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Ex Vivo Gene Therapy Using Granulocyte-Macrophage Colony-Stimulating Factor-Transduced Tumor Vaccines

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ABSTRACT

There is no standard effective therapy for metastatic renal-cell carcinoma (RCC) or prostate cancer. Both of these cancers may be immunogenic, so therapy targeted to a tumor-associated antigen may be effective. Transduction of the gene encoding granulocyte-macrophage colony-stimulating factor has shown promise in preclinical studies, and clinical trials are in their early stages. Both autologous cancer cells and partially HLA-matched allogenic cells are being studied. No dose-limiting side effects have been observed, and a few patients have had transient objective tumor regressions. Further trials with more frequent and, probably, longer immunization schedules are needed to define efficacy.

INTRODUCTION

THERE IS NO STANDARD EFFECTIVE THERAPY for metastatic renal-cell carcinoma (RCC) or hormone-refractory prostate cancer. Like melanoma, RCC is thought to be an immunogenic cancer because of its relatively high response rate to cytokine therapy. While prostate cancer has been regarded as an unlikely target for immunotherapy, recent basic studies indicate that several putative tumor-associated antigens are expressed in prostate cancer tissue or cell lines.[1] This finding suggests that immunotherapy targeted for a tumor-associated antigen may provide an effective treatment for prostate cancer.

In cancer gene therapy, various approaches, including tumor suppressor genes, prodrug in combination with a suicide gene, and gene-modified immunotherapy are now being tested in clinical studies, as reviewed by other contributors to this issue. The gene-modified tumor vaccine strategy has been the most intensively studied. In Japan, a Phase I study of a granulocyte-macrophage colony-stimulating factor (GM-CSF) gene-transduced tumor vaccine for metastatic RCC has begun as the first cancer gene therapy under the direction of a multi-institutional group at the Institute of Medical Science, University of Tokyo.

In this paper, we briefly review the *ex vivo* gene therapy approaches and early clinical trials of the GM-CSF gene-transduced tumor vaccine for RCC and prostate cancer.

PRECLINICAL STUDIES USING GM-CSF-TRANSDUCED TUMOR VACCINES

In animal models, the inoculation of cells engineered to contain cytokine genes induces tumor-specific immune responses, defined as either protection of the animal against challenge with parental tumor cells or regression of established parental tumors.[2] Among several kinds of cytokine genes, Dranoff and associates[3] demonstrated the efficacy of GM-CSF-transduced vaccines by comparing the activity of the transduced tumor cells with that of nontransduced cells in animal models of melanoma, prostate cancer, RCC, and others. Results in the Dunning rat model also support the use of GM-CSF-transfected tumor cells in prostate cancer treatment.[4] In this model, GM-CSF was shown to induce more potent antitumor activity than other molecules tested in protecting mice from subsequent lethal challenge with live melanoma cells. Those findings suggest that GM-CSF is the most promising candidate for augmenting antitumor immunity.

The action of GM-CSF involves the local spread of the cytokine at a high concentration, which activates the function of the antigen-presenting cells (APCs), including macrophages and dendritic cells. The dendritic cell is known to be the most potent APC for helper T lymphocytes. Both $CD4^+$ and $CD8^+$ T cells are activated, which results in the destruction of tumor

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cells at metastatic sites. The role of the CD4 T cells has been largely attributed to induction of tumor-specific CD8 cytotoxic T lymphocytes (CTL) in the effector phase.[3] In addition, recent analyses indicate that the GM-CSF tumor vaccine activates the CD4 T cell to simultaneous Th1 and Th2 responses.[5] Those findings suggest that multiple lymphocyte effector mechanisms including B-cell immunity, result in the potent antitumor response observed in the animal studies of GM-CSF-transduced tumor vaccines.

ONGOING CLINICAL STUDIES OF CYTOKINE GENE-TRANSDUCED VACCINES FOR RCC AND PROSTATE CANCER

Table 1 shows the clinical trials of the gene-modified tumor vaccines designed for RCC and prostate cancer in the United States and Japan.[6] The current approaches for RCC are gene-modified immunotherapy. Seven clinical trials are based on a tumor vaccine using *ex-vivo* gene-modified autologous cancer cells or partially HLA-matched allogeneic cancer cells. Four kinds of molecules—tumor necrosis factor, interleukin (IL)-2, GM-CSF, and B7.1 (CD80)—are being used to augment host immunity. An additional protocol is planned to use IL-4-transduced autologous fibroblasts in combination with untransduced autologous cells. Similarly, more than half of the gene therapies designed for prostate cancer are gene-modified immunotherapy. The *ex-vivo* gene-modified tumor vaccines using GM-CSF or IL-2 are being tested in three protocols (Table 1). For the GM-CSF-transduced tumor vaccine, autologous and allogeneic cancer cells are being studied in different protocols. To date, results from three clinical trials of the autologous GM-CSF tumor vaccine have been reported.[7–9]

RESULTS OF CLINICAL STUDIES

The vaccine dose and schedule, toxicity, and clinical response of individual trials are summarized in Table 2. The capability of eliciting an antitumor immune response was extensively monitored using several methods, as listed in Table 3.

In the RCC study, patients were randomized to treatment with escalating doses of autologous tumor vaccine with or without *ex-vivo* GM-CSF gene transfer. Cells were harvested from the patients' primary tumors and transfected with a retroviral vector carrying the GM-CSF gene.[7] The transduced and untransduced tumor cells were then irradiated with a lethal dose (15 Gy) and administered intradermally or subcutaneously. No dose-limiting side effects were observed in 16 patients. One partial response was observed in a patient with multiple lung metastases who was treated with GM-CSF gene-transduced vaccine. The patient displayed the largest delayed-type hypersensitivity (DTH) conversion in this trial; however, progression of metastases was observed 4 months after the last vaccination.[10] Histologic evaluation of the vaccination site revealed extensive infiltration of dendritic cells, macrophages, eosinophils, and T lymphocytes. In the DTH sites of patients treated with GM-CSF-transduced vaccine, the most characteristic finding was the recruitment of activated eosinophils. Eosinophil accumulation and degranulation were commonly observed in the postvaccination DTH response of melanoma and prostate cancer patients also.

In the melanoma study, not only DTH sites but also metastatic lesions resected after vaccination could be examined.[8] Diffuse infiltration of T lymphocytes and plasma cells was demonstrated in 11 of 16 patients in whom tissue could be obtained. In four patients, infiltration of eosinophils and lymphocytes was associated with the targeted destruction of tumor vasculature. Despite these promising histologically defined immune reactions, no objective clinical response was observed in the 22 patients.

In the study of prostate cancer, the low yields of cells in the primary cultures of radical prostatectomy specimens limited the number of vaccination courses.[9] Although no clinical response was noted in the eight patients, DTH site biopsies revealed infiltration of $CD45RO^+$ T cells and degranulating eosinophils consistent with the activation of Th1 and Th2 T-cell response.

In addition to these *in vivo* responses, both T-cell and B-cell

Table 1. *Ex Vivo* Gene Therapy Protocols for RCC and Prostate Cancer

Gene	*Phase*	*Target*	*Vehicle*	*Research Site*
RCC				
TNF	1	Autologous RCC	Retrovirus	NIH
IL-2	I	Autologous RCC	Retrovirus	NIH
IL-3	I	Allogeneic RCC (partially HLA matched)	Retrovirus	Memorial Sloan-Kettering
IL-4	I	Autologous fibroblasts (with untransduced RCC)	Retrovirus	U. Pittsburgh
GM-CSF	I	Autologous RCC	Retrovirus	Johns Hopkins/U.Tokyo
B7.1 (CD80)	I	Autologous RCC	Adenovirus	U. Southern California
Prostate Ca				
GM-CSF	I/II	Autologous CAP	Retrovirus	Johns Hopkins
IL-2	I	Autologous CAP	Lipid	Duke

Table 2. Summary of Phase I Students of GM-CSF-Transduced Tumor Vaccines

	Renal-cell carcinoma	*Melanoma*	*Prostate cancer*
No. pts.	Enrolled: 31 Treated: 18	Enrolled: 33 Treated: 26	Enrolled: 23 Treated: 8
Pretreatment	(-)	(-)	Radical prostatectomy
Dose and schedule	Level 1: 4×10^6 cells (N = 3) q 28d × 3 Level 2: 4×10^7 cells (N = 4) q 28d × 3 Nontransduced cells (N = 11)	Cell dose fixed at 1×10^7 Level 1: q 28d × 3 (N = 4) Level 2: q 14d × 6 (N = 7) Level 3: q 7d × 12 (N = 15)	Level 1: 1×10^7 cells (N = 5) q 7d × 6 Level 2: 5×10^7 cells (N = 3) q7 d × 6
GM-CSF production (ng/10^6 cells/24h)	42–149	84–965	143–1403
DTH conversion	All at level 2 (2–10 cm)	All evaluated (5–10 cm)	All evaluated (0.5–1.0 cm)
Objective tumor response	1 PR	None	None
Toxicities	Local: erythema, swelling pruritus Systemic: constipation (1 case) pruritus (1 case)	Local: erythema, swelling pruritus Systemic: fatigue (grade 1) occasionally	Local: erythema, swelling, pruritus Systemic: fever < grade 2 (2 cases) chills (3 cases)

antitumor responses were monitored by *in vitro* tests. Specific CTL activity against autologous tumor was detected in tumor-infiltrating lymphocytes from residual melanoma metastases.[8] Ellem and associates[11] reported an increase in the frequency of CTL precursors by the limiting dilution assay in a melanoma patient who was treated with a GM-CSF gene-transduced tumor vaccine in another clinical study. The immune response was associated with a transient partial antitumor effect. However, neither a clinical effect nor an immune response was detectable 2 months after the last vaccination.

For detecting the B-cell antitumor response, immunoblotting analyses using autologous tumor cell lysates and sera were performed in the melanoma and prostate cancer studies.[8,9] An increase in antibody titers was observed in seven melanoma and three prostate cancer patients. In prostate cancer, the induced immunoglobulin recognized the 150-kDa polypeptide commonly expressed by LNCaP and PC-3 cell lines, as well as by normal prostate epithelium and a number of other human cancer cell lines.

CONCLUSION

Although the side effects of gene-modified immunotherapy appear to be mild, the clinical efficacies shown in the clinical studies are limited. Because the primary purpose of a Phase I study is to test safety, the clinical efficacy of the GM-CSF-transduced tumor vaccine has not been fully examined as yet. Further clinical studies with more frequent and probably longer periods of vaccination are needed to define the efficacy. For the long-term treatment schedule, however, the efficiency of the tor limiting the quantity of vaccine. In prostate cancer trials, sufficient cells to provide a higher dose were recovered from surgical specimens in only three of the seven patients. The use of allogeneic tumor cells, if they can be sources of tumor antigens, will permit long-term vaccinations. Some preclinical studies suggest that HLA matching may be less critical in the application of tumor vaccines than previously thought.[12] The benefits of long-term treatment with a nontransduced allogeneic tumor vaccine were reported from clinical studies for melanoma.[13] On the basis of experiences, a Phase II clinical trial of irradiated GM-CSF-secreting LNCaP and PC-3 vaccines, using doses higher than are possible with an autologous tumor vaccine, is currently under way in the United States. There is hope that further understanding of clinical efficacy as well as the immunologic response induced by the GM-CSF-transduced tumor vaccine will be gained from the ongoing and future clinical trials.

Table 3. Immune Responses Evaluated in Clinical Trials of GM-CSF-Transduced Tumor Vaccine

In vivo
- Delayed-type hypersensitivity
- Vaccine site (histology)
- DTH site (histology)
- Metastases (histology)
- Hematology findings

In vitro
- T-cell immunity
  - Cytotoxic activity of tumor-infiltrating lymphocytes
- B-cell immunity
  - Antitumor activity of postimmunization serum

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