Clinical and Immunologic Effects of Intranodal Autologous Tumor Lysate-Dendritic Cell Vaccine with Aldesleukin (Interleukin 2) and IFN-α2a Therapy in Metastatic Renal Cell Carcinoma Patients

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Abstract

Purpose: To evaluate the clinical and immunologic outcomes of DC (dendritic cell) vaccine with interleukin (IL)-2 and IFN-α 2a in metastatic renal cell carcinoma patients.

Experimental Design: Eighteen consented and eligible patients were treated. Peripheral blood monocytes were cultured *ex vivo* into mature DCs and loaded with autologous tumor lysate. Treatment consisted of five cycles of intranodal vaccination of DCs (1×10^7 cells/1 mL Lactated Ringer's solution), 5-day continuous i.v. infusion of IL-2 ($18 MiU/m^2$), and three s.c. injections of IFN-α 2a (6MiU) every other day. Response Evaluation Criteria in Solid Tumors criteria were used for disease assessment. Correlative immunologic end points included peripheral blood lymphocyte cell phenotype and function as well as peripheral blood anti–renal cell carcinoma antibody and cytokine levels.

Results: All patients received between two and five treatment cycles. Toxicities consisted of known and expected cytokine side effects. Overall objective clinical response rate was 50% with three complete responses. Median time to progression for all patients was 8 months, and median survival has not been reached (median follow up of 37+ months). Treatment-related changes in correlative immunologic end points were noted and the level of circulating $CD4^+$ T regulatory cells had a strong association with outcome. Pre–IP-10 serum levels approached significance for predicting outcome.

Conclusions: The clinical and immunologic responses observed in this trial suggest an interaction between DC vaccination and cytokine therapy. Our data support the hypothesis that modulation of inflammatory, regulatory, and angiogenic pathways are necessary to optimize therapeutic benefit in renal cell carcinoma patients. Further exploration of this approach is warranted.

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renal cell carcinoma   tumor vaccine therapy   immunotherapy   interleukin 2
dendritic cell vaccine   IFN-α

Translational Relevance

This article summarizes a phase II trial treating patients with metastatic renal cell carcinoma with a novel autologous dendritic cell (DC) vaccine combined with standard immunotherapy. Although DCs have been hailed as the most potent antigen-presenting cells, DC vaccines in general have been quite unsuccessful. Research over the past decade has shown that these cells play a key role not only in antigen presentation, but also as gatekeepers to immune tolerance. We hypothesized that intranodal DC injection combined with interleukin-2 and IFN-α2a would have enhanced clinical results. In addition, we used autologous tumor lysate to provide an individualized and wide-ranging antigen profile. The clinical results were very impressive with a 50% overall clinical response rate. Immunologic results support these clinical observations. Some of these immunologic

insights will have effect on future immunotherapeutic treatment protocols in this disease.

Renal cell carcinoma (RCC) contributes significantly to cancer-related mortality.[14] Improved overall survival (OS) in metastatic RCC (mRCC) patients treated with interleukin (IL)-2, IFN-α, or both have been described (1, 2). Clinical and laboratory data suggest a role for the host immune system and angiogenic pathways in this disease (3–11).

The most successful therapy for mRCC has been single-agent, high-dose aldesleukin (IL-2) with durable complete remissions in a small percent of patients. Prior attempts to improve IL-2 clinical outcome with the addition of other agents or effector cells have failed (5–8, 12, 13). New directed therapies, such as bevacizumab, sorafenib, sunitinib, and temsirolimus, have had significant effect on survival of mRCC patients and are used as first-line therapy in many centers. However, they rarely induce durable complete remissions (8–11).

A major obstacle for cancer immunotherapy is tolerogenic pathways that involve regulatory cells and immunosuppressive cytokines (14–16). IL-2 mobilizes not only immune effector cells capable of destroying cancer but also expands $CD4^+CD25^+FoxP3^+$ regulatory cells, which modify the immune response. Dendritic cells (DC), the most potent antigen-presenting cells, are able to activate proinflammatory tumor–specific immune pathways as well as disrupt regulatory pathways (17–24). However, clinical responses to DC vaccine (DCV) alone given by different routes have been limited. Intranodal injection seems to be the most effective delivery method for these cells (25, 26). IL-2 and IFN-α can also contribute to overcoming other dysfunctional immune pathways. IL-2 can rectify acquired T-cell receptor signaling defects seen in cancer patients. IFN-α enhances tumor immunogenicity by inducing expression of MHC molecules and tumor-associated antigens, and can enhance DC and T-cell function (27–29). The balance between stimulatory and regulatory immune pathways may explain, in part, the low response rates observed with immunotherapy of mRCC (30–34).

We hypothesized that immunotherapy using intranodal DCV, IL-2, and IFN-α2a could exploit immune pathways to enhance therapeutic benefit. We report the results of a phase II trial using this approach.

Materials and Methods

Patients

The study was approved by Dartmouth Medical School's Committee for the Protection of Human Subjects and the U.S.A. Food and Drug Administration (IND BB 11162). Eligible patients were ages ≥18 y, had measurable disease and newly progressive metastatic or new metastatic disease, adequate end-organ function, sufficient tumor tissue for vaccine preparation, and signed informed consent. Individuals with a history of brain metastases, HIV disease, hepatitis B/C, autoimmune disease, required the use of corticosteroids or other immunosuppressive agents, or had prior treatment with IFN-α, IL-2, or autologous vaccine, were excluded.

Treatment

Treatment consisted of two induction cycles of IL-2/IFN-α 2a given on days 1 and 14, and three maintenance cycle weeks every 28 d, consistent with the well-established Negrier regimen (12). IL-2 (Chiron, Inc.) was administered by continuous infusion (18×10^6 IU/M^2 for 120 h). IFN-α 2a (6 MIU; Hofman La Roche) was given s.c. every other day for three doses (Table 1). Dose reductions followed the National Cancer Institute Common Toxicity Scoring System. IL-2 was stopped for hypotension, atrial fibrillation, renal failure, respiratory distress, mental confusion, and metabolic acidosis. IL-2 was restarted at 75% of the dose at the time of the next cycle. For recurring toxicity, the dose was cut in half (37.5% of original dose). Similar dose modifications were made for IFN-α 2a for liver and renal toxicity as well as atrial fibrillation.

View this table: In this window In a new window	**Table 1.** Treatment scheme: Days of therapy and timing of administering each component of treatment during the induction and maintenance phases

Leukapheresis for DC preparation was done before treatment and before each maintenance cycle. DCV (1×10^7 DCs in 1 mL Lactated Ringer's Solution) was given intranodally under ultrasound guidance on the day before starting IL-2/IFN-α 2a. No dose modifications were permitted for DCV. Patients were allowed to continue with IFN-α 2a and vaccine therapy if

IL-2 dose limiting toxicity occurred. Duration of vaccine treatment was limited by number of DC available after leukapheresis. Cytokines were given according to protocol up to day 103.

Vaccine preparation

Monocyte precursors were enriched from pheresis product using elutriation (patient 1-15) or an anti-CD14 antibody magnetic bead and CliniMacs platform (patient 16-18; Miltenyi Biotec, Inc.). Elutriated monocytes were cultured in AIM-V serum–free media for 9 d with 500 IU/mL granulocyte macrophage colony-stimulating factor (Berlex, Inc.) and 20 ng/mL IL-4 (R & D Systems; day 0, 3, and 6), tumor lysate (1-3 tumor cell equivalents per DC; day 5), and 50 ng/mL tumor necrosis factor α (R & D System; day 6). CliniMacs enriched monocytes were cultured in X-Vivo 15 media with 1% heat inactivated autologous serum for 8 d with 1,000 IU/mL granulocyte macrophage colony-stimulating factor and 40 ng/mL IL-4 (day 0, 3, and 6), tumor lysate (1-3 tumor cell equivalents per DC; day 5), and 50 ng/mL tumor necrosis factorα and 1 µg/mL PGE2 (Sigma; day 7). The change to CliniMacs purification allowed a closed system for DC preparation and for greater monocyte cell purification. Freeze-fractured and irradiated tumor lysate was obtained from mechanically and enzymatically treated fresh tissue. Frozen DCs from preparation of vaccine #1, stored in 90% autologous serum and 10% DMSO, were thawed and used for vaccine #2. Requirement for release of the final DC preparation included >70% viability, negative sterility test from day 6 to 7 DC culture, endotoxin test, and a negative gram stain.

Clinical assessment

Patients were assessed serially using computed tomography of chest, abdomen, and pelvis, and technetium bone scan. Response was determined by the National Cancer Institute's Response Evaluation Criteria in Solid Tumors. Follow-up for responding and stable patients occurred quarterly until progression or as clinically indicated.

Correlative immunologic studies

Lymphocyte subpopulations were characterized by standard five-color flow cytometry and analyzed with FloJo software (35). Intracellular staining was done following cell fixation and permeabilization (Il-4, IFNγ) and intranuclear staining for FoxP3 (Biolegend FoxP3 kit). Tumor specific T-cell proliferation was determined using the Dye Dilution Proliferation Assay (36). Culture conditions included lymphocytes alone or combined with tumor lysate–loaded and unloaded DCs, Con A (proliferation control), and Staphylococcus aureus B (IFN-γ control). Pretreatment, midtreatment, and posttreatment time points were evaluated for T-cell receptor function. T-cell receptor function, reported in lytic units, was determined by a standard chromium release–redirected cytotoxicity assay using FcR-positive P815 tumor cell targets, and anti-CD3 antibody (OKT3; ref. 37).

For T_{REG} function, $CD4CD25^{high}$ (T_{REG}) and $CD4CD25^{-}$ T-cells (responder cells) were isolated using the Regulatory T Cell Isolation kit (Miltenyi Biotec). T_{REG} function was determined by [^{3}H]Thymidine uptake in cocultures of responder cells mixed with autologous T_{REG} cells in the presence and absence of T-cell activation beads (anti-CD3, anti-CD28, and anti-CD2: T Cell Activation/Expansion kit; Miltenyi Biotec; ref. 38). All experimental conditions were done in triplicate.

Presence of serum IgG and IgM anti-RCC antibodies was determined by flow cytometry using allogeneic RCC cell lines (CAKI, ACHN, 769-P) and mouse anti-human IgG and IgM. Presence of cell-bound serum antibody was detected using biotinylated goat anti Human IgG or IgM secondary antibody and Streptavidin conjugated DTAF (Jackson Laboratories).

Serum was analyzed for 27 cytokines [IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, basic fibroblast growth factor, Eotaxin, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, IFN-γ, IP-10, MCP-1 (MCAF), MIP-1α, MIP-1 β, platelet-derived growth factor–BB, RANTES, tumor necrosis factor-α, vascular endothelial growth factor] using the Luminex fluorescent bead technology according to manufacturer's protocol.

Statistical methods

This phase II trial was planned as a Simon two-stage design to detect a 40% overall response rate (complete plus partial response) compared with a hypothesized response rate of 20%. Institutional Review Board approval had been granted for the original study design, but due to limitation of funds, only the first stage was completed. A 95% confidence interval (CI) for the overall response rate was determined based on the exact binomial method. Progression-free survival (PFS) was defined as the time from study enrollment until disease progression, or death. OS was defined as the time from study enrollment until death. PFS and OS were censored at the date of last follow-up. The product-limit method was used to

estimate survival curves corresponding to progression-free survival and OS.

Correlative immunologic studies were assessed using Student's *t* test. For precomparisons and postcomparisons, paired *t* test was used. Mann-Whitney Rank Sum Test was used as a nonparametric test for not normally distributed data.

Results

Patients

Eighteen mRCC patients were enrolled between January 2004 and August 2006. The patient characteristics and outcomes are summarized in Table 2. Fourteen patients received greater than or equal to three DC-vaccinations. One patient was removed from treatment before his 5th cycle due to autoimmune toxicity.

View this table: In this window In a new window	**Table 2.** Patient characteristics and response

DCV

Tumor lysate was prepared from primary tumors for 17 patients and nodal metastases for one subject (patient #12). The mature DC phenotype (n = 48 vaccines) was reflected by a mean percent positive value of 5.8 ± 10.0 for monocyte marker CD14, 60.5 ± 23.6 for DC marker CD83, and 91.8 ± 13.0, 83.1 ± 22.8, and 82.7 ± 14.6 for MHC class II, CD80, and CD86, respectively.

Toxicity

Significant clinical toxicities were related to IL-2 and IFN-α2a (Table 3). Two responding patients had autoimmune clinical syndromes (myocarditis, pneumonitis, and nephritis that resolved over the subsequent 3 to 4 months; and parotitis).

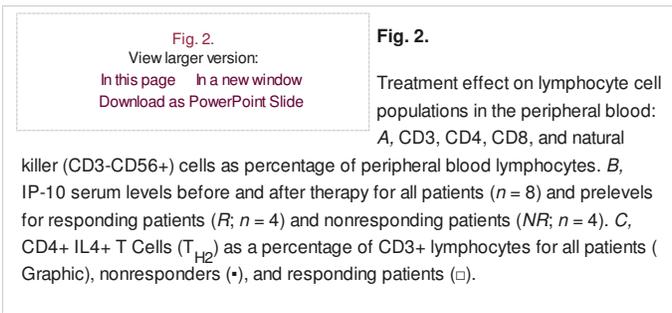|	
View this table: In this window In a new window	**Table 3.** Grade III and IV toxicity

Clinical response results

All patients were available for clinical assessment (Table 2). Follow-up ranged from 21 to 48+ months. Median PFS was 8 months (Fig. 1). The median OS has not been reached. A total of 9 objective responses were noted (50%; 95% CI, 22fs66%): three complete responses (CR), two >19.3 and >43.3 months; six partial responses (2.8-9.2 months); and six stable disease (3.2-25.9 months). Responses were seen in lung, liver, mesentery, and adrenal sites as well as a second primary renal tumor determined by biopsy after protocol therapy. Signs of clinical response were observed after the first two cycles as well as after completion of treatment. Histologic subtypes are listed in Table 2. Patients went on to receive targeted therapy as shown in Table 2.

Fig. 1. View larger version: In this page In a new window Download as PowerPoint Slide Graphic, PFS.	**Fig. 1.** Overall and progression free survival: PFS: Graphic, OS;

Correlative immunologic end points

A treatment-related increase in the precursor frequency of RCC-specific CD8$^+$IFNγ$^+$ T cells was noted in peripheral blood lymphocytes [0.027 ± 0.055 (before) versus 0.082 ± 0.098 (after); P = 0.058]. No relationship with clinical response or PFS was identified.
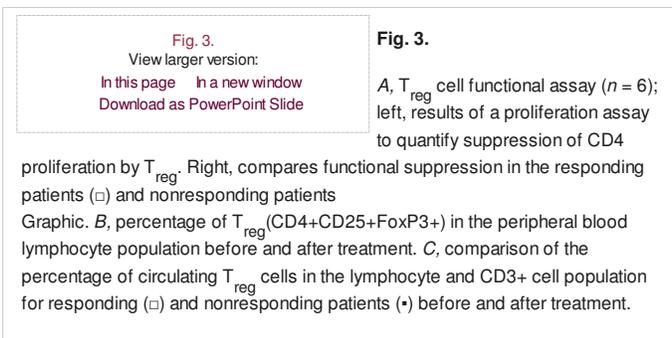
The percentage of CD4 and CD8 T cells in the peripheral blood lymphocyte population did not change as a result of treatment, but the percentage of CD3$^-$CD56$^+$ natural killer cells increased during treatment [15.1 ± 10.3% (before) versus 23.1 ± 12.0% (after); P = 0.015; Fig. 2A]. No significant changes were seen in the percentage of CD4$^+$IFNγ$^+$ Th1 cells in the T-cell population, but the percentage of CD4$^+$IL-4$^+$ Th2 cells increased with treatment in responding patients [4.7 ± 1.4% (before) versus 13.1 ± 8.0% (after); P = 0.095; Fig. 2C].

Fig. 2.

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Fig. 2.

Treatment effect on lymphocyte cell populations in the peripheral blood: *A,* CD3, CD4, CD8, and natural killer (CD3-CD56+) cells as percentage of peripheral blood lymphocytes. *B,* IP-10 serum levels before and after therapy for all patients ($n = 8$) and prelevels for responding patients (R; $n = 4$) and nonresponding patients (NR; $n = 4$). *C,* CD4+ IL4+ T Cells (T_{H2}) as a percentage of CD3+ lymphocytes for all patients (Graphic), nonresponders (▪), and responding patients (□).

Cytokine multiplex results revealed a treatment-related increase in antiangiogenic factor and TH1 cytokine IFN γ inducible protein 10 [IP-10: 126 ± 71 pg/mL (before) versus 521 ± 244 pg/mL (after); $P = 0.002$; Fig. 2B]. Responding patients had higher levels of IP-10 in pretreatment serum than nonresponding patients (169 ± 68 pg/mL versus 83 ± 45 pg/mL; $P = 0.07$).

CD8$^+$ T cell T-cell receptor function was evaluated in 11 patients who received at least 3 vaccines (4 NR, 7 R). Overall, treatment-related lytic activity increased in CD8$^+$ T cells by 33% but did not reach statistical significance.

We observed a treatment related increase in the percentage of CD4+CD25+FoxP3+ T_{REG} cells in the lymphocyte population [$2.0 \pm 1.0\%$ (before) versus $4.4 \pm 2.7\%$ (after); $P = 0.002$; Fig. 3B]. T_{REG} cells from six patients tested showed suppressive function. Surprisingly, T_{REG} cells seem to suppress less effectively in responding versus nonresponding patients, although this may reflect a less brisk proliferative response in the CD4-responding cell population (Fig. 3A).

Fig. 3.

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Fig. 3.

A, T_{reg} cell functional assay ($n = 6$); left, results of a proliferation assay to quantify suppression of CD4 proliferation by T_{reg}. Right, compares functional suppression in the responding patients (□) and nonresponding patients Graphic. *B,* percentage of T_{reg} (CD4+CD25+FoxP3+) in the peripheral blood lymphocyte population before and after treatment. *C,* comparison of the percentage of circulating T_{reg} cells in the lymphocyte and CD3+ cell population for responding (□) and nonresponding patients (▪) before and after treatment.

After two induction cycles, nonresponders ($6.5 \pm 2.7\%$) showed a significantly stronger expansion of Treg cells within the lymphocyte compartment than responding patients ($2.7 \pm 1.0\%$; $P = 0.004$). This difference was also true when Tregs were examined as a percentage of the CD3 population (Fig. 3).

Treatment increased serum IgM anti-RCC antibody levels and led to a significant increase in percent positive IgM staining on allogeneic tumor cell lines [$22.1 \pm 13.7\%$ (before) versus $38.8 \pm 21.7\%$ (after); $P = 0.022$]. However, no changes in IgG antibody levels were observed.

Discussion

Although the results of small phase II trials have significant limitations, we found a 50% overall objective response rate (95% CI, 27-74%) and a 16% CR (95% CI, 0-34%) that compares favorably to the historical observations of 16% overall objective response rate and 6% CR for high-dose IL-2 or IL-2 plus IFNα therapy (2–7, 12) and an 11% partial response rate recently reported in a small phase I/II single agent autologous tumor lysate-DCV therapy (39). The median time to progression (8 months) seen in this trial also compares favorably with recent reports of targeted therapy (8, 9). Based on historical data from the Memorial Sloan Kettering Cancer Institute and the University of California at Los Angeles Integrated Staging System, we would have predicted a median survival of 13.8 months, and a 2- and 3-year survival of 38.8% and 29%, respectively. At this time, median survival of these 18 patients has not yet been reached and the 2- and 3-year survival is 77% and 70%, respectively. OS data needs to be seen in the context of new directed therapies becoming available during the follow-up time period.

Historically, cancer immune therapies have focused on stimulation of effector cells. Interest in enhancing antitumor immune responses in cancer patients by inhibition of regulatory cells has resurged. Modulation of only one component of the immune equation induces long

lasting clinical benefit in only a small minority of patients. We hypothesized that multiple signals, which modulate both effector and regulatory functions, are necessary to improve immune therapy for cancer patients. In this study, we have shown that DCs, IL-2, and IFNα enhance tumor-specific CD8^{+} effector cells. A statistically significant differential expansion of treatment-induced peripheral blood T_{REG} cells favored a lower percentage of T_{REG} cells in responding patients. This finding of T_{REG} correlation was unexpected. This supports a T_{REG} cell threshold effect on clinical outcome (40). We also found CD4^{+} IL-4^{+} tumor-specific precursors increased in responding patients, suggesting a role for Th2 pathway. At present, it is unclear what the significance of the association of this alternative pathway and clinical outcome is and how it may be better exploited in the future.

Interestingly, we observed a treatment-related induction of IP-10, an antiangiogenic cytokine and chemoattractant for activated T cells, natural killer cells, and monocytes and a relationship between outcome and pretreatment IP-10 serum levels. IP-10 expression in RCC tumors has been described as a predictor of outcome, and shown to be induced by IL-2 and implicated as a component of the TH1 response (41, 42). Our observation reinforces the link between immune pathways and tumor angiogenesis and the potential to modulate both these systems for effective therapy.

Until recently, antigen-specific approaches have been limited by the lack of RCC-specific molecules. There are conflicting reports of clinical efficacy using immunodominant peptides derived from carbonic anhydrase-9 (43–45). By using tumor lysate, we incorporated a broader repertoire of potential tumor antigens, which may also include tumor-associated nonprotein molecules. Use of cellular products may also reduce the likelihood of tumor immune escape and expand the eligibility of participating patients.

Development of autoimmunity with immunotherapy has been reported in other studies (46–48). The induction of severe clinical autoimmune-like phenomena in one patient with a complete and durable response suggests autoimmune phenomena may be a potential surrogate marker of benefit.

Clinical responses were observed early and late in the treatment course suggesting there may be a range of predispositions toward immune responsiveness. Our prior studies have also implicated heterogeneity of patients' immune "readiness" (49). Immune profiling may ultimately define these states more clearly.

In conclusion, this study provides evidence that supports multitargeted immunotherapy for mRCC as a means to regulate effector, regulatory, and angiogenic pathways. Furthermore, elevated levels of circulating vascular endothelial growth factor have been shown to confer poor prognosis in RCC and other solid tumors, and are associated with higher numbers of circulating immature DCs and immunosuppression. Vascular endothelial growth factor blockade can enhance Type-1 cytokine response and could be a reasonable addition to the described DCV/IL-2/IFN regimen (40).

Deeper understanding of immune pathways and the interplay between different targeted therapies provide promising avenues to investigate in future trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Footnotes

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