experienced prolonged grade 3 fatigue that was temporally related to treatment. Four patients developed mild (grade 1) local reactions after injection of PA2024: patient 2 upon injections on weeks 12 and 16, patient 3 on week 8, patient 4 on weeks 12 and 16, and patient 10 on week 12. There were no adverse events related to leukopheresis, and all procedures were performed using peripheral venous access.

Response to Treatment. Twelve patients could be evaluated for the response to treatment. In patients 5, 10, and 11, PSA levels were reduced by more than one-half in the course of treatment (Fig. 2; note the logarithmic scale on the ordinate), although in patient 5, PSA declined despite the rapid progression of disease. Three patients (4, 10, and 11) experienced significant drops in circulating PAP levels (Fig. 2). No response was detected by radiography. The median time to disease progression was 135 days after registration (range, 30–274 days).

Antigen-specific T Cells. Development of T cells in response to APC8015 (i.e., PA2024-laden dendritic cells) and to soluble PA2024 was evaluated in nine patients. Data in Fig. 3 show the T-cell response measured from week 0 every 4 weeks until week 20, or until the withdrawal from trial because of disease progression. The cells from patients 1–3 could not be evaluated because they proliferated strongly in the absence of antigens; the reason for this phenomenon is unclear. Four weeks after the first treatment with APC8015, T cells from all evaluated patients proliferated in vitro in response to PA2024 ($P = 0.0001$). The change in the proliferation index differed among patients and ranged from 3 to 120. The response to PA2024 persisted for the duration of treatment or duration of monitoring (Fig. 3, top).

To determine more precisely the specificity of T cells proliferating in vitro in response to PA2024, we stimulated the cells with Leukine (recombinant GM-CSF; Fig. 3, middle) and PAP isolated from human seminal fluid (Fig. 3, bottom). Both GM-CSF and PAP induced T-cell proliferation ($P = 0.0004$ and 0.0001, respectively), in some patients more than 10 times above the baseline. Thus, infusion of APC8015 clearly induced T cells specific for GM-CSF and for PAP, the components of the fusion protein PA2024.

On week 8, we administered the first of three s.c. injections of the soluble antigen and evaluated the effects on T-cell proliferation monthly. Within the limits of resolution of the assay, soluble antigen contributed little to the antigen-specific T-cell response induced by APC8015 (compare T-cell proliferation before week 12 with the proliferation on or after week 12; Fig. 3).

Antibody Response to Dendritic Cells and Soluble Antigen. Eleven patients were evaluable for antibody responses to PA2024 (Table 3). After treatment, all patients developed antibodies to PA2024. Nine patients responded after administration of antigen-loaded dendritic cells alone (before administration of PA2024) and two patients after administration of PA2024 (week 12). We evaluated also the antibodies to GM-CSF and PAP. We found that three patients contained low titers of preexisting antibodies to GM-CSF (Table 3).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of patients enrolled in the trial</th>
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<tbody>
<tr>
<td>Age (median, range)</td>
<td>67.5 (59–84)</td>
</tr>
<tr>
<td>ECOG$^a$ performance status</td>
<td>0 (0–1)</td>
</tr>
<tr>
<td>Baseline PSA (median, range)</td>
<td>323 (19.8–920.9)</td>
</tr>
<tr>
<td>Baseline PAP (median, range)</td>
<td>22.4 (9.1–831)</td>
</tr>
<tr>
<td>Disease duration (mo)</td>
<td>72 (20–134)</td>
</tr>
<tr>
<td>Primary therapy</td>
<td>Prostatectomy 3, Radiation therapy 3, Other 6</td>
</tr>
<tr>
<td>No. of hormone manipulations</td>
<td>One 3, Two 3, Three 5, Four 1, Anemia 4, Elevated alkaline phosphatase 6, Pain level None 2, Mild 9, Moderate 0, Severe 1</td>
</tr>
</tbody>
</table>

$^a$ The table does not contain data for patient 7, who was not fully evaluable.

$^b$ ECOG, Eastern Cooperative Oncology Group.

Values are number of patients.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Dose of dendritic cells (APC8015) and soluble antigen (PA2024) delivered to patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Infusion no. 1</td>
</tr>
<tr>
<td>PA2024 (mg/dose)</td>
<td></td>
</tr>
<tr>
<td>1–3</td>
<td>248 ± 86</td>
</tr>
<tr>
<td>4–6</td>
<td>230 ± 140</td>
</tr>
<tr>
<td>7–13$^b$</td>
<td>325 ± 155</td>
</tr>
</tbody>
</table>

$^a$ No difference among groups was statistically significant.

$^b$ Patient 7 received one dose of dendritic cells and no PA2024 because of early disease progression.
Antibodies to GM-CSF became measurable in most patients, in some with high titers, mostly at weeks 16 and 20. In distinction to GM-CSF, antibodies to PAP were low in titer and occurred in only five patients. Thus, APC8015 and PA2024 raised antibodies mostly to PA2024 and GM-CSF.

To determine the effects of soluble PA2024 on circulating antibody levels, we compared the titers of antibodies to PAP in this study with the titers obtained in a similar study of prostate cancer that used APC8015 only (18). In this study, 2 of 12 evaluated patients developed titers >40 (Table 3). In the other study, 15 of 31 patients developed titers >40. This difference is statistically significant (P = 0.01, Mann-Whitney test).

Discussion

In a previous study, it was determined that APC8015 is safe at any dose that could be manufactured (18). Such dendritic cell preparations induced PAP-specific T cells, independent of the route of administration (i.v., intradermal, or intralymphatic; Ref. 20). This Phase I trial was designed to evaluate the safety and the maximum tolerable dose of PA2024 administered s.c. after two i.v. infusions of APC8015. The impetus for the study came from an analogous study in rats that showed that soluble antigen boost after infusions of antigen-loaded dendritic cells raised antibodies against the antigen (17). We speculated that a similar effect in men might be beneficial in the control of metastatic prostate disease. Immunological end points, evaluated in most patients, allowed us to assess the effects of treatment on immunity against PAP, a prostate tissue-specific antigen, and on the serum levels of PSA and PAP as surrogate markers of treatment efficacy.

Antigen-specific T cells and antibodies raised by APC8015 and PA2024 have provided important insights into the efficacy of antigen-loaded dendritic cells and the elicited immune response. Antigen-loaded dendritic cells were highly efficacious in eliciting antigen-specific T cells; in most patients, antigen-specific T cells were present within 4 weeks of the first infusion of APC8015. The immune response to the fusion protein PA2024 could be clearly dissected into immunity against the constituent PAP and GM-CSF. This is a direct demonstration that the treatment broke tolerance to a normal tissue-associated antigen (PAP) and a normal cytokine (GM-CSF).

Overall, the treatment was tolerated well. Infusions of APC8015 resulted in mild symptoms observed previously in similar studies (18). s.c. injections of PA2024 were generally harmless, with six patients experiencing mild (grade 1–2) systemic toxicity and four patients having a local grade 1 reaction at the site of s.c. injection. Interestingly, at the time of these reactions, two patients (4 and 10) demonstrated high levels of T-cell reactions to PA2024 and its components in vitro. This indicates the possibility that the s.c. administration of PA2024 induced a delayed-type hypersensitivity-like reaction.

PA2024, the fusion protein used for “pulsing” dendritic cells, contains full-length PAP and GM-CSF. The fusion molecule is endowed with the enzyme function of acid phosphatase and can stimulate growth in GM-CSF–dependent cell lines. Exposure to PA2024 allows dendritic cells to process, edit, and present antigen in the context of HLA class I and class II restriction characteristic for each individual. Consequently, we did not select patients on the basis of their HLA makeup; this decision is supported by an analysis of the ability of different HLA class I molecules to present PAP-derived peptides, which showed that most can present one or more peptides derived from PAP. Such peptides can elicit cytotoxic T cells that can effectively lyse PAP-secreting prostate tumor cells in vitro (21).

Murphy and colleagues treated prostate cancer patients with autologous dendritic cells pulsed with HLA-A0201-specific peptides derived from prostate-specific membrane antigen (11). In contrast to our experience, no patient developed

5 S. Vuk-Pavlović, unpublished data.
immunity to these peptides. In a pilot study of dendritic cells for treatment of HIV infection, we pulsed the cells with either HIV-derived HLA-A2-restricted peptides or with the whole HIV GP160 protein (8). Although dendritic cells pulsed with either the protein or the peptides elicited HIV-specific cytotoxic T cells, the protein-pulsed dendritic cells were significantly more potent. Similarly, dendritic cells pulsed with whole immunoglobulin protein elicited cytotoxic T cells in the patients with B-cell lymphoma (5) and, possibly, in multiple myeloma (22). Dendritic cells pulsed with peptides or tumor lysates...
Antibodies to GM-CSF and PAP in sera of patients treated with APC8015 and PA2024

<table>
<thead>
<tr>
<th>Patient</th>
<th>Maximum antibody titer to GM-CSF (time to maximum response, weeks)</th>
<th>Maximum antibody titer to PAP (time to maximum response, weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10,000 (16)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>20,000 (16)</td>
<td>320 (8)</td>
</tr>
<tr>
<td>3</td>
<td>40,000 (16)</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>20,000 (20)</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>320 (8)</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>640 (20)</td>
<td>10 (4)</td>
</tr>
<tr>
<td>9</td>
<td>80 (16)</td>
<td>10 (16)</td>
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<td>10</td>
<td>640 (16)</td>
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<td>1280 (16)</td>
<td>160 (20)</td>
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<tr>
<td>12</td>
<td>80 (16)</td>
<td>20 (12)</td>
</tr>
<tr>
<td>13</td>
<td>1280 (16)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Patients 7 and 8 suffered from rapidly progressive disease, and serum was not available for testing.

* Time to development of titer ≥10; for patients with preexisting antibodies, time to a 2-fold or larger increase in titer.

* Patient with preexisting antibodies to GM-CSF at titers ≥20.

* Preexisting titer was unaffected by treatment.

resulted in T-cell immune responses in the patients suffering from melanoma (7). These studies did not compare immunogenicity of dendritic cells pulsed with peptides with immunogenicity of the cells pulsed with whole proteins, but it is highly probable that tumor-cell lysates contained both HLA class I- and class II-restricted peptides.

Development of an effective immune response requires complex interactions between CD4-positive class II-restricted T cells and CD8-positive class I-restricted T cells. Possibly, protein-pulsed dendritic cells can stimulate both CD4- and CD8-positive T cells, whereas peptide-pulsed dendritic cells are designed to stimulate CD8-negative T cells only. The limited clinical data with antigen-pulsed dendritic cells suggest that breaking tolerance to hitherto unrecognized antigens such as PAP and prostate-specific membrane antigen requires stimulation of both CD4- and CD8-positive T cells (23). Under some circumstances, targeting CD8-positive T cells alone with class I-restricted peptides may suffice for stimulation of immunity, but the concurrent targeting of CD4-positive T cells may augment the response.

Although APC8015 raised T cells specific for GM-CSF and PAP with similar efficacy, the combined treatment (APC8015 and PA2024) had a different effect on raising antibodies against GM-CSF and PAP: (a) after 16–20 weeks, antibodies against GM-CSF were observed in most patients. Interestingly, antibodies against PAP were detected in only 5 (of 11) patients, invariably at low titers; (b) the titer of antibodies to GM-CSF was inversely proportional to the administered dose of PA2024; and (c) it is noteworthy that some patients harbored spontaneous antibodies to GM-CSF; however, this condition did not predict a high-antibody response to GM-CSF after treatment. In contrast to these results, we observed a higher rate of antibody responses to PAP and higher titers of anti-PAP antibodies in a study of APC8015 alone given on a schedule similar to the one used in the current trial (weeks 0, 4, and 8; Ref. 18). Comparison of the two trials of APC8015 suggests that administration of soluble antigen PA2024 in the dose and schedule used in this trial actually suppressed antibody generation. The administration of soluble antigen without adjuvant can suppress B-cell immune responses (24). However, GM-CSF is an immune adjuvant; our data and those of others demonstrate that fusion proteins containing GM-CSF elicit potent antibody responses in the absence of exogenous adjuvant.

Development of anti-GM-CSF antibodies after parenteral administration has been well documented (25, 26), but these antibodies appear to have no apparent clinical consequences. It is unlikely that antibodies to GM-CSF would affect the potency of APC8015 because dendritic cells display peptide fragments of GM-CSF and antibodies cannot recognize such peptides. Antibodies to GM-CSF could, however, limit the adjuvant effect of GM-CSF administered either as a fusion protein such as PA2024 or as a soluble protein boost.

In the reduction of the levels of circulating PSA and PAP, we found preliminary evidence of activity of this immunotherapy protocol. In one patient (5), however, PSA dropped to less than one-half of the pretreatment value at the time when the disease actively progressed; his PAP remained unchanged. This observation is in line with the concerns about the use of PSA as the sole end point for measuring the response of prostate cancer to novel treatments (27).

We did not observe any objective radiographic response to treatment. The time to disease progression after diagnosis was in the range of values reported for cytotoxic treatments for hormone-refractory prostate cancer, where progression was determined by means other than changes in PSA (28, 29). However, the treatment was well tolerated and elicited few side effects compared with chemotherapy. It raised PAP-specific immunity effectively. In addition, we observed sporadic treatment-induced decreases of circulating PSA and PAP. Consequently, we have initiated a Phase II trial with the highest dose of PA2024; the goal of the trial is to define better the response, time to progression, and survival in a cohort of men with hormone-refractory prostate cancer treated with APC8015 followed by PA2024.

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References

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Clinical Cancer Research

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Patrick A. Burch, Jami K. Breen, Jan C. Buckner, et al.


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