CONFEREECE PREVIEW:
JOINT CANCER CONFERENCE 2000
III. TRANSLATIONAL RESEARCH

1. TUMOR CELL VACCINES FOR
RENAL CELL CARCINOMA

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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. Recombinant human IL-2 is often used since a low rate of durable complete responses has been observed. 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. Patients presenting with primary RCC with a solitary metastasis frequently are treated with resection of both the primary lesion and the metastasis. Nephrectomy is often performed for the purpose of controlling symptoms (eg, pain, hemorrhage, malaise, hypercalcemia, erythrocytosis, or hypertension) related to large primary tumors. Nephrectomy is also commonly used in clinical trials to control the primary tumor prior to immunotherapy. 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. 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 vaccines and tumor-infiltrating lymphocytes.

Autologous Tumor Cell Vaccine Therapy

Despite the presence of tumor-associated antigens (TAAs) and TAA-reactive T cells, 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 per-
formed on the autologous tumor cells to enhance their immuno-
genicity.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 autolo-
gous tumor cell vaccines.19 Others
have used manipulated autologous
tumor cell vaccines including mixing with bacille Calmette-Guérin
(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 immuno-
therapy for RCC may be explained
by the fact that despite presenting
specific antigens, tumors may
induce peripheral tolerance by fail-
ing to express the T-cell co-stimula-
tory 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 sur-
face, which is an antigenic peptide
loaded onto a major histocompati-
bility complex (MHC) class I mole-
cule. The second signal is the bind-
ing 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 sig-
nal, 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 impor-
tant observation led to the finding
by Chen et al.28 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 mod-
ified cells do not require co-stimu-
lation 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 sys-
temically will bind to the IL-2 receptors on the activated CTLs, leading to proliferation and thus expansion of the num-
bers 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 resec-
tion 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 mainte-
nance of the hetero-
genicity 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. 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 $5 \times 10^6$ cells, every 28 days. For the second dose level, patients receive three injections, each containing $1 \times 10^7$ cells, every 28 days. For the third dose level, patients receive six injections, each containing $1 \times 10^7$ 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. 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 regimen is more than adequate to be effective in stimulating the proliferation of activated T cells. 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.

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References