Advances in the Biological Therapy and Gene Therapy of Malignant Disease

Evan M. Hersh and Alison T. Stopeck
Arizona Cancer Center, Department of Hematology/Oncology, Tucson, Arizona 85724

Abstract

Biological and gene therapy of cancer have become important components of clinical cancer research. Advances in this area are based on evidence for the presence of tumor antigens, antitumor immune responses, evasion of host control by tumors, and the recognition of host defense failure in cancer patients. These mechanisms are being corrected or exploited in the development of biological and gene therapy. Over the last decade, 9 biological therapies have received Food and Drug Administration approval, and another 12 appear promising and will likely be approved in the next few years. Our approach to gene therapy has been to allogene to tumors by the direct intratumoral injection of HLA-B7/β2-microglobulin genes as plasmid DNA in a cationic lipid into patients with malignant melanoma. In four Phase I studies, we found a 36% response by the local injected tumor and a 19% systemic antitumor response. In other cancers, gene transfer, expression, and an intratumoral T-cell response were seen, but no clinical response was seen. A variety of follow-up studies with HLA-B7 and other genes are planned. Evasion of host control is now a major target of gene therapy. Strategies to overcome this include up-regulation of MHC and introduction of cell adhesion molecules into tumor cells, suppression of transforming growth factor β and interleukin 10 production by tumor cells, and blockade of the fas ligand-fas interaction between tumor cells and attacking lymphocytes. With these approaches, it seems likely that gene therapy may become the fifth major modality of cancer treatment in the next decade.

Introduction

Biological therapy and gene therapy have become critically important components of the clinical cancer research effort. They have the potential to have a major impact on the survival of patients receiving conventional therapy for malignant disease. Since the introduction of IFN-α into clinical trials of human malignancy in 1979 (1) as the first molecularly defined biological therapy agent to be used in cancer, progress has been slow and steady, but it is now increasing in tempo. Presently, nine biological therapy agents are approved for use in human disease by the FDA. Another 10 are promising and, on the basis of current data, likely to be approved. Literally dozens of biological therapy agents are under development. Twenty to 30 years ago, the introduction of biological therapy agents into clinical trials was based on phenomenological and empirical observations in vitro and in animal models. There was little or no understanding of the mechanism of action of the biological agents or of appropriate molecular targets. Today, there has been a vast improvement in our understanding of the molecular biology of cancer, cancer at the cellular and tissue level, cancer invasion and metastases, host-tumor interaction, and how the tumor evades host control. Because of this, newer biological therapy agents are actually being designed specifically to target these mechanisms.

In honoring Dr. Emil J Freireich, it is important to reflect on his impact or influence on various aspects of clinical cancer research in biological therapy as it has developed. In 1962–1963, Dr. Freireich was visionary in hypothesizing on the role of tumor-host interaction in the natural history of cancer and its response to treatment. Thus, he hypothesized that: cancer patients would be immunodeficient, particularly those with advanced disease; therefore, there would be a relationship between immunocompetence and prognosis in cancer, with a better response to treatment in immunocompetent patients; and certain schedules of chemotherapy might be immunosuppressive, whereas others might not. This last hypothesis might be important because nonimmunosuppressive regimens might be associated with a better response to therapy. These concepts were the basis of early research career efforts of one of us (E. M. H.), and our joint investigations proved them to be accurate. Therefore, we are most pleased to dedicate this manuscript to honor Dr. Freireich.

Biological Therapy of Cancer

Biological therapy of cancer is defined as modification and exploitation of the cellular and molecular mechanisms of host defense and of the regulation of tissue proliferation, tissue differentiation, and tissue survival for the treatment of cancer. The major but not exclusive emphasis of biological therapy has been immunotherapy. However, it has expanded recently with the inclusion of the gene therapy approach. Gene therapy is defined as the introduction of a manufactured (cloned) gene or

1 Presented at "Foundations of Clinical Cancer Research: Perspective for the 21st Century," a symposium to honor Emil J Freireich, M.D., on the occasion of his 70th birthday, March 14–15, 1997, M. D. Anderson Cancer Center, Houston, TX. This work was supported by a grant from the Arizona Disease Research Commission.
2 To whom requests for reprints should be addressed, at Arizona Cancer Center, Department of Hematology/Oncology, 1515 North Campbell Avenue, P.O. Box 245024, Tucson, AZ 85724.

The abbreviations used are: FDA, Food and Drug Administration; IL, interleukin; GM-CSF, granulocyte macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; TGF-β, transforming growth factor β; DMRIE, 1,2-dimyrystoisoxypoly-3-dimethylhydroxyethyl ammonium; DOPE, dioleoyl-phosphatidylethanolamine bromide; β2m, β2-microglobulin.
gene fragment, antisense molecule, or ribozyme into cells or tissues of the body for the treatment of disease. Gene therapy may replace a missing gene, restore a gene function that is altered by mutation, increase the expression of a gene, introduce a new gene function, or abrogate the expression of a gene.

In cancer, approaches to gene therapy include: gene-modified tumor cells as vaccines; adoptive cellular therapy with gene-modified effector cells; tumor modification with prodrug-activating enzyme genes, followed by treatment with the prodrug; introduction of multidrug resistance genes into bone marrow or stem cells; introduction of wild-type tumor suppressor genes into tumors with mutated and, therefore, nonfunctional tumor suppressor genes or lost genes; and introduction of oncogene antisense. Down-regulation of gene function can also be carried out by ribozymes that degrade specific mRNA.

The commonly used biological therapies include: antitumor cytokines or immunomodulatory cytokines; antitumor or immunomodulatory monoclonal antibodies; bone marrow growth factors; host defense cells such as lymphokine-activated killer cells or tumor-infiltrating lymphocytes, generated in vivo in cancer; and infused in vitro; tumor vaccines; immunomodulators; anti-growth factors; and, as outlined above, gene therapy.

The era of biological therapy began around 1969, with the initiation of studies of tumor cell vaccines and Bacillus Calmette-Guérin therapy in leukemia by George Mathé and his group at the Institute of Tumorology and Immunogenetics (Paris, France; Ref. 5). Although numerous studies were done with Bacillus Calmette-Guérin and its derivatives over the next decade, the modern era of biological therapy actually began in 1979, with the first studies of natural IFN-γ in patients with various malignancies (1). Since then, advances in modern biotechnology, genetic engineering of proteins, monoclonal antibodies, and other techniques in molecular biology have created a large number of different agents in different classes for cancer treatment. At present, nine biological therapy agents are approved and licensed for the treatment of human disease by the FDA (Table 1). For example, IFN-γ is approved for the treatment of hairy cell leukemia, chronic myelogenous leukemia, Kaposi’s sarcoma, and malignant melanoma (5).

Another 12 biological therapy agents appear promising and, in our view, are likely to be approved in the next few years (Table 2). For example, IL-12 is the single most potent cytokine in animal tumor models. Microgram doses of this drug can regress established tumors (7). IL-12 directly activates CD4- and CD8-positive lymphocytes and down-regulates T helper 2 cells, thus shifting the balance to T helper 1 cells. The latter produces IL-2 and IFN-γ upon stimulation and drives the development of effective antitumor immunity (8). Clinical trials of IL-12 are now in progress. Tumor necrosis factor α has been shown to be highly active in inducing complete remissions in inoperable malignant melanoma or sarcoma of the extremities when it is administered as part of a regimen of isolated limb perfusion (9).

The investigation of tumor vaccines languished, with minimal interest, for many years. However, this field has become highly active in the last few years, mainly because of the developments of molecular biology and the identification of specific tumor antigens in many human tumors (10). A variety of tumor antigens that can induce active T cell-mediated immunity, including such melanoma antigens as Mage 1, Mart 1, tyrosinase, and gpl00, have been identified (11). In addition, the adenocarcinoma antigen MUC 1 is also active in the induction of T-cell immunity (12). At present, randomized, controlled Phase III studies are being performed with conventional cancer vaccines, consisting of tumor cells, tumor extracts, or tumor antigens coupled to adjuvants; these are being compared to no treatment or standard therapy. Phase I and II studies are being done with purified tumor antigens or tumor antigens in the form of DNA vaccines.

Although there are no FDA-approved monoclonal antibody therapies for cancer, treatment of malignant lymphoma with immunotoxins or radioimmunonconjugates appears to induce high remission rates, even in patients with advanced refractory disease, and may soon be approved (13). In the realm of supportive care, both thrombopoietin and IL-11 (14, 15) appear to induce rapid platelet recovery after chemotherapy in a similar fashion to the rapid granulocyte recovery that is seen with G-CSF or GM-CSF therapy. They are likely to be approved for use as an adjunct to cancer chemotherapy in the near future. These are just some examples of the promising biologicals that are now in clinical development.
Cellular and Molecular Targets for Biological Therapy

It is now well established that human tumors contain unique antigens that are not present in normal tissues, that these antigens may be immunogenic, and that host defense plays a role in the natural history of cancer. As our understanding of how tumors evade host control has improved, a number of exciting new targets for biological therapy and gene therapy have been identified. Combining an attack on these factors with established biological or gene therapy approaches to augment the responsiveness of antigen-presenting cells and the T-cell systems gives a fairly large spectrum of new strategies for biological therapy and gene therapy of cancer. The premise here is that tumors express tumor rejection antigens that can be recognized by the host immune system and that induce a CTL response. The failure of these mechanisms in human cancer is due to factors that have now been identified and can be directly manipulated.

Some of these factors include the following. Many tumors down-regulate the MHC class I and II molecules, thus failing to present antigen to the T-cell system (16, 17). These can be up-regulated with IFN-γ or even by the transfection of the MHC antigen genes (18). Solid tumors often do not express the co-stimulatory molecules, such as B7.1, which is found on antigen-presenting cells (19). Therefore, they fail to provide the necessary second stimuli to the precursors of CTLs for their proliferation and differentiation. B7.1 transfection into solid tumor cells has been shown to be a potent stimulator of tumor immunity and to induce resistance to subsequent challenge with wild-type tumor (20). In lymphoma, we have demonstrated that patients whose tumors express B7.1 have a more vigorous T-cell infiltration of their tumors and a better prognosis than do patients whose tumors do not express B7.1 (21).

Solid tumors secrete a variety of potent immunosuppressive molecules. These include TGF-β (22) and IL-10 (23). They also express immunosuppressive molecules on their surface, such as the fas ligand (24). TGF-β directly suppresses T-cell proliferation, differentiation, and CTL generation. We have shown that, when TGF-β secretion by murine breast cancer cells is down-regulated by antisense treatment, the transduced cells show decreased tumorigenicity. Animals that reject the transformed cells develop resistance to challenge with wild-type tumor (25). IL-10 acts to shift the balance from T helper 1 to T helper 2 and away from the generation of CTLs (26). The recently discovered fas ligand system appears to be critical in host defense failure and evasion of host control by tumors. Progressing tumors express fas ligand. This interacts with the fas molecule on the activated tumor-infiltrating lymphocytes and induces them to undergo apoptosis (24). This mechanism should be readily interrupted with antisense molecules, ribozymes, or with specific monoclonal antibody to fas ligand.

Impaired antigen presentation could be addressed by activation of macrophages or dendritic cells with cytokines such as GM-CSF, IFN-γ, or the flt-3 ligand or their genes (27). Finally, impaired lymphocyte proliferation and differentiation in the tumor or in the regional draining lymph nodes can be activated and restored with cytokines or genes that are involved in this process, including IL-2, IL-7, IL-12, and IFN-γ (28). Thus, each aspect of host tumor interaction and its failure can be addressed.

Gene Therapy

The rationale for cancer gene therapy by vaccination with gene-modified tumor cells is the following. Human tumors have been shown to contain tumor-associated and tumor-specific antigens and to be immunogenic in the tumor-bearing patient (29). Even vaccination with unaltered tumor cells has resulted in an immune response and tumor regression in at least a small fraction of patients with a variety of human tumors (30). There is an extensive animal model literature that shows that vaccination with gene-modified tumor cells results in a much higher level of tumor resistance than does vaccination with wild-type unmodified tumor cells (31). In a typical murine gene therapy model, tumor cells are transduced using a retrovirus vector and are selected on the basis of neomycin resistance to express the gene of interest, such as B7.1, allogeneic MHC class I or II molecules, IFN-γ, IL-2, GM-CSF, and so on. These transduced selected tumor cells lose their tumorigenicity when they are inoculated into syngeneic animals, or they grow transiently and then regress. This lack of tumorigenicity is associated with the development of tumor-specific cytotoxic CD8-positive cells and can be overcome by the administration of antibody to CD8. Animals that have rejected these transformed tumor cells are resistant to challenge with the wild-type tumor. This resistance can also be overcome with antibody to CD8. Finally, vaccination with irradiated, transformed cells also effectively induces specific tumor immunity. In general, two genes, one that increases the immunogenicity of the tumor and one that drives lymphocyte proliferation, are more effective than one gene (32). A low level of transient gene expression may be sufficient to elicit an effective immune response. Various vectors and approaches to gene delivery have been shown to be effective in the animal models and are possible in the human. These include in the in vitro (ex vivo) in vivo approach, the direct intratumoral approach, and, also, delivery of the vectors by the intraarterial i.v. or intracavitary routes.

Clinical Trials

The approach we have taken to gene therapy is to use gene-modified tumor cells as vaccines. This approach can use the in vitro (ex vivo) in vivo approach, whereby the tumor is removed, disaggregated, transformed in vitro, and reintroduced as a vaccine. Alternatively, one can use the in vivo approach, whereby the genes are directly introduced into the tumor with the appropriate vector. The tumor vaccine approach overcomes some of the current limitations on gene therapy, in that transformation of a limited fraction of tumor cells and only transient gene expression may still provide an adequate stimulus to induce a strong immune response. Our clinical strategy to gene therapy of human cancer is to deliver genes of interest with cationic lipids intratumorally. When plasmid DNA is admixed with cationic lipids, complexes form with the double-stranded DNA between and within tubules of the lipid bilayer. In vitro administration of plasmid DNA, admixed with the cationic lipids DMRIE and DOPE, results in enhanced transfection compared to naked DNA (33).
The initial preclinical study of this approach was done by Nabel's group at the University of Michigan (34). In the murine CT 26 tumor, they administered an allogeneic MHC class I plasmid DNA in cationic lipids intratumorally at the site of prior inoculation of wild-type tumor cells. Tumor was allogenized, tumor growth was markedly retarded, and animals were also resistant to subsequent wild-type tumor challenge. The mechanism was hypothesized to be an allogeneic response with secondary cytokine cascade and induction of specific tumor immunity to the tumor antigens of the wild-type tumor. Parker and her colleagues at Vical Inc. (the sponsor of most of the clinical trials of this approach; Refs. 35 and 36), using a plasmid expressing the IL-2 gene, confirmed and extended Nabel's initial approach. In B16 melanoma and in the Renca renal tumor models in the mouse, intratumoral injection of plasmid DNA with the IL-2 gene and DMRIE/DOPE resulted in retardation of tumor growth or tumor regression and prolonged survival, compared to saline or empty vector controls. In the Renca model, there was a clear-cut dose response, with increasing efficacy with increasing dosage. In all models, CTLs that were reactive against wild-type tumor were generated. In another study, HLA-B7, the gene planned for the subsequent clinical trials, was transferred intrarterially into pigs (37). A local inflammatory response was observed. A long-lasting CTL response to HLA-B7 was noted among the peripheral blood lymphocytes of the recipient animals. This proved that the approach was, indeed, a powerful and long-lasting immunogenetic stimulus.

Clinical trials were initiated by Nabel et al. in 1993 (38). He initially treated five HLA-B7-negative patients with malignant melanoma by repeated intratumoral injections of microgram amounts of HLA-B7 in DMRIE/DOPE into multiple tumor nodules (38). In posttreatment biopsies, plasmid DNA, specific RNA, and protein were detected in the injected nodules. Two of two patients tested showed development of CTLs against autologous tumor, and one of five had a partial remission, with regression of injected and uninjected nodules.

On the basis of this study, Vical Inc. sponsored three Phase I clinical trials of HLA-B7 gene therapy in HLA-B7-negative patients. Patients received a single injection of 10, 50, or 250 mg of plasmid DNA in DMRIE/DOPE or two or three serial injections of 10 μg into a single tumor nodule. The DNA:lipid ratio was 5:1. The HLA-B7 and B2M genes were driven by a single Rous sarcoma virus promoter (Allovectin). Studies were done in metastatic malignant melanoma at the Arizona Cancer Center (39), in metastatic renal cell carcinoma at the University of Chicago (40), and in metastatic colon cancer at the Mayo Clinic (41). In the majority of patients, the treatment was well tolerated. The transferred DNA and HLA-B7 protein were found in the majority of posttreatment biopsy samples. A cellular infiltrate of the tumor with CD8-positive T cells was also noted after gene injection. The plasmid DNA could be detected in the tumor as long as 8 weeks after the single injection, which was the timing of the last biopsy. In addition, the majority of patients showed a lymphocyte-proliferative response to HLA-B7, indicating a successful allogenization of the tumor (Table 3).

Clinical responses were seen only in malignant melanoma. Seven of 14 patients, all of whom had advanced refractory disease, had a local regression of the injected nodule of at least 25% reduction in tumor volume. One of these regressions of a retrocaval node was complete. Two of the lung nodules regressed over 75%. Overall, four of the seven were deemed to have substantial clinical benefit. The median survival of the 14 was 8.1 months. Subsequently, Nabel's group (42) treated an additional 10 patients with malignant melanoma with Allovectin and observed responses in two (one of which qualified as a partial remission). In another study, Silver and coworkers (43) treated seven patients with multiple injections of Allovectin. Of these, three patients were HLA-B7 positive. Silver et al. (43) hypothesized that, although the patients' normal tissues expressed MHC class I molecules, the tumor might not because melanomas frequently down-regulate MHC expression. Therefore, by transfection with HLA-B7, the tumor would regain its ability to express MHC class I molecules, permitting them to present tumor antigen on the cell surface. Posttreatment biopsies confirmed that tumor in these three patients converted from HLA-B7 negative to positive after transfection in vivo. Of the seven treated patients, three responded; two of these were HLA-B7 positive. Overall, 36 patients with melanoma were treated in these Phase 1 studies. Thirty-six % experienced local tumor regression in the injected nodule, and 19% had regression of distant, uninjected nodules (Table 4).

We have initiated a Phase I study of the IL-2 gene (Leuvecin; Ref. 44). The plasmid contains the IL-2 gene under the control of the cytomegalovirus promoter. Patients thus far have received increasing doses of plasmid DNA in DMRIE/DOPE, in doses ranging from 10 to 300 μg per dose, into one tumor nodule weekly for 6 weeks. Five patients were assigned to each dosage group. A variety of tumor types were treated, and all patients had advanced, chemotherapy-refractory disease. DNA was transferred and expressed in over 80% of the cases. However, only 5 of the 23 treated patients showed any regression of the injected tumor nodule. Because of this, we continue to

### Table 3 Biological and clinical activity of intratumoral transfer of the HLA-B7/β2M genes in DMRIE/DOPE

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>% gene transfer</th>
<th>% protein expression</th>
<th>% immune response</th>
<th>Clinical response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer</td>
<td>15 87 86</td>
<td>100</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td>15 90 79</td>
<td>95</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Renal cancer</td>
<td>14 78 86</td>
<td>93</td>
<td>0</td>
<td>14</td>
</tr>
</tbody>
</table>

* MR/PR, minor response/partial response.  
* S/P, stable/progression.

### Table 4 Summary of early Phase II studies of gene therapy by intratumoral transfer of HLA-B7/β2M in DMRIE/DOPE for malignant melanoma

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Ref.</th>
<th>Local response/total (%)</th>
<th>Systemic response/total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nabel et al.</td>
<td>38</td>
<td>1/5</td>
<td>1/5</td>
</tr>
<tr>
<td>Nabel et al.</td>
<td>42</td>
<td>2/10</td>
<td>1/10</td>
</tr>
<tr>
<td>Stopeck et al.</td>
<td>39</td>
<td>7/14</td>
<td>3/14</td>
</tr>
<tr>
<td>Silver et al.</td>
<td>43</td>
<td>3/7</td>
<td>2/7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13/36 (36%)</td>
<td>7/36 (19%)</td>
</tr>
</tbody>
</table>
multiple animal model studies have shown that immunotherapy by the transfer of two genes is also a factor as is discussed above. This includes transcription and translation of the transfection efficiency was low in terms of the percentage of tumor cells transformed (approximately 10% of the tumor cells in the biopsy samples were HLA-B7 positive on immunohistochemistry), biological activity of some type was detected in virtually all patients. This included transcription and translation of the transferred DNA in the majority of patients, induction of a CD-8-positive T-cell infiltrate of the tumor, and development of anti-HLA-B7 immune reactivity. In addition, the majority of patients receiving the IL-2 plasmid had mild-to-moderate systemic symptoms, suggesting the systemic release of IL-2. In addition, some of antitumor activity was seen in about 36% of the patients receiving HLA-B7 and in about 20% of the patients receiving IL-2. However, it was disappointing that none of the patients with renal cell carcinoma or colon carcinoma showed a tumor regression in response to HLA-B7.

The conclusions from these studies are mildly encouraging. Intratumoral administration of plasmid DNA in cationic lipids is clinically feasible, safe, and well tolerated. Although the transfection efficiency was low in terms of the percentage of tumor cells transformed (approximately 10% of the tumor cells in the biopsy samples were HLA-B7 positive on immunohistochemistry), biological activity of some type was detected in virtually all patients. This included transcription and translation of the transferred DNA in the majority of patients, induction of a CD-8-positive T-cell infiltrate of the tumor, and development of anti-HLA-B7 immune reactivity. In addition, the majority of patients receiving the IL-2 plasmid had mild-to-moderate systemic symptoms, suggesting the systemic release of IL-2. In addition, some of antitumor activity was seen in about 36% of the patients receiving HLA-B7 and in about 20% of the patients receiving IL-2. However, it was disappointing that none of the patients with renal cell carcinoma or colon carcinoma showed a tumor regression in response to HLA-B7.

Explanations and possible strategies for improvement are suggested by these data. The low transfection efficiency could be addressed by increasing the DNA dose, by using more efficient lipids, or by using other vectors. Because all patients had advanced refractory disease, host defense failure may have played a role, and systemic immunomodulation with cytokines such as IL-2 or IL-12 would be a relevant strategy. Intratumoral immunosuppression is also a factor as is discussed above. This can also be addressed. Multiple animal model studies have shown that immunotherapy by the transfer of two genes is superior to the introduction of one gene in inducing tumor resistance (45-47). Thus, there are several strategies that are available to address these obstacles.

Our immediate plans include three major approaches. For the use of the IL-2 plasmid, a protocol is underway to increase the dose of DNA to 1500 µg per injection. This is based on the animal model observation of a dose response for the IL-2 gene. Second, a protocol is being designed for the concurrent administration of the HLA-B7 and IL-2 genes. This should, on the one hand, make the tumor more immunogenic (HLA-B7) and, on the other, drive lymphocyte proliferation (IL-2). Finally, a protocol is under development to combine intratumoral gene therapy with the HLA-B7/B2M plasmid with systemic immunomodulation with daily low-dose IL-2 treatment. It has been demonstrated in patients with bone marrow transplants that immunorestoration is accomplished by the administration of approximately 1 million units of IL-2 daily over a prolonged period of time (48).

### Conclusions

There are a variety of other approaches to gene therapy being investigated around the world. In a recent survey of the literature, we were able to find 22 reports of clinical trials of gene-modified tumor cells being used as vaccines (49). A variety of genes were under study, including B7.1, IFN-γ, IL-2, IL-7, IL-12, and GM-CSF (Table 5). Overall, the response of metastatic disease, including minor response, partial remission, and complete remission, was approximately 22%. There were eight reports of the introduction of herpes simplex virus thymidine kinase into tumors (mainly brain tumors), followed by systemic drug treatment with ganciclovir. About 21% of patients showed some degree of response. Finally, we could find three reports of the introduction of wild-type p53 into tumors expressing mutant P53. Responses were reported in 30% in these few studies. For a field in its infancy, these results are encouraging.

The major obstacle to the field of gene therapy is the need for and concurrent lack of nonimmunogenic vectors that can deliver genes of interest with high efficiency, targeted to every tumor cell in the body, with regulatable and tissue-specific long-term expression. Thus, the obstacles to gene therapy include: the transferred genes may be rapidly degraded; transfection of fresh tumor cells in vitro or in vivo may be limited; gene delivery to all tumor sites cannot be achieved with current methodology; the tumor may mutate and circumvent the action of the therapeutic gene; the tumor may not express the tumor-associated antigens; and the host may be immunoincompetent.

### Table 5 Recent studies of gene therapy of cancer

<table>
<thead>
<tr>
<th>Approach</th>
<th>Studies (n)</th>
<th>Tumors</th>
<th>Genes</th>
<th>Vectors</th>
<th>Response/total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunization with gene modified cells</td>
<td>22</td>
<td>Colon cancer, glioblastoma, head and neck, melanoma, neuroblastoma, renal, various</td>
<td>IL-2, IL-4, IL-12, IFN-α, GM-CSF, HLA-B7</td>
<td>AV, Li, RV</td>
<td>35/162 (22%)</td>
</tr>
<tr>
<td>Prodrug activating gene plus prodrug (HSV-TK + ganciclovir)</td>
<td>8</td>
<td>Glioblastoma, mesothelioma, ovarian cancer</td>
<td>HSV-TK</td>
<td>AD, RV</td>
<td>11/52 (21%)</td>
</tr>
<tr>
<td>Wild-type p53 in mutant p53 tumors</td>
<td>3</td>
<td>Colon cancer, hepatoma, NSCLC p53</td>
<td>p53</td>
<td>RV, plasmid</td>
<td>7/23 (30%)</td>
</tr>
</tbody>
</table>

*AV, adenovirus; Li, cationic lipids; RV, retrovirus; HSV-TK, herpes simplex virus thymidine kinase; NSCLC, non-small cell lung carcinoma.*
Also, data that show that immunization with gene-modified tumor cells is superior in the clinical setting to immunization with unmodified tumor cells with adjuvant are lacking. Another factor that applies to all attempts to immunize against cancer is that the tumor burden may exceed the immune response capacity for control. Finally, the animal models may not be entirely appropriate. They mainly use a preimmunization design, the animals are young and fully immunocompetent, and the experimental tumors are highly immunogenic.

In December 1995, the NIH Gene Therapy Panel also considered obstacles to gene therapy. These include low frequency of gene transfer, and the lack of quantitative assessment; only qualitative assessment of gene transfer and expression was feasible. Some of the protocols lacked suitable controls, and there was a lack of well-defined biochemical and disease end points. The NIH Gene Therapy Panel recommendations included a focus on basic aspects of gene transfer and expression and the development of improved vectors. These vectors should deliver genes optimally, should achieve and maintain high levels of expression, should achieve tissue specific and regulated expression, and should be able to target to specific cells and tissues. Extensive research is being done to address all of these points. It seems likely that these problems of delivery and expression will be solved within the next decade. With that, gene therapy may very well take its place as the fifth major modality of cancer treatment.

Acknowledgments

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References


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