Original Article

Dendritic Cell Vaccines Presenting Autologous Tumor Antigens from Self-renewing Cancer Cells in Metastatic Renal Cell Carcinoma

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Abstract

Background and objectives: Metastatic renal cell cancer is typically resistant to chemotherapy but does respond to vascular endothelial growth factor receptor signal transduction inhibition and to a variety of immunotherapies, including interleukin (IL)-2 and monoclonal antibodies that inhibit immune checkpoints. Enhanced immune recognition of tumor antigens may improve clinical outcomes. The objective of this study was to investigate the effects of a patient-specific approach utilizing autologous dendritic cell vaccines and self-renewing autologous tumor cells in patients with metastatic renal cell carcinoma.

Methods: Short-term cell lines were established from resected renal cell cancer specimens. Autologous dendritic cells were derived from peripheral blood mononuclear cells cultured in granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4. Antigen loading was accomplished by incubating irradiated tumor cells with dendritic cells. The vaccine was admixed with GM-CSF and injected subcutaneously weekly for 3 weeks, then monthly for 5 months.

Results: Short-term cell lines were established for 28 patients. Dendritic cells were produced for 11 patients. Nine patients were referred for treatment. The nine patients received 58 injections, and 6 received all 8 planned doses. Treatments were well-tolerated, other than mild to moderate injection site reactions. One patient experienced an anaphylactoid reaction attributed to microaggregates in GM-CSF. Three patients had conversion from negative to positive for a delayed-type hypersensitivity test to intradermal injection of one million autologous irradiated tumor cells. Two of seven patients had delayed complete regression of measurable oligometastatic disease. Three patients were still alive and disease-free after 5 years.

Conclusions: This patient-specific vaccine approach is feasible, well-tolerated, and associated with encouraging long-term survival in some patients. Larger trials of dendritic cells with autologous tumor antigens derived from self-renewing tumor cells, rather than bulk tumor, may be warranted.

Introduction

Prior to the new millennium, metastatic renal cell cancer (mRCC) was a highly lethal disease with a 5-year survival rate of less than 10%.[1] Chemotherapy was considered ineffective, and the standard therapies were interferon (IFN)-α and high-dose interleukin (IL)-2, but the latter was not an option for many patients because of its severe side effects. IFN-α produced response rates of only 5% to 10%, but in a 360-patient randomized trial it was associated with a higher response rate, longer median survival (8.5 months *vs*. 6 months), and greater 1-year survival (43% *vs*. 31%) compared to medroxyprogesterone acetate hormonal therapy.[2] IL-2 received regulatory approval for mRCC in May 1992 based on a response rate of 15% among 255 patients enrolled in seven different trials, and especially the durabil-

ity of those responses.[3] Subsequent reports described response rates of 19% and 23%,[4,5] and as high as 48% in more favorable subsets.[6] However, at a national level neither of these agents significantly improved survival for the population of mRCC patients.

Treatment of mRCC changed dramatically in 2005 after the introduction of small molecule, targeted, tyrosine kinase inhibitors (TKIs), which for several years have been the standard treatments of choice as initial and salvage therapy in mRCC.[7] These agents all block signal transduction pathways involving vascular endothelial growth factor, in addition to other pathways. The first of these to be approved was sorafenib, in December 2005. Currently available similar agents include sunitinib (January 2006), pazopanib (October 2009), axitnib (January 2012), and cabozantinib (April 2016). Another class of agents approved after 2005 for use in mRCC is the inhibitors of the mammalian target of rapamycin (commonly known as mTOR). These mTOR inhibitors include temsirolimus (May 2007) and everolimus (March 2009). Recently, the TKI lenvatinib (May 2016) was granted a marketing indication for use in combination with everolimus. Also approved was the anti-vascular endothelial growth factor monoclonal antibody bevacizumab (August 2009), which is marketed for use in combination with IFN-α. In large randomized trials both sunitinib and temsirolimus were clearly superior to IFN-α.[8,9] None of these agents has been compared directly to high-dose IL-2, and it is doubtful there ever will be such a comparison because of the more rigorous baseline health requirements needed for IL-2 therapy.

Availability of these agents has impacted survival for mRCC patients treated in the community practice of oncology. For instance, 5-year survival rates for patients with metastatic kidney cancer at diagnosis doubled from 7.5% ($n = 40$) during 1998–2003 to 15.8% ($n = 54$) during 2004–2009, median survival increased from 8 to 10 months, and survival for patients with regionally advanced disease at diagnosis increased from 56% ($n = 43$) to 69% ($n = 51$).[10] National data from the Surveillance Epidemiologic End Result program revealed a relative survival rate of 12% for distant-disease stage patients with cancer of the kidney and renal pelvis who were diagnosed during 2007–2013.[11] These data suggest that despite the approval of so many new agents, primarily on the basis of response rates and improved progression-free survival (PFS), mRCC is still a highly lethal disease, and there is still an unmet need for agents that can improve survival further.

There is great optimism that advances in immunotherapy beyond IFN-α and IL-2 will result in additional survival benefit. The monoclonal antibody nivolumab that binds to the programmed death molecule-1 immune checkpoint, was approved for the treatment of mRCC in November 2015. In an 821-patient randomized trial of nivolumab versus everolimus in patients with previously-treated mRCC, the programmed death molecule-1 inhibitor was associated with a higher response rate and longer overall survival.[12] Recently, in a large trial of first-line therapy in mRCC, the combination of nivolumab and ipilimumab (the cytotoxic T-lymphocyte antigen-4 inhibitor) was found to be superior to sunitinib, in terms of objective response rate (42% *vs.* 27%), PFS (11.6 *vs.* 8.4 months), and 18-month overall survival (75% *vs.* 60%) with fewer grade 3 and 4 adverse events (46% *vs.* 63%).[13] Unfortunately, because of the toxicity anticipated for the combination of nivolumab and ipilimumab, the patients enrolled in this trial were younger and probably healthier than the general population of patients with mRCC. Furthermore, among the 550 patients treated with the combination immunotherapy, there were eight treatment-related deaths and 22% of patients discontinued treatment because of treatment-related toxicity. Thus, despite the improvements in survival, it is coming at the expense of significant toxicity, and even greater expense when combination therapies are used. Many

practicing oncologists would prefer to treat with a programmed death molecule-1 inhibitor such as nivolumab or pembrolizumab alone rather than with a more toxic and costly combination.

It had been hoped that vaccine approaches would result in clinical benefit similar to that seen with these newer agents, but so far that has not been the case. Still, because of their lack of toxicity and different mechanism of action, there may be a niche for therapeutic vaccines, especially in combination with other therapies.[14] Dendritic cells (DCs) are the most effective antigen presenting cells, and patient-specific autologous tumor cell antigens may be the best source of antigens for a vaccine therapy.[15] The objective of this study was to investigate, in patients with mRCC, the effects of a patient-specific therapeutic vaccine approach utilizing autologous DCs loaded with antigens from autologous tumor cells that had been self-renewing in a short-term cell culture prior to irradiation.

Methods

Regulatory assurances

Patients were treated according to a clinical trial that conformed to the ethical guidelines of the 1975 Declaration of Helsinki. All patients gave written informed consent prior to enrollment for treatment. The clinical protocol was approved by the Hoag Hospital Institutional Review Committee for the Protection of Human Subjects, the U.S. Food and Drug Administration (BBIND8554), and reviewers for the National Cancer Institute's Physician Data Query (clinical trial number: NCI-V01-1646). The ClinicalTrials.gov Identifier is NCt00014131.

Design

This study was designed as an open-label, single arm trial with an adaptive trial design based on evidence of delayed type hypersensitivity (DTH) reactions to intradermal (i.d.) injections of their irradiated tumor cells. The initial intent was to enroll up to 40 patients who had measurable disease at the time of enrollment, and up to 40 patients who did not have measurable disease at that time. The primary endpoints were overall survival, PFS, and objective response rates in those patients who had measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST).[16] Secondary objectives were identification of adverse events (AEs) and results of tumor DTH tests.

Autologous tumor cell lines

Surgically resected cancer specimens (kidney primary or metastatic lesion) were submitted on behalf of patients with mRCC. Tumors were transported in a prepared kit containing physiologic media supplemented with fetal bovine serum, penicillin, streptomycin, gentamicin and fungizone. After arrival in the lab, tumors were mechanically and enzymatically dissociated into single-cell suspensions and either cryopreserved or immediately put into tissue culture as previously described.[17,18] Fibroblast contamination was eliminated by a combination of nutritional protein starvation and a preferential attachment procedure.[19] Initial success was defined by expansion to 50 million cells. The patient's managing physician was notified once a cell line was successful, and if there was continued interest in possible treatment, the culture was expanded to 200 million cells. A portion of the cells were transferred

into serum-free media to avoid any contamination with bovine proteins, for use in the i.d. skin tests. The remaining cells were incubated with IFN-γ at a concentration of 1,000 U/mL for 72 hours, to increase expression of histocompatibility antigens and to induce damage associated molecules that attract DCs.[20] The cells were then irradiated in a 100 Gy cesium irradiator. The irradiated tumor cells (ITCs) were cryopreserved for potential incubation with DCs.

Autologous DCs

DCs were derived from peripheral blood mononuclear cells (PBMCs) obtained during a single leukapheresis procedure processing 1.5 to 2 L blood volume. Cellular components in the PBMC product were estimated using a Coulter counter. Monocytes were enriched from the apheresis PBMC product using Ficoll density gradient centrifugation. Monocytes were differentiated into DCs over 6 days by culturing in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 as previously described.[17,18,20] Flow cytometry to measure fluorescence from monoclonal antibody binding to surface antigens was used to measure certain cell markers.[20,21]

Autologous dendritic cell vaccine (DCV)

As previously described for melanoma patients,[20] a range from 150 to 350 million DCs were incubated overnight with 100 million ITCs for phagocytosis and antigen loading. Ten aliquots were prepared, with each DCV dose containing 3 to 33 million cells. Quality control sterility and endotoxin tests were performed on the final DCV product. For each patient, 10 individual DCV doses were cryopreserved in liquid nitrogen.

Patient eligibility

Key eligibility criteria for treatment were: (1) histologic diagnosis of RCC; (2) availability of a short-term autologous tumor cell line; (3) availability of monocytes derived from the patient's PBMCs; (4) referral by the managing physician for vaccine therapy; (5) willingness to travel to Newport Beach, CA, USA for DCV injections; (6) Karnofsky performance status ≥70, Eastern Cooperative Oncology Group performance level of 0–2; and, (7) satisfying usual phase II clinical trial criteria for hematologic, metabolic, renal and hepatic chemistry tests. At the time of treatment, patients had to be 16 years of age or greater. Patients with brain metastases were eligible if known tumors had been controlled with therapy and they were not taking pharmacologic doses of corticosteroids. Treatment was deferred until at least 1 month after any immunosuppressive therapy, including high-dose corticosteroids, chemotherapy, or radiation therapy. Patients were tested for reactivity to common recall antigens (Candida and trichophyton), but anergy was not an exclusion criterion. Presence of measurable disease at the time of treatment was not an eligibility requirement, but patients were stratified for analysis based on whether they had measurable disease per RECIST.

Treatment schedule

At the time of each treatment, a cryopreserved vial of DCV was thawed and suspended in 500 micrograms of GM-CSF and injected within 5 hours of thawing. As in related trials,[20,22] subcutaneous injections were administered during weeks 1, 2, 3, 8, 12, 16, 20 and

24. Concurrent anticancer therapy was not allowed.

Follow up information

After completion of vaccine injections, patients were followed-up in person and/or by telecommunication every 3 months to collect information regarding administration of additional anticancer therapies, any delayed severe adverse events (SAEs) that might be attributable to vaccine treatment, approximate date of disease progression, or date and cause of death if deceased. Follow up was continued for up to 5 years from the date of enrollment for surviving patients.

Survival

Tumor responses were defined according to RECIST for patients with objectively measurable disease. Patients without measurable disease were monitored for disease progression at a maximum of 3-month intervals by physical examination and photon emission tomography or computerized tomography scans and, if symptomatic, whole-body bone scan and brain magnetic resonance imaging. Managing physicians and clinical trial staff identified the first site of disease progression and a date of disease progression to estimate PFS. Overall survival was calculated from the date of baseline DTH test (1 week before the first DCV injection) to the date of death.

DTH to autologous tumor cells

At baseline, and following completion of the first three weekly vaccinations, patients underwent i.d. tests for DTH to one million ITCs that had not been exposed to IFN-γ and had been maintained and cryopreserved in serum-free medium. Within 48 to 72 hours of injection, the test was interpreted by nursing staff as negative (no induration), weakly positive (5 to 9 mm induration), or positive (≥1 cm induration). The protocol was amended to add a third tumor DTH test after 5 months of therapy, just prior to the last vaccine injection for patients who received all eight injections.

Safety

AEs were assessed at each visit for a vaccine injection, and 4 weeks after the last injection. AEs and SAEs were classified and graded 0 to 5 per National Cancer Institute Common Toxicity Criteria for Adverse Events, version 3.0.

Statistics

Overall survival and PFS were estimated using the methods of Kaplan-Meier. The 95% confidence intervals were determined for proportions. Means were compared using the Student's *t*-test. Proportions were compared using Fisher's exact test.

Results

Tumor cell lines

From January 2000 to December 2006, short-term tumor cell lines

Table 1. Percentage of cells positive for HLA1 or HLA-2 and the MIF per flow cytometry, before and after incubation with IFN-γ

n = 21	Pre IFN-γ	Post IFN-γ
HLA-1 %	97 ± 7	99 ± 1
HLA-1 MIF	359 ± 282	833 ± 403
HLA-2 %	10 ± 15	84 ± 24
HLA-2 MIF	75 ± 118	168 ± 153

Abbreviations: HLA, human lymphocyte antigen; IFN-γ, interferon-gamma; MIF, median intensity of fluorescence.

were established for 28 of 119 samples submitted to the laboratory. More specifically, these were characterized as 28 successes, 54 failures to grow and 9 poor quality samples, and 28 samples were discontinued. Efforts to establish cell lines were discontinued because of ineligibility for the clinical trial ($n = 17$), due to physician decision ($n = 5$), death of patients ($n = 3$), patient request ($n = 2$), and technical processing error ($n = 1$). Thus, the estimated success rate was 28/72 (38.8%, 95% CI: 27.6% to 50.2%).

The time to cell line success took 0.5 to 10.3 months, with an average of 4.0 ± 2.3 months to reach 50 million cells. The success rates were similar whether the cultures were started immediately after processing, or following cryopreservation: 32.8% (19/58) for fresh samples, and 37.5% (9/24) success rate from cryopreserved samples. The length of time to a successful culture was faster for fresh tumors, with a mean of 3.5 ± 2.35 months compared to 5.2 ± 1.60 months from cryopreserved samples ($p = 0.032$). It took an average of 5.76 ± 2.69 months to prepare a cell line and complete quality control testing of cells for potential incubation with DCs.

Twenty-one cell lines were incubated with 1,000 units/mL of IFN-γ for 72 hours. As can be seen in Table 1, incubation with IFN-γ resulted in an 8-fold increase in the percentage of cells considered positive for HLA-2 expression, and an increase in median intensity of fluorescence for both HLA-1 and HLA-2.

DCs

DCs were successfully generated for 11/11 patients who underwent leukapheresis to collect PBMCs. All 11 were incubated with ITCs to prepare the DCV containing DCs loaded with autologous tumor antigens. Table 2 summarizes the data for 9 patients who were subsequently treated with DCV and for two DCV products that were prepared but never administered because the patients were not referred for treatment. The apheresis PBMC products

averaged 70% lymphocytes, 19% monocytes, and 11% granulocytes. The apparent decrease in viability in the final product was due to the presence of residual ITCs that were not phagocytized. Table 3 summarizes the phenotypes after incubation of DCs with ITCs. The decrease in CD11c positivity was due to dilution by residual nonphagocytosed ITCs, which did not express CD11c. The increase in median intensity of fluorescence for CD83 and CD80 was consistent with some DC maturation after incubation with ITCs.

DCV therapeutic product

DCV consisted of autologous DCs and residual ITCs that were not phagocytosed during overnight coincubation. Frozen aliquots of DCV were cryopreserved in liquid nitrogen. At the time of treatment one aliquot was thawed, and viability and cell count were determined. The cells were then washed and suspended in GM-CSF for injection. Table 4 shows the averages and ranges for the first three injections for all 9 patients and for the 6 patients who received an eighth dose. There was little variation in number and viability of cells among doses for each individual patient, but there was substantial variability between patients in numbers of cells injected. The 20% to 25% nonviable cells was the result of residual ITCs that had not been phagocytosed. It took a median of 5.0 months and a mean of 4.75 ± 3.36 months (range: 2.00 to 9.25 months) from the time of initiation of cell culture to prepare the patient-specific vaccine product, including quality control testing on the DCV product.

Clinical trial

The first patient enrolled in October 2001 and the final patient in March 2006. One patient started treatment in 2001, three in 2002, two in 2003, one in 2004, one in 2005, and one in 2006. Enrollment was discontinued in 2007 because of poor accrual. Accrual likely was negatively impacted by the increasing availability and efficacy of small molecule TKIs, such as sorafenib and sunitinib. The study was closed to follow up in 2009, when the last of the long-term surviving patients had been followed for 5 years.

Patient characteristics

The clinical characteristics of the 9 patients enrolled for treatment are summarized in Table 5. There were 8 men and 1 woman, with a range in ages from 29 to 74 years. All had distant mRCC. Five

Table 2. Relationship of apheresis PBMCs to DCs before and after incubation with autologous ITCs for the final treatment product

	Used for treatment, n = 9	Not used for treatment, n = 2
PBMCs from apheresis	$3.9 ± 1.1 × 10^9$	$10.2 ± 6.9 × 10^9$
Lymphocytes	70.4 ± 6.2%	57.9 ± 1.6%
Monocytes	19.4 ± 4.2%	24.5 ± 0.6%
DCs pre-ITCs antigen loading	$2.1 ± 0.6 × 10^8$	$5.0 ± 2.8 × 10^8$
Viability of DC pre-ITC antigen loading	92.6 ± 5.7%	99 ± 0.0%
DCs post-ITCs antigen loading	$2.2 ± 1.0 × 10^8$	$5.5 ± 4.2 × 10^8$
Viability of DCs post-ITCs antigen loading	85.0 ± 4.8%	96.0 ± 5.7%

Abbreviations: DC, dendritic cell; ITC, irradiated tumor cells; PBMC, peripheral blood monocyte.

Table 3. Phenotypic characterization of DCs by positivity and MIF pre and post incubation with ITCs

	Treated patients, *N* = 9		Untreated patients, *N* = 2	
	Pre-ITC incubation, *N* = 6*	Post-ITC incubation, *N* = 8*	Pre-ITC incubation, *N* = 2	Post-ITC incubation, *N* = 2
HLAII %	100	100	100	100
CD80 %	36 ± 21	29 ± 17	42 ± 23	38 ± 32
CD80 MIF	95 ± 19	162 ± 55	87 ± 40	130 ± 100
CD11c %	100 ± 0.5	86 ± 16	100 ± 0	95 ± 5
CD11c MIF	4,061 ± 1,893	4,590 ± 2,443	6,745 ± 680	4,380 ± 3,080
CD83 %	7 ± 5	13 ± 18	7 ± 0	5 ± 4
CD83 MIF	93 ± 28	190 ± 105	56 ± 1.4	117 ± 33
CD86 %	86 ± 15	70 ± 20	56 ± 11	55 ± 22
CD86 MIF	2,660 ± 1,186	2,986 ± 1,113	2,098 ± 275	1,576 ± 1,064

Abbreviations: DC, dendritic cell; ITC, irradiated tumor cell; MIF, median intensity of fluorescence. *Two of the treated patients did not have phenotype testing pre-ITC loading, and one treated patient did not have phenotype testing post-ITC loading.

Table 4. Numbers and viability of cells in dendritic cell vaccine products

	Average # cells × 10^6	Range of cell # × 10^6	Average viability	Range of viability
Dose #1, *n* = 9	15.0	4.5 to 31.2	76%	67–92%
Dose #2, *n* = 9	15.6	5.0 to 26.4	78%	61–88%
Dose #3, *n* = 9	15.1	5.0 to 27.0	78%	64–88%
Dose #8, *n* = 6	12.9	3.7 to 23.0	77%	56–96%

of the nine had metastatic disease at the time of initial diagnosis; the other four had recurred with mRCC after an earlier diagnosis. Three had previously had no treatment other than surgery.

Treatment and outcomes

Treatment and outcomes are summarized in Table 6. Initial DTH and anergy tests were performed between 16 October 2001 and 17 March 2006. These 9 patients received 58 injections, with 6 patients completing all eight planned injections. Three patients experienced conversion of their DTH tests from negative to positive; all three survived beyond 2 years, but only 1 of the 3 patients who

survived 5 years had conversion of their DTH test.

Patient summaries

Patient #1-RR had spinal compression from metastases with leg weakness at the time of initial diagnosis. He discontinued treatment after three vaccinations because of recurrent problems in the area of prior spine surgery, which necessitated another operation.

Patient #2-HS had lung and bone lesions at the start of treatment that did not progress during treatment but did progress about 11 months after completing the series of injections. He died from progressive disease 11 months later.

Table 5. Patient characteristics at baseline

Patient ID	Age in years	Sex	ECOG	Prior Rx in addition to nephrectomy	Measurable disease
1-RR	57	M	2	RT, IFN-α	Adrenal, pelvis, bone, brain
2-HS	72	M	0	None	None
3-DD	67	M	1	RT, IL-2, CE	Lung, Bone
4-CG	53	F	1	None	Neck nodes
5-RK	49	M	0	ALT	Abdominal nodes
6-DC	62	M	1	IL-2	None
7-JO	74	M	0	None	Lung
8-JM	29	M	1	RT	Lung, abdomen
9-LL	65	M	2	IFNα + Bev, Sorafenib	Lung, bone

Abbreviations: ALT, autologous lymphocyte therapy; Bev, bevacizumab; CE, chemoembolization; ECOG, Eastern Cooperative Oncology Group; IFN-α, interferon-alpha; IL-2, interleukin-2; RT, radiation therapy.

Table 6. Summary of treatment and clinical results for patients treated with autologous DCV

Patient ID	# DCV	Ave # cells per dose × 10⁶	Baseline DTH	Post DCV DTH	Obj Resp	First PD site	PFS in months	OS in months
1-RR	3	15.5	Neg	Neg	PD	Bone	1.4	25.5
2-HS	8	28.2	Neg	Pos	–	Lung, bone	16.4	27.4
3-DD	4	21.9	Neg	Neg	PD	Lung, bone	1.9	13.1
4-CG	8	11.5	Neg	Neg	PD	Neck, RP	1.7	10.4
5-RK	8	12.8	Neg	Neg	SD*	Brain	10.9	>60
6-DC	8	4.8	Neg	Neg	–	None	>60	>60
7-JO	8	20.5	Neg	Pos	SD*	Brain	22.4	>60
8-JM	3	12.4	Neg	Neg	PD	Abdomen	1.5	5.7
9-LL	8	8.0	Neg	Pos	PD	Lung	1.9	26.1

Abbreviations: AE, adverse event; DTH, delayed type hypersensitivity test to autologous tumor cells; neg, negative; Obj Resp, objective response; OS, overall survival; PD, progressive disease; PFS, progression-free survival; Pos, positive; RP, retroperitoneum; SD, stable disease. *Subsequent delayed complete response.

Patient #3-DD had presented with spinal compression from metastases at the time of initial diagnosis. He went off-study after four injections because of progressive disease in the bone and lung, and died about 11 months later.

Patient #4-CG had presented with left cervical adenopathy that persisted at the start of DCV. Scans showed progression of cervical and retroperitoneal lymph nodes during DCV therapy but she completed all eight doses. She subsequently was treated with IL-2 but discontinued because of toxicity. She died 15 months from the original diagnosis, and 5 months after her last DCV injection.

Patient #5-RK had recurred 4 years after original nephrectomy and adjuvant auto-lymphocyte therapy.[23] At baseline, he had measurable pulmonary lymph node metastases that remained stable throughout treatment. These eventually resolved several months after completing the series of DCV injections. He subsequently progressed in the brain and was treated with gamma knife. After that, he remained disease free and was still alive at 5 years.

Patient #6-DC developed bilateral lung metastases 4 years after the original diagnosis. These responded to IL-2, and a residual lung lesion was resected and was the source of ITCs for the vaccine. He had no measurable disease at the time of treatment. He remained disease free after 5 years.

Patient #7-JO had metastatic disease that included several lung nodules (largest 3.1 cm in the right basilar area), and cytologically-proven malignant pleural effusion. Following nephrectomy for a 3.5 cm tumor primary tumor on 06/09/03, a computed tomography scan on 07/30/03 showed pleural effusion and several lung nodules (largest 3.0 cm in the right basilar area and others measured as 2.6 cm, 2.2 cm, and 1.7 cm. On 12/01/03 these were measured as 2.6, 1.7, and 1.4 cm, and the pleural effusion had resolved. When he started the vaccine in May 2004, a 1.0 cm lung lesion persisted as the only site of measurable disease. This lesion remained stable initially then slowly resolved such that 7 months after starting therapy, in November 2004, his scans were interpreted as showing no metastatic disease and, therefore, in complete remission. He subsequently was diagnosed with a brain metastasis that was treated with gamma knife. He remained disease free and was still alive at 5 years.

Patient # 8-JM had familial RCC. More than 2 years after undergoing nephrectomy, he presented with pulmonary symptoms and was found to have multiple lung metastases and recurrence in the renal bed that were present when DCV was initiated. DCV was discontinued after three doses because of progression. He subsequently was treated with high-dose IL-2. He died 2.5 years from

the original diagnosis, 9 months after the diagnosis of recurrent and metastatic disease, and within 6 months of starting DCV.

Patient #9-LL had extensive lung metastases when originally diagnosed. He had some response to sorafenib prior to starting DCV. He progressed during DCV treatment but completed all 8 doses. He subsequently again briefly responded to sunitinib; after progression, a trial of inhaled GM-CSF was discontinued because of toxicity. He survived an additional 8 months without therapy.

Cutaneous tests for immunological response

DTH testing

Two of the nine patients (#2-HS, #8-JM) were anergic at baseline based on skin tests for Candida and trichophyton, and none of the nine reacted to the autologous tumor DTH test at baseline. Two patients converted to a positive (>10 mm) test at 4 weeks (#2-HS, #9-LL), and another patient converted at the completion of 6 months of therapy (#7-JO). The third patient (#3-DD) had an intermediate skin test result at week 4. Thus, 4/9 (44.%, 95% CI: 11.9% to 76.9%) showed some immune response to autologous tumor that was not present at study entry. As shown in Table 6, among the 3 patients whose tumor DTH test converted from negative to positive, all survived more than 2 years. However, of the 3 5-year survivors, only 1 had conversion of the tumor DTH test.

Toxicity

The vaccine was well-tolerated except for mild to moderate pain and erythema at the injection site in 5/9 patients (3 grade-2, 2 grade-1), and 1 patient (#5-RK) experienced an anaphylactoid reaction. The highest grade of toxicity experienced by any individual patient was 3 with grade 0, 1 with grade 1, 4 with grade 2, 1 with grade 3, and 0 with grade 4. One grade 2 AE was bone discomfort that was attributed to GM-CSF since the patient had no bone metastases.

#5-RK experienced a grade 3 anaphylactoid reaction after his eighth and final injection on 06/24/03. This was reported as an SAE because management included hospitalization. He had experienced mild hives and pruritus on the neck and palms of hands during the prior week, which was responding to diphenhydramine. After the final vaccination, he left the facility in stable condition,

but called clinical trial personnel 15–20 minutes later and reported increased rash, shortness of breath, and numbness of lips and feet. On his arrival back in the Clinic, he reported continuing numbness of lips and feet, as well as chest tightness. He was treated with oxygen and intravenous medications and then admitted to the hospital. Specific medications used in treatment were dexamethasone, diphenhydramine, cimetidine, ranitidine, and insulin. The patient responded and by the following morning had returned to baseline with mild residual rash; so, he was discharged home where he had no further sequelae. It was eventually determined this was likely due to microaggregates in the GM-CSF preparation related to manufacturing changes after the product had been transferred from Immunex to Amgen to Berlex.

Discussion

The original objectives of this study included manufacturing feasibility, immune response based on tumor DTH skin tests, safety observations, and anticancer efficacy including response rate, PFS, and overall survival. The results of this abbreviated clinical trial are being reported because it is evident that despite advances in targeted therapies, including oral TKIs targeting signal transduction pathways, mTOR inhibitors, and monoclonal antibodies to immune checkpoints, there is still an unmet need for nontoxic complementary therapies for patients with mRCC. This report on patient-specific therapeutic DCV demonstrates feasibility, limited toxicity, and data consistent with anticancer effect in several patients with mRCC.

With regard to feasibility, the first requirement is to have a short-term cell culture as a source of antigen from self-renewing autologous tumor cells. However, the success rate in this era was only 39%. This is well below the 71% (56/79) success rate previously reported from this laboratory.[22] One reason for this may have been more rigorous criteria for determining that a cell line was malignant renal cancer cells. The laboratory had previously recognized that some putative renal cell cancer cell lines generated from primary kidney masses in the past consisted of normal renal cells rather than malignant renal cells, based on differences in the ability to tolerate freezing and thawing. Reliable, rapid establishment of cell lines would be important for potential commercialization of this approach. So, while this trial confirmed feasibility, it did not establish the reliability or practicality of the approach, at least with the cell culture strategies in use at the time of this trial. On the other hand, satisfactory apheresis PMBC products were collected for each patient and DCs were produced for all patients. The final product was intended to be all DCs, but in fact contained sizeable numbers of non-phagocytosed ITCs. It may be desirable to have a purer DC product.

Toxicity does not appear to be an issue for this or any other DCVs reported to date. In this trial the DCV was well-tolerated, other than the one anaphylactoid reaction which was attributed to the adjuvant GM-CSF. As in most studies of DCVs, the most common AEs were reactions at the injection site and various symptoms that can be characterized as flu-like, including fever, malaise, myalgias, arthralgias, bone discomfort and nausea. This constellation of symptoms has been reported regardless of the antigens being presented.[24–26] Bone discomfort is a well-known side effect of GM-CSF that is caused by expansion of granulocytes and monocytes in the bone marrow, and is probably not related to the DCV itself.

There was some suggestion of efficacy in this study. One patient, who was already experiencing a spontaneous regression of metastatic disease, had a delayed complete regression of the only measurable lesion that persisted. Another patient had delayed regression of pulmonary adenopathy. Neither of these occurred in a time frame to be classified as objective responses per RECIST. Similar to what has been seen in melanoma patients treated with such patient-specific DCV, there appeared to be no improvement in PFS but an apparent impact on OS. This is consistent with the hypothesis that tumor-initiating cells are being preferentially targeted with this approach, rather than antigens expressed on the more numerous terminally differentiated tumor cells that predominate in any measurable tumor lesion. As a consequence, even when such a vaccine is working, there likely will still be apparent disease progression before there might be stabilization and then regression.[15] The 33% actual 5-year survival rate is of note, in as much as the national figure was less than 10% for patients with mRCC during the years this trial was conducted.[1]

We previously reported results for 25 renal cell cancer patients treated with ITCs as a tumor cell vaccine (TCV) that was administered with a variety of different adjuvants, primarily GM-CSF and IFN-γ.[27] However, 8 of those patients had only a large primary lesion or locally advanced disease that had been resected. In that study, the 14 patients with measurable disease had a median survival of 7.5 months, and only 2 (14%) survived beyond 3 years. In contrast, in the trial reported here, among the 7 patients with measurable disease, median survival was 25.5 months, and 2 (29%) patients survived beyond 5 years. Although the numbers are small, this is consistent with other clinical data that suggests the DCV approach is superior to the TCV approach. The latter depends on *in vivo* rather than *ex vivo* antigen loading of DCs. These results are consistent with those seen in a randomized trial that compared DCV to TCV in patients with metastatic melanoma, in which the median survival was twice as long for patients treated with DCV as opposed to TCV (43.4 *vs.* 20.5 months), with a 70% reduction in the risk of death.[28]

Others have conducted small studies of autologous DCVs in mRCC, as summarized elsewhere.[24,25,29,30] Antigen sources have included shared peptides,[31] allogeneic cell lines,[32] autologous tumor cells,[33] autologous tumor lysate,[34] and autologous tumor RNA.[35] The treatment used in this report differs in the use of autologous tumor cells that are self-renewing *ex vivo*, which may preferentially present antigens associated with tumor stem cells and early progenitor cells and/or rapidly proliferating cells. One review article identified 197 patients treated with DCVs in 14 phase I/II trials, but included patients treated with allogeneic DCs.[24] A more recent review that limited analysis to those reports that included at least 6 patients and only autologous DCs came up with 12 trials involving 186 patients.[25] Similar to this report, most of the trials involved small numbers of patients with substantial variability in extent of metastatic disease. Objective response rates were estimated at 6% to 13% in the two meta-analyses. Most AEs were local injection site reactions and flu-like symptoms; less common were myalgias, fatigue, bone or articular pains. Few toxicities were detected above grade 2.

The largest experience with a DCV for mRCC has not been published but information is available from press releases. The product is a DCV with the generic name rocapuldencel-T, previously referred to as AGS-003 (Argos Therapeutics). Autologous DCs are transfected with amplified mRNA from autologous tumors and RNA for a synthetic truncated human CD40 ligand. This DCV is injected i.d. The phase III randomized trial (known as 'ADAPT') tested the DCV as front-line treatment in patients with mRCC. The trial enrolled 462 patients from 107 institutions between May 2013 and July 2015. The patients were randomized 2:1 to receive rocapuldencel-T plus standard therapy (primarily sunitinib) or standard therapy alone. The primary endpoint of the

study is overall survival. In February 2017, it was announced that an Independent Data Monitoring Committee had recommended halting follow up based on a futility analysis at a time when 75% of the projected 290 deaths had occurred.[36] Final analysis is projected in 2018.

The obvious weakness of our study was that it could not be completed as planned due to poor enrollment; therefore, the results are for only a small cohort of heterogenous patients. A major objective of this effort was to treat as many patients as possible for whom a tumor cell line had been generated. Therefore, it was anticipated that this patient cohort would be heterogenous in terms of extent of disease at the time of treatment because of variation in clinical courses from the time a sample of metastatic tumor was obtained to the time of actual treatment. Also, other than the tumor DTH skin testing, there was no evaluation of immune responses based on changes in lymphocytes or cytokines. As noted earlier, successful commercial application of this approach would necessitate a higher and faster rate of success in establishing autologous tumor cell lines.

Hypothesis/Future Direction/Prospective and Prediction

Cancer heterogeneity leads to the hypothesis that the optimal approach to personalized therapeutic vaccines is via autologous tumor antigens. Autologous DCs presenting such antigens appear to offer advantages over injections of irradiated autologous tumor cells themselves. Clinical results with autologous DCVs are encouraging but it is unclear what the optimal DC product is, and it is unclear whether strategies that enrich for tumor initiating cells as the source of antigen are superior to whole tumor lysate or mRNA as sources of autologous tumor antigens. Additional investigation and clinical trials are warranted.

Conclusions

In this study, there was evidence of clinical activity for a patient-specific DCV on the basis of two delayed complete remissions, and an observed 5-year survival rate of 33%. This was achieved with minimal toxicity. Larger trials of DCs with autologous tumor antigens derived from self-renewing tumor cells, rather than bulk tumor, may be warranted, especially as an adjunct to other therapies, but the autologous tumor cell lines must be produced more rapidly and reliably for this to be practical.

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Conflict of interest

RO Dillman is currently an employee of AIVITA Biomedical, Inc.

C Depriest has no conflicts of interest.

Author contributions

Dr. Dillman designed the clinical trial, directed the laboratory that manufactured the investigational products, analyzed the data, and wrote the manuscript. Ms. Depriest coordinated the clinical trial including product administration, monitoring of patients, and collection of data, and she reviewed the manuscript.

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