

CONFERENCE PREVIEW: JOINT CANCER CONFERENCE 2000 III. TRANSLATIONAL RESEARCH

1. TUMOR CELL VACCINES FOR RENAL CELL CARCINOMA

Scott J. Antonia, MD, PhD

*From the Division of Medical Oncology, Clinical Investigations Program,
H. Lee Moffitt Cancer Center & Research Institute, Tampa, Fla.*

Introduction

Therapeutic options for patients with metastatic renal cell carcinoma (RCC) are limited, and it is unlikely that refining conventional treatment modalities will have a significant impact on this disease. Therefore, it is important to develop novel treatment modalities such as immunotherapy. We are in the process of conducting a clinical trial investigating the use of a B7-1 gene-modified autologous tumor cell vaccine in combination with systemic interleukin-2 (IL-2).

Therapy for Metastatic RCC

There is no standard approach to the treatment of patients with metastatic RCC. Chemotherapy outside the setting of a clinical trial is generally not used because no single agent or combination chemotherapy has been shown to affect the survival of these patients.¹ Recombinant human IL-2 is often used since a low rate of durable complete responses has been observed.² Although still controversial, increasing evidence shows that low-dose, subcutaneous IL-2 regimens on an outpatient basis have response rates similar to high-

dose, bolus, intravenous regimens.³ Patients presenting with primary RCC with a solitary metastasis frequently are treated with resection of both the primary lesion and the metastasis.⁴ Nephrectomy is often performed for the purpose of controlling symptoms (eg, pain, hemorrhage, malaise, hypercalcemia, erythrocytosis, or hypertension) related to large primary tumors.⁵⁻⁷ Nephrectomy is also commonly used in clinical trials to control the primary tumor prior to immunotherapy.⁸⁻¹¹ This approach is controversial because it has not been proven to improve the efficacy of immunotherapy. It has been shown, however, that nephrectomy in carefully selected patients does not preclude the subsequent delivery of immunotherapy in the majority of patients.^{12,13} Nephrectomy has also been used in a variety of different clinical trials to obtain tissue for the production of biological therapy such as tumor cell vaccines¹⁴ and tumor-infiltrating lymphocytes.¹⁵

Autologous Tumor Cell Vaccine Therapy

Despite the presence of tumor-associated antigens (TAAs) and TAA-reactive T cells,¹⁶ the immune system is not effective in rejecting

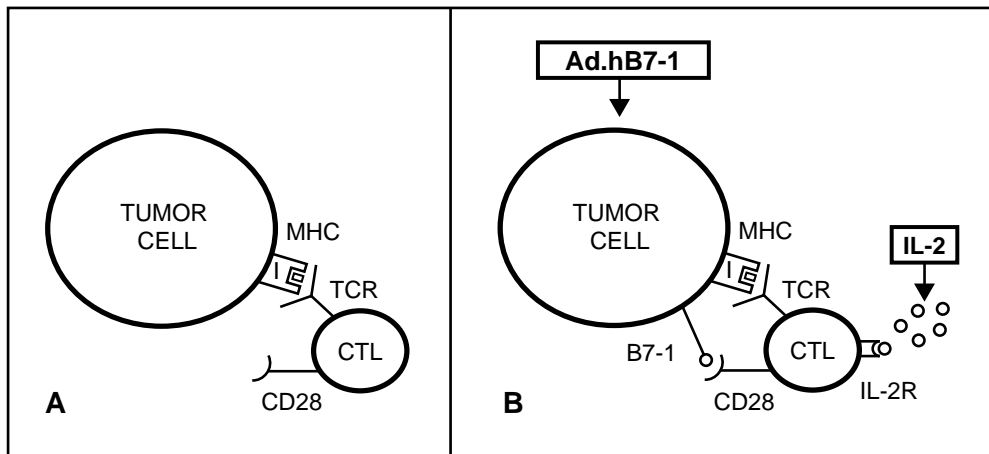
tumor cells in cancer patients. Several approaches have been designed in an attempt to enhance the immunogenicity of tumor cells. One approach has been to use autologous tumor cell vaccines.¹⁷ This has the advantage of exploiting the full complement of TAAs of a patient's tumor without specifically identifying these TAAs, and there is a complete HLA match. With this approach, various manipulations including transfection of genes of immunologic importance are performed on the autologous tumor cells to enhance their immunogenicity.¹⁸ In the case of RCC, several clinical trials employing the use of autologous tumor cell vaccines have been conducted. Some trials have used unmanipulated autologous tumor cell vaccines.¹⁹ Others have used manipulated autologous tumor cell vaccines including mix-

ing with bacille Calmette-GuJrin (BCG),^{14,20-23} mixing with IL-2 gene-transfected fibroblasts²⁴ and directly transfecting autologous tumor cells with the granulocyte-macrophage colony-stimulating (GM-CSF) gene.²⁵

B7-1 Gene-Modified Tumor Cell Vaccines

The limited results obtained with prior attempts at immunotherapy for RCC may be explained by the fact that despite presenting specific antigens, tumors may induce peripheral tolerance by failing to express the T-cell co-stimulatory molecule B7-1. T cells require two signals to become activated.²⁶ The first is signaling through the T-cell receptor (TCR) when it binds to its ligand on the tumor cell surface, which is an antigenic peptide

loaded onto a major histocompatibility complex (MHC) class I molecule. The second signal is the binding of the T-cell surface molecule CD28 to its ligand B7-1. When a T cell receives the first signal in the absence of the co-stimulatory signal, it is rendered anergic.²⁷ Since tumor cells fail to express T-cell co-stimulatory molecules, reactive cytotoxic T lymphocytes (CTLs) encounter TAAs on the tumor cell surface in a tolerizing rather than an activating context. This important observation led to the finding by Chen et al²⁸ that when murine tumor cells are forced to express the T-cell co-stimulatory molecule B7-1, previously tumorigenic cells are efficiently rejected. This could occur because activated T cells once primed by the B7-1 gene modified cells do not require co-stimulation for effector function.



Summary of rationale for immunization strategy. (A) Tumor cells present TAA-derived peptides on surface MHC class I molecules. TCRs of TAA-specific CTL can bind MHC/peptide complexes but fail to become activated since they are not co-stimulated by B7-1-negative RCC cells. (B) Autologous RCC cells are forced to express surface B7-1 molecules by infection with a recombinant adenovirus containing the human B7-1 gene ex vivo. When injected into patients, it is hypothesized that TAA-specific CTLs will be activated since the required signaling through the TCR and CD28 can occur. In addition to being rendered functionally activated, IL-2 receptors will be expressed. Recombinant IL-2 delivered systemically will bind to the IL-2 receptors on the activated CTLs, leading to proliferation and thus expansion of the numbers of RCC-reactive CTLs. Once activated, the CTLs can migrate to metastatic deposits and kill the unmodified tumor cells since B7-1 is not required for effector function.

Autologous B7-1 Gene-Modified RCC Tumor Cell Vaccines

Patients who require a palliative resection of their primary tumor or a symptomatic metastasis are eligible in our study. Frequently, these resected lesions are bulky and provide a large number of tumor cells. This has the advantage of requiring only a short period of time in culture, which allows for the maintenance of the heterogeneity of the tumor cell population, and the

target cell number can be reached before the cell cultures undergo senescence. The tumors are mechanically and enzymatically disrupted, and the resultant cell suspension is adapted to short-term cell culture, usually less than two to three weeks. Once an adequate number of cells are present within the culture, they are infected with a replication defective recombinant adenoviral vector that contains the human B7-1 cDNA under the direction of the constitutively active cytomegalovirus promoter.²⁹ Sterility, identity of the cells as RCC cells, and expression of the transgene are all confirmed. The final gene-modified tumor cell suspension is radiated, and aliquots are stored frozen in liquid nitrogen.

Phase I Clinical Trial

As described above, several clinical trials involving the use of autologous RCC tumor cell vaccines have been completed and reported. These studies have demonstrated the lack of significant systemic toxicity associated with these vaccines, and they have provided a guide for the dose escalation scheme we have used in this trial. In our B7-1 gene-modified tumor cell vaccine trial, cohorts of patients receive escalating doses of the vaccine by increasing the number of cells given in each injection and by increasing the frequency of administration of the cells. Patients at all dose levels receive injections of cells over a three-month period. For the first dose level, patients receive three injections, each containing 5×10^6 cells,

every 28 days. For the second dose level, patients receive three injections, each containing 1×10^7 cells, every 28 days. For the third dose level, patients receive six injections, each containing 1×10^7 cells, every 14 days.

Systemic IL-2 as an Immunomodulatory Agent

Resting T cells, when activated by signaling through the TCR and co-stimulated by the B7-1/CD28 interaction, up-regulate the expression of IL-2 receptors. The binding of IL-2 to these receptors results in T-cell proliferation.³⁰ The rationale for administering exogenous IL-2 to patients as immunotherapy is to expand the numbers of specific T cells that have been activated by their encounter with antigen. Based on the pharmacokinetics of recombinant human IL-2 and the binding affinity of IL-2 to relevant IL-2 receptors on T cells, it has been demonstrated that the amount of IL-2 given with a subcutaneous, moderate-dose IL-2 outpatient regimen³ is more than adequate to be effective in stimulating the proliferation of activated T cells.³¹ In our protocol, we administer exogenous, recombinant IL-2 systemically during the final six weeks of the immunization period with the intention of providing a proliferative stimulus to the T cells activated by the vaccine in order to expand the tumor cell-reactive effector T cells. The use of IL-2 has a dual role: even if this hypothesized synergy with the

vaccine does not occur, IL-2 as a single agent has known activity in RCC and thus would be expected to be at least additive with the vaccine effect.

Outcome Measures

As in any phase I clinical trial, the primary goal of this trial is to determine the safety of this novel therapy. However, we also have the measurable surrogate endpoint of immunogenicity. Traditional assays of immune function have not been useful in evaluating immune responses in tumor vaccine clinical trials due to lack of adequate sensitivity. However, recent assays for determining the number of specific activated T cells by staining for cytokines secreted by individual cells (enzyme-linked immunospot [ELISPOT] assays) have been shown to be useful. Since we have unmanipulated tumor cells available from each patient, we also are able to perform *in vitro* restimulation of the patients' lymphocytes to further enhance the sensitivity of these assays.

To determine if there is an induction of an antitumor antibody response, we perform cell enzyme-linked immunosorbent assays (ELISAs) using serum obtained from the patients before and after vaccine administration. The target cells are the autologous RCC tumor cells. The final step in assessing immunogenicity is by delayed-type hypersensitivity (DTH) skin testing. All patients receive intradermal injections of unmodified,

irradiated, autologous tumor cells before and after vaccine administration. The degree of induration and erythema that is present 48 hours after injection is measured. In addition, the DTH test site undergoes biopsy and is subjected to immunohistochemical analysis to determine if there is an influx of cells of the immune system.

Appreciation is expressed to the investigators at the University of Pennsylvania for providing the recombinant adenoviral vector containing the B7-1 gene referred to in this report.

References

1. Yagoda A, Abi-Rached B, Petrylak D. Chemotherapy for advanced renal-cell carcinoma: 1983-1993. *Semin Oncol.* 1995;22:42-60.
2. Fyfe G, Fisher RI, Rosenberg SA, et al. Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. *J Clin Oncol.* 1995;13:688-696.
3. Yang JC, Rosenberg SA. An ongoing prospective randomized comparison of interleukin-2 regimens for the treatment of metastatic renal cell cancer. *Cancer J Sci Am.* 1997;3 (suppl 1):S79-S84.
4. O'Dea MJ, Zincke H, Utz DC, et al. The treatment of renal cell carcinoma with solitary metastasis. *J Urol.* 1978;120:540-542.
5. Bukowski RM, Novick AC. Clinical practice guidelines: renal cell carcinoma. *Cleve Clin J Med.* 1997;64 (suppl 1):S11-S44.
6. Motzer RJ, Bander NH, Nanus DM. Renal-cell carcinoma. *N Engl J Med.* 1996;335:865-875.
7. Linehan WM, Shipley W, Parkinson D. Cancer of the kidney and ureter. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology.* Philadelphia, Pa: JB Lippincott; 1993: 1023-1051.
8. Gleave ME, Elhilali M, Fradet Y, et al. Interferon gamma-1b compared with placebo in metastatic renal-cell carcinoma. Canadian Urologic Oncology Group. *N Engl J Med.* 1998;338:1265-1271.
9. Walther MM, Yang JC, Pass HI, et al. Cytoreductive surgery before high dose interleukin-2 based therapy in patients with metastatic renal cell carcinoma. *J Urol.* 1997;158:1675-1678.
10. Novick AC. Current surgical approaches, nephron-sparing surgery, and the role of surgery in the integrated immunologic approach to renal-cell carcinoma. *Semin Oncol.* 1995;22:29-33.
11. Rackley R, Novick A, Klein E, et al. The impact of adjuvant nephrectomy on multimodality treatment of metastatic renal cell carcinoma. *J Urol.* 1994;152:1399-1403.
12. Fallick ML, McDermott DF, LaRock D, et al. Nephrectomy before interleukin-2 therapy for patients with metastatic renal cell carcinoma. *J Urol.* 1997;158:1691-1695.
13. Levy DA, Swanson DA, Slaton JW, et al. Timely delivery of biological therapy after cytoreductive nephrectomy in carefully selected patients with metastatic renal cell carcinoma. *J Urol.* 1998;159:1168-1173.
14. Chang AE, Aruga A, Cameron MJ, et al. Adoptive immunotherapy with vaccine-primed lymph node cells secondarily activated with anti-CD3 and interleukin-2. *J Clin Oncol.* 1997;15:796-807.
15. Figlin RA, Pierce WC, Kaboo R, et al. Treatment of metastatic renal cell carcinoma with nephrectomy, interleukin-2 and cytokine-primed or CD8(+) selected tumor infiltrating lymphocytes from primary tumor. *J Urol.* 1997;158:740-745.
16. Romero P, Dunbar PR, Valmori D, et al. Ex vivo staining of metastatic lymph nodes by class I major histocompatibility complex tetramers reveals high numbers of antigen-experienced tumor-specific cytolytic T lymphocytes. *J Exp Med.* 1998;188:1641-1650.
17. Pardoll DM. Cancer vaccines. *Nat Med.* 1998;4:525-531.
18. Roth JA, Cristiano RJ. Gene therapy for cancer: what have we done and where are we going? *J Natl Cancer Inst.* 1997;89:21-39.
19. Weidmann E, Logan TF, Yasumura S, et al. Evidence for oligoclonal T-cell response in a metastasis of renal cell carcinoma responding to vaccination with autologous tumor cells and transfer of in vitro-sensitized vaccine-draining lymph node lymphocytes. *Cancer Res.* 1993;53:4745-4749.
20. Galligioni E, Francini M, Quايا M, et al. Randomized study of adjuvant immunotherapy with autologous tumor cells and BCG in renal cancer. *Ann NY Acad Sci.* 1993;690:367-369.
21. Chang AE, Yoshizawa H, Sakai K, et al. Clinical observations on adoptive immunotherapy with vaccine-primed T-lymphocytes secondarily sensitized to tumor in vitro. *Cancer Res.* 1993;53:1043-1050.
22. Galligioni E, Quايا 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.
23. Fenton RG, Steis RG, Madara K, et al. A phase I randomized study of subcutaneous adjuvant IL-2 in combination with an autologous tumor vaccine in patients with advanced renal cell carcinoma. *J Immunother Emphasis Tumor Immunol.* 1996;19:364-374.
24. Veelken H, Mackensen A, Lahn M, et al. A phase-I clinical study of autologous tumor cells plus interleukin-2-gene-transfected allogeneic fibroblasts as a vaccine in patients with cancer. *Int J Cancer.* 1997;70:269-277.
25. Simons JW, Jaffee EM, Weber CE, et al. Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by ex vivo granulocyte-macrophage colony-stimulating factor gene transfer. *Cancer Res.* 1997;57:1537-1546.
26. Schwartz RH. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell.* 1992;71:1065-1068.
27. Gimmi CD, Freeman GJ, Gribben JG, et al. Human T-cell clonal anergy is induced by antigen presentation in the absence of B7 costimulation. *Proc Natl Acad Sci U S A.* 1993;90:6586-6590.
28. Chen L, Ashe S, Brady WA, et al. Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4. *Cell.* 1992;71:1093-1102.
29. Boxhorn HK, Smith JG, Chang YJ, et al. Adenoviral transduction of melanoma cells with B7-1: antitumor immunity and immunosuppressive factors. *Cancer Immunol Immunother.* 1998;46:283-292.
30. Cantrell DA, Smith KA. The interleukin-2 T-cell system: a new cell growth model. *Science.* 1984;224:1312-1316.
31. Smith KA. Rational interleukin-2 therapy. *Cancer J Sci Am.* 1997;3(suppl 1):S137-S140.