Vaccine Therapy for Renal Cell Carcinoma

Robert J. Amato, DO

Scott Department of Urology, Baylor College of Medicine, Houston, TX

Several potential vaccines have been evaluated for the treatment of patients with renal cell carcinoma (RCC). They include dendritic cells pulsed with tumor lysate, a dendritic cell–tumor cell hybrid, irradiated tumor cells admixed with adjuvants, and a heat shock protein–peptide complex. Promising results have been obtained in several early clinical trials, but issues of tumor immunosuppression and lack of identified tumor-associated antigens must be addressed before vaccine therapy can be applied successfully in advanced RCC. In this patient population, vaccine therapy will likely be required in combination with other forms of immunotherapy, such as interleukin-2 and thalidomide. In contrast, vaccine therapy alone may be sufficient for high-risk patients in the adjuvant setting.

[Rev Urol. *2003;5(2):65–71*]

© *2003 MedReviews, LLC*

Key words: Dendritic cells • Heat shock proteins • Renal cell carcinoma • Vaccines

Renal cell carcinoma (RCC) is treated by curative nephrectomy in early-stage disease, but 20%–30% of patients ultimately develop metastatic lesions.[1] Moreover, distant metastases may already be present at the time of diagnosis in up to 50% of patients. Once metastatic disease is present, the prognosis is poor, with median survival of 7–11 months. Adjuvant therapy has not proved effective in RCC patients at high risk for progression. In advanced RCC, cytotoxic chemotherapy and radiotherapy are generally ineffective, and immunotherapy with interleukin-2 (IL-2) or interferon alpha (IFN-α) produces objective responses in only 10%–15% of patients.[2,3] Even when these cytokines are administered in combination

with chemotherapy, the response rates are only marginally higher, and survival does not appear to be prolonged. Moreover, severe systemic toxicity often results from the large cytokine doses needed for treatment responses. Accordingly, more effective and safer treatment regimens are needed.

The activity of IL-2 and IFN-α implies that RCC may be sensitive to other immune-based strategies.

Distant metastases may already be present at the time of diagnosis in up to 50% of patients.

Under normal immune surveillance, the immune system recognizes and destroys tumor cells through the coordinated actions of dendritic cells and lymphocytes. The dendritic cells are "professional" antigen-presenting cells that recognize tumor-associated antigens, process them into small peptides, and then present these peptides to T lymphocytes within the context of the major histocompatibility complex (MHC). If this presentation is made in the presence of appropriate co-stimulatory molecules, then it leads to clonal expansion of activated T lymphocytes and generation of cytotoxic T lymphocytes that mediate specific tumor immunity. Unfortunately, tumor cells are able to evade immune surveillance by creating a microenvironment that suppresses cytotoxic T-cell mechanisms or by altering or masking expression of tumor-associated antigens. Tumor vaccines have been developed and evaluated in an effort to make tumor cells more immunogenic and thereby overcome their defense mechanisms. This article reviews the current status of vaccine therapy in RCC.

Dendritic Cells

Dendritic cells are found naturally in low numbers, accounting for only 0.15%–0.7% of circulating mononuclear cells.[4] They are derived from at least two precursor populations: $CD34^+$ bone marrow stem cells and circulating $CD14^+$ monocytes. The development of culture methods for differentiating and expanding dendritic cell populations has been an important step, allowing clinical evaluation of dendritic cell vaccines. The $CD14^+$ monocytes can be differentiated into dendritic cells by incubation with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4, whereas the $CD34^+$ stem cells generally require additional cytokines, including tumor necrosis factor-alpha (TNF-α), c-fms-like tyrosine kinase ligand 3 (flt3-ligand), and CD40 ligand.[5] Both sources of dendritic cells appear to be equally effective for use in vaccine therapy. The dendritic cells may then be pulsed in culture with tumor-associated antigens or a tumor lysate and then adoptively transferred back into the patient.

Several studies demonstrate that functional dendritic cells can be isolated consistently by these culture techniques. These cells have cytoplasmic veils characteristic of dendritic cells, and they express phenotypic dendritic cell markers, including MHC class II molecules, CD1a, CD4, intercellular adhesion molecule-1 (ICAM-1), and the co-stimulatory molecules B7-1 and B7-2.[6] In contrast, they do not express markers for monocytes, T cells, or B cells. Functionally, the cultured dendritic cells are potent co-stimulators of phytohemagglutinin-induced proliferation of autologous tumor-infiltrating lymphocytes (TILs). When pulsed with an autologous RCC tumor lysate and cocultured with autologous renal TILs, the dendritic cells acted synergistically with low-dose IL-2 in stimulating growth expansion of the TILs, up-regulating $CD4^+$ and $CD8^+$ T-cell populations, enhancing cytokine production by the TILs, and enhancing autologous tumor cell lysis.[7] Functional dendritic cells have also been prepared directly from nonfractionated peripheral blood mononuclear cultures.[8]

Dendritic Cell Vaccines with Tumor Lysates or Irradiated Tumor Cells

Dendritic cell vaccines have been evaluated in several clinical studies of patients with advanced RCC (see Table 1). Investigators at the University of Innsbruck in Austria isolated dendritic cells by culturing adherent peripheral blood mononuclear cells with GM-CSF and IL-4, pulsing them with autologous tumor lysate and keyhole limpet hemocyanin (KLH), and finally activating the cells by incubation with TNF-α and prostaglandin E_2.[9,10] The activated dendritic cells were then administered via three consecutive monthly intravenous infusions. Treatment was well-tolerated, with moderate fever being the only side effect. Immunologic activity against both the tumor lysate and the KLH was evident after the vaccinations, but only 1 of 7 patients achieved a

Severe systemic toxicity often results from the large cytokine doses needed for treatment responses.

partial clinical response. The responding patient developed a Th1-predominant response that was associated with a strong delayed-type hypersensitivity (DTH) reaction against KLH.

Kugler and colleagues[11] at the University of Göttingen in Germany used an electrofusion technique to generate a hybrid vaccine consisting of allogeneic dendritic cells and irradiated autologous tumor cells. Seventeen patients with RCC were given the vaccine by subcutaneous injection followed 6 weeks later by a second injection. All patients without evidence of disease progression received further booster injections every 3 months. The hybrid vaccine was well-tolerated, with mild to moderate fever and tumor pain reported. After a mean follow-up of 13 months, 4 patients completely rejected all metastatic tumor lesions and 2 additional patients had tumor mass reductions of more than 50%. All patients with objective responses developed DTH reactions to the autologous tumor. Notably, vaccination with the dendritic cell–tumor cell hybrid led to induction of cytotoxic T-cells reactive with the mucin 1 (Muc1) tumor-associated antigen. These promising results need to be confirmed in larger randomized trials.

Other Tumor Cell and Lysate Vaccines

Investigators at the University of Michigan recently evaluated vaccine-primed lymph node cells in patients with metastatic RCC.[12] Patients were first vaccinated intradermally with irradiated autologous tumor cells admixed with bacillus Calmette-Guérin (BCG), and then had lymph node cells harvested 7–10 days later. The lymph node cells were activated ex vivo with anti-CD3 and this population was then expanded with IL-2. Intravenous infusion of the activated cells was followed by administration of 0.36 mIU IL-2 every 8 hours for up to 5 days. Patients with responses or stable disease were retreated 2 to 3 months later. Of 33 evaluable patients, 4 responded completely and 4 responded partially, for an objective response rate of 24%. Complete responses lasted 11 to 48 months or more, partial responses 4 to 59 months or longer. These results suggest that adoptive transfer of vaccine-primed lymph node cells plus IL-2 administration produces durable objective responses in patients with metastatic RCC.

In another study, patients with metastatic RCC were vaccinated with autologous tumor cells admixed with GM-CSF, followed by adoptive lymphocyte infusion.[13] The vaccine was injected intradermally at four sites, and a second vaccination was given 2 weeks later. Lymphocytes were collected by leukophoresis, then activated and expanded with anti-CD3 and IL-2, and then reinfused. Finally, 3 mIU of IL-2 was given subcutaneously for 5 days. Up to four courses of immunotherapy were administered.

Table 1
Summary of Vaccine Trials in Metastatic or Advanced RCC

Vaccine	Trial	No. Evaluable	Efficacy, no. (%)
Dendritic cell/tumor lysate vaccines	Holtl et al 1999[9] Rieser et al 2000[10]	7	PR: 1 (14)
	Kugler et al 2000[11]	17	CR: 4 (24) PR: 2 (12)
	Chang et al 2002[12]	33	CR: 4 (12) PR: 4 (12)
	Gurjal et al 2001[13]	16	OR: 1 (6)
	Dillman et al 2001[16]	26	Patients without evidence of disease at time of treatment, OR: 5 (19) Patients with measurable disease at time of treatment, OR: 0
Heat shock protein vaccines	Amato et al 1999[24]	29	CR: 1 (3) PR: 3 (10)
	Amato et al 2000[25]	25	CR: 1 (4) PR: 1 (4)

RCC, renal cell carcinoma; PR, partial response; CR, complete response; OR, overall response

Of the 16 patients, there was one case of sepsis and one death due to the lymphocyte infusion. One patient had an objective response to treatment but died during nephrectomy at 9 months. After a median follow-up of 5 months, five patients had stable disease; in one, it remained stable for 31 months.

An autologous tumor lysate vaccine was evaluated in the adjuvant setting in a multicenter phase III trial conducted in Germany.[14] Patients with pathologic stage T2-3N0-3M0 RCC were randomly assigned to undergo radical nephrectomy followed either by tumor lysate vaccine or no adjuvant treatment. Six injections of the vaccine were given at 4-week intervals. Of the 365 patients, only 35 had positive lymph node findings. Three-year, progression-free survival was 84.7% in the group receiving vaccine and 80.9% in the group without adjuvant treatment; the advantage of the adjuvant vaccine was more pronounced in patients with T3 (74.4% vs 65.9%) than in those with T2 (89.7% vs 85.7%) tumors. In contrast, in a prospective study of 120 consecutive RCC patients, adjuvant therapy with a vaccine consisting of irradiated autologous tumor cells admixed with BCG increased DTH responses to autologous tumor, but it did not improve 5-year disease-free and overall survival compared with a control group that did not receive the vaccine.[15]

Another vaccine approach used short-term cell cultures established from primary RCC tumors or metastatic lesions.[16] A total of 27 patients received irradiated autologous tumor cells admixed with various adjuvants, administered subcutaneously each week for 3 weeks and then monthly for 5 months. Of 10 patients without evidence of disease at the time of treatment, 9 were still alive after 1 to 8 years and 5 had conversion of their DTH response to the tumor cells from negative to positive. In contrast, none of the 16 patients with measurable metastatic disease at the time of treatment responded to treatment. This group had a median survival of 5 months, and only 1 patient had a conversion of the DTH response. This study suggests that vaccine therapy with short-term cultures of autologous tumor cells is feasible in the adjuvant setting but is not effective in patients with advanced disease.

One study suggested that vaccine therapy with short-term cultures of autologous tumor cells is feasible in the adjuvant setting but is not effective in patients with advanced disease.

Dendritic Cell Vaccines with Tumor-Associated Antigens

The preceding trials used tumor cells or a cell lysate, because specific tumor-associated antigens have not yet been identified in RCC (see Table 1). Nevertheless, the observation that Muc1 cytotoxic T-cells were generated following administration of the hybrid vaccine suggests that Muc1 glycoprotein may be a possible antigen for use in dendritic cell vaccines. Preliminary results were reported recently from a phase I study of dendritic cells pulsed with a mannan-conjugated recombinant Muc1 fusion protein.[17] Plastic-adherent peripheral blood mononuclear cells were isolated by leukophoresis from patients with Muc1 solid tumors, then cultured with GM-CSF and IL-4, and pulsed with the Muc1 fusion protein. The Muc1 dendritic cell vaccine was given as two intradermal injections of 10 million cells each, and any remaining cells were injected subcutaneously. The procedure was repeated two more times at 4-week intervals. All 4 patients who completed three cycles demonstrated strong Th1 cellular responses and DTH reactions. Two of these patients had stable disease for >10 weeks, whereas the other 2 patients progressed.

Another candidate tumor-associated antigen in RCC is G250. On the basis of studies with a G250 monoclonal antibody, this protein appears to be expressed on 85% of primary and metastatic RCC but not on normal kidney cells. When dendritic cells were loaded with G250-derived peptides and cultured with autologous $CD8^+$ T cells, they generated cytotoxic T cells capable of lysing target cells expressing G250.[18] Recently, a G250–GM-CSF fusion protein was shown to be more effective than GM-CSF in activating dendritic cells and enhancing T-cell–mediated anti-tumor activity against RCC.[19] The activity of dendritic cell vaccines with Muc1 or G250 in RCC patients remains to be established.

An alternative strategy for augmenting dendritic cell function is to block CTLA4, a molecule that normally interferes with co-stimulatory signals provided by the interaction of B7 on dendritic cells and CD28 on T cells. In a recent study, peripheral blood mononuclear cells were collected from patients receiving idiotype-pulsed dendritic cell vaccines.[20] Gradient centrifugation was used to enrich for dendritic cell precursors and T cells, which were then pulsed with idiotype and control proteins in the presence or absence of blocking antibodies to CTLA4. Idiotype-specific activation of $CD4^+$ and $CD8^+$ populations was greatly augmented by blocking CTLA4, and the populations of these cells could be expanded while

maintaining tumor cytolytic activity. Thus, blocking CTLA4 may improve tumor targeting by dendritic cells.

Tumor-associated antigens can also be applied to the surface of cell-sized microspheres and then used as an immunogen. In a phase I/II study, a vaccine consisting of tumor cell membrane protein attached to 10 million microspheres was administered to patients following palliative resection of metastatic RCC or melanoma.[21] The vaccine was given either alone, in combination with cyclophosphamide, or in combination with cyclophosphamide and IL-2. Two doses of vaccine were administered at 4-week intervals. Cyclophosphamide was given 1 week before the first vaccine dose, whereas IL-2 was administered for 1 week starting 5 days after each vaccination. The first 13 patients in the study included 4 with metastatic RCC. One patient with resected metastatic RCC who was treated with the vaccine plus cyclophosphamide and IL-2 remained free of disease at 6 months after therapy.

Heat Shock Protein Vaccines

Heat shock proteins (HSPs) isolated from cancer cells provide protective immunity that is specific to that malignancy, whereas those derived from normal tissues do not show antitumor activity.[22] The specificity of cancer-derived HSPs has been attributed to HSP-associated peptides. Whereas these peptides and HSPs are nonimmunogenic separately, the peptides chaperoned by HSPs are able to elicit antigen-specific $CD8^+$ cytotoxic T lymphocytes. Although these findings suggest that some HSP-associated peptides may be unique to cancer, it has not been possible to identify them against the background of peptides common to both normal and cancerous cells. Moreover, the specificity of the HSP-peptide complex for one cancer relative to another suggests that a large and complex array of peptides may be involved. These peptides are likely generated by random mutations that accumulate in cancer cells due to continuous cell division and genetic instability, thus leading to an antigenic fingerprint unique to each tumor.

HSP-peptide complexes have been shown to elicit specific immunity to murine tumors.[23] The proteins gp96 and hsp70 were highly and equally immunogenic in these models, whereas a third protein, hsp90, was much less immunogenic. The lower immunogenicity of hsp90 was attributed to a lack of measurable adenosine triphosphatase activity, needed for transferring the peptide to acceptor molecules on antigen-presenting cells. Mechanistically, it appears that antigen-presenting cells possess HSP receptors that take up the HSP-peptide complex and then process and present the chaperoned peptides within the context of MHC I molecules.[22] Moreover, exposure of antigen-presenting cells to the HSP-peptide complex leads to secretion of inflammatory cytokines and expression of co-stimulatory molecules. These findings suggest that HSP-peptide complexes derived from tumors may represent an active immunotherapy option for cancer patients.

Tumor-mediated suppression of the immune system represents the major hurdle facing vaccine therapy for RCC.

Two clinical trials have been conducted on RCC patients using a vaccine consisting of autologous tumor-derived HSP 96-peptide complex (HSPPC-96) (see Table 1). In the first study, patients received one of three vaccine doses—2.5, 25, or 100 μg—given weekly for 4 weeks, starting 4–6 weeks after surgery, to obtain the tumor sample.[24] A follow-up vaccine dose was administered every other week at 12 or 20 weeks to patients with disease regression (administration at between 12 and 20 weeks) or stabilization (administration at 20 weeks). One patient had a complete response at 100 μg. Three patients had partial responses, and 3 additional patients showed disease stabilization lasting for more than 1 year, all at 25 μg. (Of these, 2 with partial responses are still holding and the 3 with stabilized disease have had failure of therapy.)[22] The HSPPC-96 vaccine was safe and well tolerated, with 59% of the patients overall receiving the vaccine for 3 months or longer.

In the second study, RCC patients received weekly vaccinations with 25 μg HSPPC-96 for 4 weeks followed by two additional doses every 2 weeks.[25] Patients were reevaluated at 10 weeks, and those with responses or stable disease received four additional doses of vaccine at 2-week intervals. Patients with disease progression continued to receive the vaccine as well, but they also received 11 mIU of IL-2 given subcutaneously 5 days per week for 4 consecutive weeks. Of 8 patients completing a course of HSPPC-96 alone, one patient had a complete response, one patient had a partial response, and the remaining 6 patients had stable disease after 18 weeks. Of 9 patients who received the vaccine plus IL-2, 6 patients had stable disease at 18 weeks and the other 3 patients were still receiving treatment at the time of the communication. Significant toxicity was not observed with the

vaccine alone or with vaccine in combination with IL-2.

The results of these phase II trials are consistent with those of studies in murine tumor models. The HSPPC-96 vaccine was highly effective in the adjuvant setting but less so in animals with progressive tumors, in which only disease stabilization was achieved.[26] On the basis of these clinical findings, a randomized, multicenter phase III study has been initiated in RCC patients in the adjuvant setting.

Patients in the high-risk adjuvant setting and those with minimal disease are most likely to benefit from vaccine therapy.

The identity of the peptides in HSPPC-96 that are responsible for enhancing cytotoxic T-cell activity, leading to objective treatment responses, is unknown. A TIL isolated from a melanoma patient who had dramatic tumor regression following HSP immunization was recently shown to recognize the HLA-A2–restricted cytotoxic T-cell epitopes in tyrosinase-related protein-2 (TRP-2) and NY-ESO-1 melanoma antigen.[27] However, peripheral blood mononuclear cells from this patient showed high reactivity against these epitopes before immunization, but not against the antigens used for the immunization. The immune response against the latter antigens was less pronounced, suggesting that TRP-2 and NY-ESO-1 may be of importance in causing cytotoxic T-cell–mediated tumor destruction and subsequent disease regression in melanoma. Similar analyses will need to be conducted on RCC patients with complete responses following HSPPC-96 or other vaccines. However, it should be noted that NY-ESO-1 is not expressed in RCC cell lines or in specimens from patients with RCC, and studies of TRP-2 in RCC have not been reported.[28,29]

Future Directions

Tumor-mediated suppression of the immune system represents the major hurdle facing vaccine therapy of RCC. The key is to identify the subset of patients in whom vaccine therapy will be most beneficial. Patients in the high-risk adjuvant setting and those with minimal disease are most likely to benefit, because they are least likely to mount tumor suppression of the immune system. In contrast, patients with advanced disease are likely to have significant tumor immunosuppression, and therefore, combination strategies will be needed. Unfortunately, combination approaches involving IL-2 or GM-CSF have not been effective in addressing tumor immunosuppression. Other combination strategies involving active regimens will need to be considered. One possibility is to administer a vaccine, such as the dendritic cell tumor lysate, in combination with a background of IL-2 and thalidomide therapy.

The application of vaccine therapy in advanced RCC is also hindered by the lack of information about tumor-associated antigens. This explains why many studies used tumor lysates or irradiated tumor cells in vaccine therapy. Even HSPPC-96 involves a vast array of antigenic peptides, some of which are specific for cancer cells and others not.

In summary, significant progress has been made in vaccine therapy for RCC, but there is still a long way to go. Once tumor-associated antigens specific for RCC are identified, and the issue of tumor immunosuppression is

Main Points

- Adjuvant therapy has not proved effective in renal cell carcinoma (RCC) patients at high risk for progression.
- Tumor cells suppress normal immune mechanisms or alter or mask antigen expression; vaccines may make these cells more immunogenic to overcome their defense mechanisms and let the immune system recognize and destroy them.
- Vaccine sources include dendritic cells pulsed with tumor antigens or lysate or hybridized with irradiated autologous tumor cells; irradiated tumor cells coupled with adjuvant therapy; vaccine-primed lymph node cells; and heat shock protein–peptide complexes.
- Vaccine therapy with short-term cultures of autologous tumor cells appears feasible in the adjuvant setting but not for advanced disease.
- Significant tumor immunosuppression is more likely in advanced disease; combination strategies may overcome this problem, but those tried so far have not proved effective.
- Identification of tumor-associated antigens specific to RCC and a means of overcoming tumor immunosuppression are necessary to make vaccine therapy a viable treatment option for RCC.

solved, it will be possible to take the next step forward in making vaccine therapy a viable treatment option. ■

References

1. Godley PA, Taylor M. Renal cell carcinoma. *Curr Opin Oncol*. 2001;13:199–203.
2. Amato RJ. Chemotherapy for renal cell carcinoma. *Semin Oncol*. 2000;27:177–186.
3. Bukowski RM. Cytokine therapy for metastatic renal cell carcinoma. *Semin Urol Oncol*. 2001;19:148–154.
4. Fearnley DB, Whyte LF, Carnoutsos SA, et al. Monitoring human blood dendritic cell number in normal individuals and in stem cell transplantation. *Blood*. 1999;93:728–736.
5. Meidenbauer N, Andreesen R, Mackensen A. Dendritic cells for specific cancer immunotherapy. *Biol Chem*. 2001;382:507–520.
6. Radmayr C, Bock G, Hobisch A, et al. Dendritic antigen-presenting cells from the peripheral blood of renal-cell-carcinoma patients. *Int J Cancer*. 1995;63:627–632.
7. Mulders P, Tso CL, Gitlitz B, et al. Presentation of renal tumor antigens by human dendritic cells activates tumor-infiltrating lymphocytes against autologous tumor: implications for live kidney cancer vaccines. *Clin Cancer Res*. 1999;5:445–454.
8. Hinkel A, Tso CL, Gitlitz BJ, et al. Immunomodulatory dendritic cells generated from nonfractionated bulk peripheral blood mononuclear cell cultures induce growth of cytotoxic T cells against renal cell carcinoma. *J Immunother*. 2000;23:83–93.
9. Holtl L, Rieser C, Papesh C, et al. Cellular and humoral immune responses in patients with metastatic renal cell carcinoma after vaccination with antigen pulsed dendritic cells. *J Urol*. 1999;161:777–782.
10. Rieser C, Ramoner R, Holtl L, et al. Mature dendritic cells induce T-helper type-1-dominant immune responses in patients with metastatic renal cell carcinoma. *Urol Int*. 2000;63:151–159.
11. Kugler A, Stuhler G, Walden P, et al. Regression of human metastatic renal cell carcinoma after vaccination with tumor cell–dendritic cell hybrids. *Nat Med*. 2000;6:332–336.
12. Chang AE, Li Q, Jiang G, et al. A phase II trial of anti-CD3 activated, vaccine-primed lymphocytes in stage IV renal cell cancer [abstract]. *Proc Am Soc Clin Oncol*. 2002;21:3a.
13. Gurjal A, Dansey RD, Klein JL, et al. Phase II trial of specific cellular immunotherapy in renal cell carcinoma (RCC) [abstract]. *Proc Am Soc Clin Oncol*. 2001;20:222b.
14. Doehn C, Richter A, Lehmacher W, et al. Multicenter phase III trial to compare radical nephrectomy plus adjuvant autologous tumor cell-lysate vaccine versus radical nephrectomy without adjuvant treatment for renal cell carcinoma stages pT2-3bpN0-3M0: a 3-year analysis [abstract]. *Proc Am Soc Clin Oncol*. 2002;21:180a.
15. Galligioni E, Quaia M, Merlo A, et al. Adjuvant immunotherapy treatment of renal carcinoma patients with autologous tumor cells and bacillus Calmette-Guerin: five-year results of a prospective randomized study. *Cancer*. 1996;77:2560–2566.
16. Dillman RO, Barth NM, VanderMolen LA, et al. Treatment of kidney cancer with autologous tumor cell vaccines of short-term cell lines derived from renal cell carcinoma. *Cancer Biother Radiopharmaceut*. 2001;16:47–54.
17. Desai J, Mitchell P, Loveland B, et al. A phase I trial of dendritic cells pulsed with MUC1 peptide in patients with solid tumours [abstract]. *Proc Am Soc Clin Oncol*. 2002;21:15b.
18. Vissers JLM, De Vries JM, Schreurs MWJ, et al. The renal cell carcinoma–associated antigen G250 encodes a human leukocyte antigen (HLA)–A2.1-restricted epitope recognized by cytotoxic T lymphocytes. *Cancer Res*. 1999;59:5554–5559.
19. Tso C-L, Zisman A, Pantuck A, et al. Induction of G250-targeted and T-cell-mediated antitumor activity against renal cell carcinoma using a chimeric fusion protein consisting of G250 and granulocyte/monocyte-colony stimulating factor. *Cancer Res*. 2001;61:7925–7933.
20. Hsu FJ, Komarovskaya M. Idiotype-specific T-cell activation/amplification using CTLA4 blockade and targeting of tumor to dendritic cells [abstract]. *Proc Am Soc Clin Oncol*. 2002;21:25a.
21. Okazaki I, Mescher M, Curtsinger J, et al. An autologous large multivalent immunogen (LMI) vaccine for the treatment of metastatic melanoma and renal cell carcinoma [abstract]. *Proc Am Soc Clin Oncol*. 2002;21:20a.
22. Srivastava PK, Amato RJ. Heat shock proteins: the "Swiss Army Knife" vaccines against cancers and infectious agents. *Vaccine*. 2001;19:2590–2597.
23. Udono H, Srivastava PK. Comparison of tumor-specific immunogenicities of stress-induced proteins gp96, hsp90, and hsp70. *J Immunol*. 1994;152:5398–5403.
24. Amato RJ, Murray L, Wood L, et al. Active specific immunotherapy in patients with renal cell carcinoma (RCC) using autologous tumor derived heat shock protein–peptide complex–96 (HSPPC-96) vaccine [abstract]. *Proc Am Soc Clin Oncol*. 1999;18:332a.
25. Amato RJ, Wood LS, Savary C, et al. Patients with renal cell carcinoma (RCC) using autologous tumor-derived heat shock protein-peptide complex (HSPPC-96) with or without interleukin-2 (IL-2) [abstract]. *Proc Am Soc Clin Oncol*. 2000;19:454a.
26. Tamura Y, Peng P, Kang L, et al. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. *Science*. 1997;278:117–120.
27. Khong HT, Rosenberg SA. Pre-existing immunity to tyrosinase-related protein (TRP)-2, a new TRP-2 isoform, and the NY-ESO-1 melanoma antigen in a patient with a dramatic response to immunotherapy. *J Immunol*. 2002;168:951–956.
28. Neumann E, Engelsberg A, Decker J, et al. Heterogeneous expression of the tumor-associated antigens RAGE-1, PRAME, and glycoprotein 75 in human renal cell carcinoma: candidates for T-cell-based immunotherapies? *Cancer Res*. 1998;58:4090–4095.
29. Jungbluth AA, Chen YT, Stockert E, et al. Immunohistochemical analysis of NY-ESO-1 antigen expression in normal and malignant human tissues. *Int J Cancer*. 2001;92:856–860.