Immune modulation as cancer treatment using gene therapy

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Modulation of the immune system as an approach to attack cancer has been explored for the past 50 years. Efficacy of this treatment has been achieved in a limited number of patients. However, a major obstacle of immune therapy is the toxicity related to the nonspecific nature of immune activation. Efforts to improve the specificity of the immune response have been investigated through the use of gene therapy. Clinical trials involving gene therapy for melanoma, lung cancer, and head and neck cancer have been conducted by US Oncology at the Mary Crowley Medical Research Center–Baylor University Medical Center. These studies represent one of the most active gene therapy programs in the USA. Preliminary results have helped define the mechanism of action, safety, and potential efficacy of immune stimulatory treatment approaches in oncology.

Modulation of the immune system to treat cancer has been tested extensively in oncology. Cancers thought to be most sensitive to immunotherapy include melanoma, renal cell carcinoma, colorectal cancer, and non–small cell lung cancer. Common immunotherapy approaches have involved the use of interleukin (IL)-2 (1), interferon (IFN)-alpha-2b (2), antibody therapy with muromonab-CD3 (3), lymphokine-activated killer cell infusions (4), tumor-infiltrating lymphocyte infusions (5), dendritic cell infusions (6), peptide-stimulated vaccine infusions (7), and various combinations of these. A limited number of successes have been observed (approximately 20% in patients with metastatic melanoma). However, toxicity related to nonspecific immune activation limits dosing and the effectiveness of these approaches. Thus, more recently, a strategy of tumor-specific immune activation has been explored involving gene therapy.

Key components of the immune system critical to understanding the therapeutic approaches described in this review may require definition. Antigens are targets for immune response that are located on the surface of malignant cells. Antigen-presenting cells (i.e., dendritic cells) are cells that roam the body in search of foreign antigens. Antigenic peptides are fragments of antigens that can be processed inside antigen-presenting cells and redisplayed
on the cell surface through linkage to major histocompatibility complex (MHC) molecules that bind to antigen peptides for the purpose of cell surface display (Figure 1). T lymphocytes contain receptors that recognize MHC/antigenic peptides. These cells become activated upon recognition and binding. Lymphokines such as IL-1, IL-2, IFN, and GM-CSF (granulocyte-macrophage colony-stimulating factor) are released by lymphocytes when stimulated and induce proliferation and activation of other lymphocytes (B cells) and monocytes. B lymphocytes recognize circulating antigens without MHC binding and produce antigen-specific antibodies, which mediate antitumor effects through binding to tumor antigens.

Some therapeutic approaches involve the following:

1. Improving expression (surface display) of antigens
2. Improving the number and sensitivity of antigen-presenting cells
3. Creating antigenic peptides in order to stimulate antigen-presenting cells (in vitro)
4. Altering MHC so that antigenic peptides can be exposed, thereby uncloaking malignant cell identity to immune effector cells attempting to attack the cancer

Education of the immune system to recognize malignant cells intensifies the cellular interaction and provides a focus for the immune attack. Other immunotherapy options available today focus on the induction of cytokines or the addition of appropriate cytokines to maximize anticancer effects. Malignant cells evade the immune system, in some cases, by decreasing MHC levels and/or producing factors that inhibit antigen-presenting cell function or T-cell or B-cell function.

One method of providing tumor antigen stimulation is to surgically harvest a tumor growing in a patient and to modify the tumor ex vivo (in the laboratory) so that it will no longer grow but still displays its surface antigens to stimulate the immune system when replaced in the body. This is known as vaccine therapy. Tumor vaccines have been shown to be efficacious in some clinical trials, primarily those involving melanoma, renal cell carcinoma, or colorectal cancer (8–11).

The vaccine effect can be enhanced by several methods. Coincubation of the defective antigenic autologous tumor cells with bacillus Calmette-Guérin (a nonpathogenic bacterial species) can enhance the inflammatory response to the vaccine, thereby improving the antitumor systemic effect. Vaccine approaches can also use cancer cell lines that are expected to have antigens similar to the patient's tumor. These cell lines are lysed following incubation with oncolytic viruses and are then injected into the patient to stimulate an antitumor response. Tumor vaccines have been shown to be effective in animal tumor models.

The mechanism for the antitumor effect includes induction of antigen-specific antitumor immunity mediated by CD4 and CD8 T cells. A comparison of vaccine approaches is shown in Figure 2. Most recently, gene therapy has been used to increase antigen surface expression in tumor cells via intratumoral injection rather than resection of the malignant tissue for ex vivo processing.
Insertion of immune-modulating cytokine genes (such as IL-2, IL-4, gamma-IFN, tumor necrosis factor [TNF]-alpha, M-CSF [macrophage colony-stimulating factor], or GM-CSF) into tumor cells is another form of gene therapy designed to enhance an immune response against implanted human tumors (12–26). Subcutaneous injection of cytokine-transduced tumor cells in animal tumor models has been shown to induce prolonged tumor-specific immune responses and improve survival from lethal tumor challenges. One of the earliest studies showing such an effect involved injection of the TNF gene into murine sarcoma tumors, which were then implanted subcutaneously into nude mice (27). Implantation of tumor cells transduced with genes (neomycin) that do not affect immune responsiveness did not change tumor growth. However, implantation of TNF-transduced tumor cells induced significant regression of tumors compared with controls (Table 1). Tumor regression related to the implantation of TNF-transduced sarcoma cells appeared to be mediated by CD4- and CD8-positive lymphocytes. Data also revealed that implantation of identical sarcoma cells not transduced with any genes following implantation of the TNF-transduced cells was associated with regression of implanted secondary tumors, suggesting long-term systemic antitumor activity. Implantation of tumor cells (i.e., breast cancer cells) not used as the initial vaccine did not induce an immune response, thereby suggesting that the induced systemic approach was specific against the tumor used as the gene-transduced vaccine. Clinical testing would need to confirm that enhanced targeting of the immune system against the cancer with gene therapy will increase immunogenicity and reduce systemic toxicity. Defects that limit immune recognition of cancer are listed in Table 2. New therapeutic approaches, including gene therapy, are designed to target those defects.

### Table 1. Gene therapy with TNF in a murine sarcoma model*

<table>
<thead>
<tr>
<th>Study group</th>
<th>Tumor area on day 16 (mm²)</th>
<th>Ratio of mice with detectable tumor on day 23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified cells</td>
<td>75</td>
<td>12/12</td>
</tr>
<tr>
<td>Neomycin-transduced cells</td>
<td>70</td>
<td>12/12</td>
</tr>
<tr>
<td>TNF-transduced cells</td>
<td>5</td>
<td>1/1</td>
</tr>
<tr>
<td>TNF-transduced cells + MoAb to CD4</td>
<td>70</td>
<td>6/6</td>
</tr>
<tr>
<td>TNF-transduced cells + MoAb to CD8</td>
<td>70</td>
<td>6/6</td>
</tr>
<tr>
<td>TNF-transduced cells + MoAb to Thy1</td>
<td>15</td>
<td>2/6</td>
</tr>
</tbody>
</table>

*TNF indicates tumor necrosis factor, MoAb, monoclonal antibody

### Table 2. Defects in immune recognition of cancer

- Poor antigen stimulation
- Lack of class I expression
- Lack of adhesion molecule expression
- Decreased lymphoid number
- Poor lymphoid function
- Poor antigen processing
- Inadequate activity of dendritic cells or monocytes
- Immunosuppressive cytokines (transforming growth factor β)
- Altered response to activating cytokines
- Poor tumor vascular access

Results of the in vitro and in vivo studies described above were felt to be encouraging, thus
justifying development of clinical trials within our program initially using retroviral vector gamma-IFN gene. The published results are summarized below.

**Autologous Vaccination of Immune-Modulating Genes**

Our first trial involving gene therapy to enhance antitumor immunity was performed in 1994 as part of the Baylor/PRN/Mary Crowley gene therapy program. Sixty-four patients with advanced melanoma were entered into the trial in order to harvest autologous tumor tissue and create a vaccine by introducing the gene for gamma-IFN (via a retroviral vector) into the tumor cells (Figure 3) (28). Seventy surgical tumor specimens were processed from 58 evaluable patients. The median age of treated patients was 53 years (range, 17–83 years). Of the 58 patients who underwent tumor resection, 12 tumor samples could be transduced with the gamma-IFN gene. Adequate transgene expression (gamma-IFN protein), production of functional gamma-IFN protein, and up-regulation of MHC-I and MHC-II molecules on transduced tumor cells were confirmed in the 12 tumor cell lines.

The majority of patients were, however, unable to achieve autologous tumor cell line expansion, generally because of overgrowth of fibroblasts, which were mixed in with the malignant tissue at the biopsy. Additional difficulties with processing involved the time to achieve the targeted minimum of 1 X 10⁷ cells prior to transduction. The duration from harvest of tumor tissue to release for clinical treatment ranged from 61 to 168 days. During this time, several patients had disease progression despite standard care management and were unable to participate in the treatment phase of the trial. However, 5 patients achieved sufficient cell numbers and gamma-IFN expression to undergo treatment (subcutaneous injection every 2 weeks) with the retroviral vector gamma-IFN gene vaccine. One patient received 13 injections, 1 patient received 10 injections, and 3 patients received <=5 vaccine injections. The patients receiving the fewest injections had a poor survival (35–72 days). However, of the 2 patients who received 10 or 13 injections, 1 survived 885 days, and the other remains alive without evidence of disease after >5 years. This patient had metastatic disease to his liver, brain, and adrenal gland. All lesions were surgically resected creating a minimum disease state, and the vaccine was administered every other week for 13 injections. Clinical toxicity to the gene-transduced vaccine was not observed in any of the 5 treated patients. Furthermore, no evidence of retroviral vector contamination was detected in the released cell product or in patients following treatment.

Given the advanced stage of disease, it was difficult to evaluate efficacy. However, it is highly unlikely that patients with brain and solid organ metastases survive more than 1 year, although a patient in our study has survived 5 years without disease recurrence. Nevertheless, spontaneous complete remission occurs at a 0.1% frequency in melanoma (29), so additional trials were needed.

Researchers at Duke University have looked at retroviral gamma-IFN gene-transduced autologous melanoma cell injection for treatment of metastatic melanoma, using an approach identical to ours, by processing autologous harvested melanoma tissue and transducing it with retroviral gamma-IFN gene (30). In our trial, all treated patients received low-dose IL-2 (1.1 X 10⁶ U) following vaccine injection, whereas in the Duke trial IL-2 was
SINGLE-COURSE INTRATUMORAL INJECTION OF GAMMA-IFN RETROVIRAL VECTOR IN PATIENTS WITH METASTATIC MELANOMA

In our program's second trial, 13 patients with metastatic melanoma were treated with intratumoral injection of the retroviral gamma-IFN gene vector rather than removal of the tumor and transduction of the gamma-IFN gene ex vivo. Each patient had lesions accessible to intratumoral injection and distant lesions evaluable to measure systemic response. Patients were entered into 1 of 3 treatment arms. The dose of the gene-transduced vaccine was identical for each arm (1.5 X 10^8 PFU/dose administered at a volume of 0.3 mL for 5
consecutive days). Preclinical data suggested that gene transduction efficiency varied depending on the cation (hexadimethrine bromide or protamine sulfate) mixed with the vector during injection. In this trial, 4 patients received hexadimethrine bromide, 4 patients received protamine sulfate, and 5 patients received no cation (36). Successful insertion of the gamma-IFN gene into the tumor cell was based on the enzyme-linked immunospot and polymerase chain reaction assays and was confirmed in each arm. Toxicity to the vector was not observed, and there was no evidence of viable retrovirus contamination (65 samples tested). Coadministration with cations did not appear to alter transgene expression following injection into the tumors. Patients with evidence of transgene expression had a median survival of 528 days compared with patients who had no evidence of transgene expression (median survival, 333 days).

From this study we concluded that treatment via a single intratumoral injection was safe. Efficacy evaluation was limited by the removal of the gene-transduced tumor 8 days after injection in order to assess transgene expression. No responses in injected or distal lesions were observed; however, prolonged survival of patients with identifiable transgene expression was interesting. Further clinical research using multiple courses of therapy, combination therapy with systemically administered cytokines (IL-2), and/or treatment of patients with earlier stages of less extensive disease is indicated.

**GAMMA-IFN RETROVIRAL VECTOR ADMINISTERED INTRATUMORALLY WITH MULTIPLE COURSES IN PATIENTS WITH METASTATIC MELANOMA**

Multiple courses of direct intratumoral injection of adenoviral and retroviral vector preparations have been shown to be effective in the induction of regional tumor regression in several mouse tumor models (37–44) and in patients receiving retroviral vectors involving the p53 gene (45).

In our third trial with gamma-IFN retroviral vector in patients with metastatic melanoma, we administered multiple intratumoral injections of gamma-IFN retroviral vector at escalating doses without removing the injected lesion for a single 5-day injection course (group A, n = 9) or for multiple 5-day injection courses (group B, n = 8). One cycle consisted of doses of 0.3, 0.5, and 1.5 mL per injection of gamma-IFN retroviral vector (1 X 10^7 PFU/mL) for 5 consecutive days. Patients received either 1 cycle or 6 cycles administered every 2 weeks.

Enzyme-linked immunosorbertent assays were performed to assess induction of tumor-specific antibodies against 4 melanoma tumor cell lines (DM 92, DM 93, DM 252, and DM 400), 2 nonmelanoma cell lines, and nonmalignant tissue (fibroblast lines). Studies to determine evidence of replication-efficient retrovirus were also performed from patient samples prior to administration. No significant toxicity was observed, and 10 of the 17 patients treated showed evidence of elevated antitumor antibody response titers following injection. These were generally specific for the melanoma cell lines and were highest in patients who received the higher dose levels (46). Two patients achieved a complete histologic response, and 2 patients achieved a partial response of the injected lesion (>=50% reduction of disease but not complete). Three of the 8 patients who received multiple doses of gamma-IFN...
retroviral vector achieved a response compared with only 1 of 9 patients who received a single cycle. Furthermore, survival in patients entered into the multicycle treatment regimen suggested improvement compared with survival of patients who received only a single cycle (Figure 4, \( P = 0.027 \) log rank). Consistent with prior data, gamma-IFN retroviral vector intratumoral injection was well tolerated, and there was no evidence of replication-competent retrovirus in the product or patient samples. These continue to be analyzed in surviving patients >4 years after initiation of investigation with gamma-IFN retroviral vector.

This trial is the first to attempt multiple courses of direct intratumoral injections of gamma-IFN retroviral vector. Animal studies suggest that the antitumor effect related to gamma-IFN retroviral vector injection is correlated with the number of injections and the titer of the retroviral vector preparation (44, 46). Further research involving multiple injections is indicated.

Systemic effects have been infrequent in phase I studies with immune-modulating gene vectors, although results of animal studies have demonstrated systemic antitumor effects following injections with immune-modulating genes, such as gamma-IFN, HLA-B7, or IL-2, in association with improved survival (12, 14, 21, 24, 26, 44, 48). Data suggest that transfer of gamma-IFN gene into tumor cells may alter tumor antigen expression and induce a localized immune response. However, clinical effects of a systemic response may be difficult to evaluate in patients with bulky advanced disease who have an expected survival of <6 months. It takes several months to build up the immune response against the tumor. Before the immune system sufficiently responds to the vaccine, the malignant progress is still occurring and may override the patient's immune response if the disease is too far advanced when immunotherapy is initiated. Thus, given the potential systemic activity to local injection, many trial designs consider initial treatment with chemotherapy or treatment of patients with early stage disease since toxicity is minimal.

**OTHER IMMUNE-MODULATING VACCINE APPROACHES PERFORMED IN THE MARY CROWLEY MEDICAL RESEARCH FACILITY**

Studies exploring vaccines transduced with the GM-CSF gene (GVAX) using autologous tumor cells and allogeneic cell lines are being explored in patients with prostate cancer and non–small cell lung cancer. Also, plasmid DNA for HLA-B7 (Allovectin-7) complexed with a cationic lipid is being examined in melanoma patients. Phase I investigation reveals a low toxicity profile.

The GVAX vaccine has been studied (phase I) in patients with melanoma, renal cell cancer, and prostate cancer. GVAX vaccine is an allogeneic vaccine, which does not require surgical harvest of autologous tumor tissue. Currently, we are performing trials in patients with hormonal naive prostate cancer and hormonal refractory prostate cancer in which the GM-CSF gene is placed into allogeneic prostate cell lines containing antigens most likely to be expressed in autologous tumors. Patients receive multiple intradermal injections of the prostate GVAX vaccine for as long as stable disease is maintained or induced response is observed. Gene delivery uses an adenoviral vector for GM-CSF as opposed to a retroviral
vector, which was used for the gamma-IFN trials described.

Trials investigating the HLA-B7 gene use a plasmid DNA complex with a cationic lipid mixture. Lipid-complexed DNA plasmids have a higher transduction-efficiency rate than retroviral vectors, but intracellular release of the transgene product from the lipid complex may have some limitations. Trials investigating intratumoral injection of Allovectin-7 include phase II studies in patients with refractory progressive melanoma and a phase III investigation comparing patients receiving Allovectin-7 combined with chemotherapy (dacarbazine) with patients receiving chemotherapy alone. Response rates of 35% in injected lesions and 15% in systemic (not injected) lesions were observed in phase I/II trials.

**CONCLUSION**

Gene therapy offers a unique opportunity to modulate the immune system and potentially enhance antitumor effects. Regardless of the delivery vehicle used, safety has been confirmed with these approaches. Phase III trials are ongoing nationally and are being performed at the Mary Crowley Medical Research Center to determine whether or not gene therapy offers an advantage over standard treatment. Additionally, combining these approaches with standard approaches may also benefit patients with advanced cancer. Investigation of immune enhancement is one of several clinical research areas we are pursuing.

**References**


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Antigen identification → Antigen presentation to dendritic cell → Antigen processing → Antisense education of new lymphocytes

Figure 1

a) Tumor

b) Tumor/bacillus Calmette-Guérin

c) Tumor/oncolytic virus

d) GM-CSF gene-transduced tumor

Figure 2
Figure 3

Figure 4