Dendritic Cell–Tumor Fusion Vaccines for Renal Cell Carcinoma

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ABSTRACT
Renal cell carcinoma is a malignant disease that demonstrates resistance to standard chemotherapeutic agents. A promising area of investigation is the use of cancer vaccines to educate host immunity to specifically target and eliminate malignant cells. Dendritic cells (DCs) are potent antigen-presenting cells that are uniquely effective in generating primary immune responses. DCs that are manipulated to present tumor antigens induce antitumor immunity in animal models and preclinical human studies. A myriad of strategies have been developed to effectively load tumor antigen onto DCs, including the introduction of individual peptides, proteins, or tumor-specific genes, as well as the use of whole tumor cells as a source of antigen. A promising approach for the design of cancer vaccines involves the fusion of whole tumor cells with DCs. The DC-tumor fusion presents a spectrum of tumor-associated antigens to helper and cytotoxic T-cell populations in the context of DC-mediated costimulatory signals. In animal models, vaccination with DC-tumor fusions resulted in protection from tumor challenge and regression of established metastatic disease. We have conducted phase 1 dose escalation studies in which patients with metastatic breast and renal cancer underwent vaccination with DC-tumor fusions. Twenty-three patients underwent vaccination with autologous DC-tumor fusions. Vaccination was well tolerated without substantial treatment-related toxic effects. Immunologic responses and disease regression were observed in a subset of patients. Future studies will explore the effect of DC maturation and cytokine adjuvants on vaccine potency.

IMMUNOTHERAPY FOR RENAL CANCER
Renal cell carcinoma (RCC) is a life-threatening malignant disease for which available therapy is inadequate. In one review of 690 patients enrolled in therapeutic trials for metastatic disease, the median survival was 10 months, with a 2-year survival of only 45% for those in the best prognostic category (1). RCC demonstrates high levels of resistance to standard chemotherapeutic and hormonal agents, with response rates typically less than 10%. In contrast, RCC has demonstrated particular susceptibility to immune-based treatment strategies. In a combined series of 255 patients, patients undergoing therapy with high-dose interleukin 2 (IL-2), demonstrated a 4% complete response rate and an 11% partial response rate. Tumor responses have also been observed after nonmyeloablative allogeneic transplantation because of a graft-versus-tumor effect (2, 3). However, toxicity from both of these approaches is substantial, and therapy is, therefore, limited to highly selected patients treated by experienced medical personnel.

Investigators have explored the use of tumor-specific immunotherapy in patients with RCC in an effort to enhance treatment efficacy while limiting toxicity. A variety of renal carcinoma-associated antigens have been identified that are recognized by patient-derived CTLs (4–8). Expression of RAGE-1, PRAME, Her-2/neu, G250, and gp-100 have been demonstrated in RCC cell lines and patient-derived specimens. Telomerase-specific T cells also effectively target renal carcinoma cells (9, 10). The MUC-1 tumor antigen was also found in most patient-derived specimens with increased expression in more advanced disease. Peptide-specific CTLs generated in vitro lyse antigen-expressing RCC cells in a major histocompatibility complex (MHC)-restricted fashion.

Despite the presence of tumor-specific antigens with the capacity to be recognized by CTLs, clinical responses are muted and ineffective in eradicating disease. The development of immune tolerance toward malignant cells is in part due to the likelihood that tumor antigens are presented to the immune system in the absence of crucial costimulatory signals necessary for the initiation of T-cell responses (11, 12). Therefore, T cells with the capability of recognizing these antigens become inactivated. A major focus of cancer immunotherapy has been the attempt to reverse tumor-induced anergy through the presentation of antigen in the context of appropriate secondary signals. In this way, native immunity directed against antigens selective for, or overexpressed in, malignant cells may be amplified and may result in tumor rejection.

BIOLOGY AND ROLE OF DENDRITIC CELLS AS CANCER VACCINES
Dendritic cells (DCs) are antigen-presenting cells that are uniquely capable of inducing primary immune responses (13, 14). DCs derive their potency from the prominent expression of costimulatory and adhesion molecules necessary for the activation of naïve T-cell populations (15–17). DC function depends on the level of maturation. Immature DCs reside at sites of antigen capture (i.e., skin, bronchoepithelium) and are functional in the internalization and processing of exogenous antigens. After antigen capture, DCs migrate to sites of T-cell traffic, lose phagocytic capacity, and, in concert with a mature phenotype, up-regulate expression of costimulatory molecules (18, 19). DCs have been generated in vitro by cytokine stimulation of precursor populations in the bone marrow, peripheral blood, or cord blood (11, 20–23).
A major area of investigation in cancer immunotherapy involves the design of DC-based cancer vaccines. DCs effectively induce antitumor immune responses through the presentation of tumor antigens in the context of DC-mediated costimulation. A myriad of strategies have been examined to introduce tumor-associated antigens into DCs. Murine studies have demonstrated that immunization with DCs, pulsed with tumor-specific peptides or proteins, protect animals from subsequent challenge with tumor cell lines and eradicate disease in cancer-bearing animals (24–26). Effective tumor vaccines have also been generated by the transduction of tumor genes into DCs through the use of retroviral and adenoviral vectors, as well as the pulsing of DCs with tumor-specific RNA (27–30).

Clinical trials have demonstrated immunologic and clinical responses after immunization with DCs pulsed with tumor-specific peptides (9, 31, 32). In one study, patients were immunized with CD34−-derived DCs pulsed with melanoma-derived peptides (32). Most patients demonstrated an increase in peptide-induced T-cell interferon-γ production after vaccination. Cellular immunity to at least two peptides was associated with clinical response. Investigators have demonstrated that immunization of lymphoma patients with idiotype-pulsed DCs has resulted in disease regression and the induction of idiotype-specific cellular immunity (33). Patients tolerated the therapy without difficulty and did not manifest signs of autoimmunity.

All of the above mentioned strategies involve the targeting of known tumor-associated antigens. Use of individual tumor antigens enhances the specificity of the resultant immune response and allows for the monitoring of cellular immune responses directed against defined peptide targets. However, immunotherapeutic approaches that rely on induction of immunity against a particular antigen are potentially subject to tumor cell resistance mediated by the down-regulation of that single gene product. In addition, tumor cells are likely to express a variety of specific antigens that have not yet been identified. The use of single gene products for DC-based immune strategies also limits the clinician to a small group of potential antigens of uncertain immunogenicity. Strategies to circumvent this limitation is the pulsing of DCs with tumor lysates, whole tumor RNA, or apoptotic bodies (34–37).

DENDRITIC CELL VACCINE STUDIES IN RENAL CARCINOMA

Several studies have examined the ability of DC-based vaccines to generate tumor-specific immune responses in patients with renal carcinoma (38–43). In one study, 12 patients were vaccinated with DCs pulsed with autologous tumor lysate and the immunogenic protein, keyhole-limpet hemocyanin (KLH; ref. 38). KLH and tumor-specific immune responses were demonstrated. In another study, 15 patients underwent vaccination with lysate-pulsed DCs administered into lymph nodes or adjacent tissue by ultrasound guidance (39). One patient experienced a partial response and seven demonstrated decreased capacity to stimulate allogeneic T-cell proliferation (51). However, fusion cells generated with both DC populations were equally effective in generating tumor-specific immune responses. In a murine myeloma model, fusions generated with mature, compared with healthy animals demonstrated decreased capacity to stimulate allogeneic T-cell proliferation (51). However, fusion cells generated with both DC populations were equally effective in generating tumor-specific immune responses. In a murine myeloma model, fusions generated with mature, compared with immature, DCs were more effective in generating antitumor CTL responses and protection from tumor challenge (52).

DC-tumor fusion vaccines have been generated by chemical means with PEG or with physical techniques such as electroporation (53). Fusion of DCs with a murine melanoma line that expresses β-galactosidase was accomplished by the delivery of a series of electrical pulses to align and fuse the cell membranes.

DENDRITIC CELL–TUMOR FUSIONS: ANIMAL STUDIES

A novel strategy for inducing effective antitumor immunity is through the use of hybridomas created by the fusion of DCs with tumor cells. In this approach, an immunogenic cell is created that expresses tumor antigens and DC-derived costimulatory molecules. Multiple tumor antigens, including those yet unidentified, can, therefore, be simultaneously presented in the appropriate HLA context. This strategy is likely to induce a polyclonal CTL response that would optimize the possibility of inducing tumor rejection.

Work from our laboratory has demonstrated that fusion cells are effective in inducing antitumor responses (44). In an animal model, murine MC38 adenocarcinoma cells were fused by coculture with syngeneic bone marrow-derived DCs in the presence of polyethylene glycol (PEG). The MC38 line was transfected with the human MUC-1 gene to serve as a tumor-specific marker. Fusion cells demonstrated dual expression of MUC-1 and the DC-derived costimulatory molecules B7-1 and B7-2. Vaccination with fusion cells was protective against an otherwise lethal challenge of tumor cells and induced disease regression in tumor-bearing animals. Harvested splenocytes demonstrated CTL-mediated killing of tumor targets that was inhibited by in vivo depletion of helper or cytotoxic T-cell populations.

Other studies have confirmed the effectiveness of DC-tumor fusion vaccines in the treatment of established B16 melanomas, Lewis lung carcinomas, RMA-S lymphoma, P815 mastocytoma, and other tumor types (45–47). In a murine transgenic model for human MUC-1, immunization with DC-tumor fusion cells that express MUC-1 resulted in the reversal of MUC-1 unresponsiveness and the generation of antitumor immunity without evidence of autoimmunity directed against MUC-1-expressing epithelial tissue (48). In a murine multiple myeloma and glioblastoma model, the efficacy of fusion cell vaccination was significantly enhanced by the administration of IL-12 as a cytokine adjuvant (49, 50). In another study, DCs generated from tumor-bearing compared with healthy animals demonstrated decreased capacity to stimulate allogeneic T-cell proliferation (51). However, fusion cells generated with both DC populations were equally effective in generating tumor-specific immune responses. In a murine myeloma model, fusions generated with mature, compared with immature, DCs were more effective in generating antitumor CTL responses and protection from tumor challenge (52).
A fusion efficiency of greater than 40% was observed, with the resultant population coexpressing β-galactosidase and DC-derived costimulatory molecules. In a treatment model, vaccination in conjunction with IL-12 resulted in disease regression and improved survival. In one study, (54) fusions of DCs with mammary carcinoma cells generated with PEG or electrical pulsed demonstrated similar protection in a tumor challenge model. A recent report described the transient creation of highly immunogenic fusion cells with a viral fusogenic membrane glycoprotein. The fusion cells efficiently migrated to draining lymph nodes (55).

**DENDRITIC CELL–TUMOR FUSIONS: PRECLINICAL HUMAN STUDIES**

Subsequent in vitro studies with primary human tumors have demonstrated that fusion cells effectively stimulate tumor-specific immune responses. In one model, fusions were generated with breast carcinoma cells and autologous DCs (56). Fusion cells coexpressed the MUC-1 tumor-associated antigen and DC-derived costimulatory molecules. The fusion cells maintained the functional potency of DCs and stimulated autologous T-cell proliferation. Significantly, these studies showed that autologous T cells are primed by the fusion cells to induce MHC class I-dependent lysis of autologous breast tumor cells. Similar results have been obtained with fusions generated with primary ovarian carcinoma cells (57). In another study, fusions generated with patient-derived chronic lymphocytic leukemia cells and autologous DCs were more effective than lysate-pulsed DCs in stimulating tumor-specific CTL responses (58).

**DENDRITIC CELL–TUMOR FUSIONS: CLINICAL STUDIES**

We are currently conducting clinical trials to study the safety profile and explore the immunologic impact of vaccination with DC–tumor fusion cells in patients with solid tumors and hematologic malignant diseases. We have completed studies in patients with breast and renal carcinoma (59). Patients with accessible tumor tissue whose tumor cells could be obtained without requiring major surgical intervention were potentially eligible. Sites of tumor acquisition included malignant effusions, soft tissue masses, superficial lymph nodes, and peripheral lung nodules accessible by thoracoscopy. Single-cell suspensions were generated by mechanical and chemical digestion. Tumor cells uniformly expressed cytokeratin, and most expressed MUC-1. Autologous DCs were generated from adherent peripheral blood mononuclear cells obtained by leukapheresis and cultured for 1 week with granulocyte macrophage colony-stimulating factor (GM-CSF), IL-4, and autologous plasma. DCs uniformly expressed DR and CD86. DCs and tumor cells were cocultured with PEG, and the resultant population underwent immunocytochemical and/or flow cytometric analysis to quantify the number of fusion cells as defined by coexpression of DCs and tumor antigens.

Twenty-three patients were treated (10 breast cancer patients and 13 renal cancer patients). Subcutaneous vaccinations were administered to each patient at 3-week intervals for a total of three doses. Patients were vaccinated with $1 \times 10^6$ DCs pulsed with KLH protein at the time of the first fusion vaccination. At a separate site, patients were concurrently vaccinated with fusion cells. In the breast cancer study, successive cohorts of patients received $1 \times 10^5$, $3 \times 10^5$, and $1 \times 10^6$ fusion cells. In the renal cancer study, successive cohorts of patients received $1 \times 10^6$, $2 \times 10^6$, and $4 \times 10^6$ fusion cells.

No substantial treatment-related toxic effects were observed, and full dose escalation was reached in each of the studies. Adverse events judged to be potentially related to vaccine therapy across studies included transient pain at sites of tumor, injection site reactions, transient fever, muscle aches, flu-like symptoms, pruritus, fatigue, peripheral swelling, rash, and transient elevations of antinuclear antibody titers in the absence of associated symptoms of autoimmunity.

A subset of patients demonstrated evidence of enhanced KLH-specific immunity after vaccination as manifested by increased proliferation of peripheral blood mononuclear cells in response to coculture with KLH protein. Tumor-specific immunity was assessed by measuring intracellular interferon-γ expression by T cells after in vitro exposure to tumor lysate. Of 18 evaluable patients, vaccination resulted in a 2-fold or greater increase in lysate-reactive CD4 and CD8 T cells in 10 and 7 patients, respectively. Two patients with metastatic breast cancer demonstrated disease regression, and six patients (five renal cancer patients and one breast cancer patient) demonstrated disease stabilization for 3 to 9 months after the completion of vaccination.

**ALLOGENEIC DENDRITIC CELL/RENAL CARCINOMA HYBRID VACCINES**

Investigators have explored the use of allogeneic DCs to generate the DC/tumor fusion vaccine. A potential advantage of this strategy is that DCs generated from normal donors, as compared with cancer patients, may demonstrate greater functional activity. Conversely, T-cell responses to allogeneic DC/tumor fusions are dependent on the inconsistent expression of MHC class I molecules by the tumor. In addition, the absence of class II expression by tumor cells may prevent the development of an antigen-specific helper T-cell response. In one report, disease regressions were reported after vaccination with allogeneic DC/tumor fusions (60). This study was subsequently retracted because of concerns regarding inaccuracies with primary data and insufficient documentation of methodology. In another study, eight patients underwent vaccination with allogeneic DC/renal carcinoma fusions (61). Stabilization of disease and the induction of antitumor immunity was seen in a subset of patients. In a recent preliminary report of a phase I study, 24 patients underwent serial vaccination with allogeneic DC/tumor hybrids. Vaccination was well tolerated, and antitumor immunity was observed in a subset of patients. Two patients experienced a partial response, and eight patients demonstrated stable disease (62).

**CONCLUSION**

DC vaccines represent a promising immunotherapeutic strategy for patients with malignant disease. DC-tumor fusions potently stimulate antitumor immunity in preclinical animal and human studies. We have initiated clinical trials to examine the
safety and the immunologic and clinical efficacy of this treatment approach. Future strategies will examine the role of cytokine adjuvants and mature DCs in enhancing vaccine potency. We will be initiating a study in which patients with RCC who are undergoing debulking nephrectomy will undergo vaccination with mature DC-tumor fusions.

OPEN DISCUSSION

**Dr. Robert Figlin**: In your design of future trials, would you consider vaccinating patients who are only CA-IX+?

**Dr. David Avigan**: That is something we should probably look at in the study to see whether we can tease out any difference in response.

**Dr. Figlin**: Is the GM-CSF in that study going to be given at the site of vaccination or at another site?

**Dr. Avigan**: At the site of vaccination.

**Dr. Figlin**: Have you learned anything about regulatory T cells (T-regs) and kidney cancer in all of your efforts when you look at the cell population that you are removing? How many T-regs are there in this population of cells?

**Dr. Avigan**: In terms of the vaccine itself, we do an adherence step in which we remove the large majority of T cells, if not all of them, so the product that we give back is relatively depleted of T cells. In terms of the phenotype of the T-cell populations that are stimulated in vivo by the vaccine, that is a very important point that we want to study as part of these upcoming vaccine trials.

**Dr. Richard Childs**: So the data you showed with some vaccinated patients having up to 10% of their T cells secreting γ interferon against peripheral blood mononuclear cells pulsed with lysate is obviously a huge percentage of cells.

**Dr. Avigan**: Remember, we are doing an in vitro stimulation with lysate.

**Dr. Childs**: So that is not fresh out of the blood?

**Dr. Avigan**: Unlike a tetrimeric study, it is a functional study that requires some degree of ex vivo stimulation.

**Dr. Childs**: In terms of T-cell antitumor reactivity, have you ever looked just fresh out of the blood?

**Dr. Avigan**: We have not. We are attempting to expand a minority population through an in vitro exposure. By comparing values seen at pre- and postvaccination, one is able to get some quantitative sense of the impact of vaccination.

**Dr. Childs**: In the 12 of 13 tumors in which the renal cells were DR+, are you sure those were renal cells? Were you studying hemopoietic cells? I very rarely ever see a renal cell that is DR+.

**Dr. Avigan**: We carefully characterized the cell populations by immunophenotyping. We made sure that our vaccine dose correlated only with cells that had distinctive features of both tumor and dendritic cells.

**Dr. James Yang**: How did you finally solve this conundrum about mature DCs being a better stimulus but not migrating? Why are there mature CCR7 increases after maturation?

**Dr. Avigan**: CCR7 expression increases with the onset of DC maturation. It does appear that the fusion procedure induces maturation in the DC fusion partner, potentially resulting in increased CCR7 expression. To definitively demonstrate migration patterns, one would need to tag the cells and determine whether they migrate to draining lymph nodes.

REFERENCES


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