REVIEW

Gene Therapy for Cancer: What Have We Done and Where Are We Going?

*Jack A. Roth, Richard J. Cristiano**

Gene-based therapies for cancer in clinical trials include strategies that involve augmentation of immunotherapeutic and chemotherapeutic approaches. These strategies include ex vivo and in vivo cytokine gene transfer, drug sensitization with genes for prodrug delivery, and the use of drug-resistance genes for bone marrow protection from high-dose chemotherapy. Inactivation of oncogene expression and gene replacement for tumor suppressor genes are among the strategies for targeting the underlying genetic lesions in the cancer cell. A review of clinical trial results to date, primarily in patients with very advanced cancers refractory to conventional treatments, indicates that these treatments can mediate tumor regression with acceptably low toxicity. Vector development remains a critical area for future research. Important areas for future research include modifying viral vectors to reduce toxicity and immunogenicity, increasing the transduction efficiency of nonviral vectors, enhancing vector targeting and specificity, regulating gene expression, and identifying synergies between gene-based agents and other cancer therapeutics. [J Natl Cancer Inst 1997;88:21-39]

The concept of gene therapy follows logically from the observation that certain diseases are caused by the inheritance of a single functionally defective gene. Theoretically then, diseases caused by a known monogenic defect, such as adenosine deaminase deficiency or Gaucher's disease (also called glucosylceramide lipidosis) could be treated and potentially cured by the insertion and expression of a normal copy of the mutant or deleted gene in host cells. This idea of gene-replacement therapy represents the basic framework for the therapeutic approach to monogenic diseases. If a renewable population of cells (e.g., bone marrow stem cells) could be transduced with normal copies of the target gene, only a single or limited number of treatments would be necessary. Otherwise, the gene would need to be repeatedly administered to the patient over his or her life span. The recent identification of bone marrow stem cell populations and the ability to efficiently transduce other long-lived hematopoietic cells has made this approach feasible enough for testing in clinical trials (*1*,*2*). Recent reviews (*3*-*7*) have summarized progress in gene therapy.

The evolution of gene therapy has taken a somewhat unexpected course on the basis of these rather conceptually simple beginnings. Most of the approved protocols for what is now called gene therapy involve cancer patients. This would not have been anticipated because cancer seems to be a particularly unsuitable target for the classical approach of gene-replacement therapy. Cancer generally arises as the culmination of a multistep process that involves a variety of somatic gene alterations. At first blush, it might appear necessary to be able to correct all of the genetic abnormalities in the cancer cell, which is daunting since all of these are not known. It would also seem necessary to restore normal gene function to every cancer cell, which is beyond the capabilities of the vectors currently available for use in gene therapy. As it turns out, these considerations may not limit strategies involving gene replacement for therapy of cancer (more on this below). For these reasons, cancer gene therapy has focused instead on using recombinant DNA constructs to augment existing therapies. The treatment strategies that have evolved include the use of recombinant vaccines as immunotherapeutics, the protection of bone marrow during chemotherapy by transducing a drug-resistance gene into marrow stem cells, and the use of expression vector constructs that bring about the conversion of inactive prodrugs into active drugs. Some individuals may contend that these interventions do not constitute gene therapy but that they are instead recombinant DNA therapeutics that do nothing to restore normal functioning genes to the cancer cell. There is some merit in this distinction, since in some instances, the genes being introduced into the cells have no direct therapeutic function. Certainly, in situations where the introduced DNA is for diagnosis or prevention, the term therapy should not be used (*8*). On the basis of this classification, many of the interventions to date in cancer would be classified as gene therapeutics as distinct from gene-replacement therapy.

* *Affiliations of authors:* J. A. Roth (Departments of Thoracic and Cardiovascular Surgery and Tumor Biology), R. J. Cristiano (Department of Thoracic and Cardiovascular Surgery), The University of Texas M. D. Anderson Cancer Center, Houston.

Correspondence to: Jack A. Roth, M.D., Department of Thoracic and Cardiovascular Surgery, The University of Texas M.D. Anderson Cancer Center, Box 109, 1515 Holcombe Blvd., Houston, TX 77030.

See ''Notes'' following ''References.''

Current Approaches to Gene Therapeutics and Gene Therapy

Immunotherapy Using Recombinant DNA Constructs Expressing Cytokines and Lymphokines

An immune response against syngeneic tumors can be generated in animal models using a variety of tumors induced by chemical carcinogens and viruses. Tumor regression can result from manipulating the human immune response with interleukin 2 (IL-2). The response rates of cancer patients to these immune manipulations is low and primarily confined to patients with melanoma and renal cell cancer. In addition, cytokines secreted by tumor cells into which cytokine gene-expressing recombinant DNA constructs have been inserted have elicited antitumor immune responses in preclinical (i.e., animal model) studies (*9-19*). This suggests that the results of immunotherapy could be improved by the use of recombinant DNA tumor cell vaccines or by adoptive transfer of genetically engineered lymphocytes. A major advantage of this approach is the potential to generate a systemic immune response against the tumor.

Current protocols based on this idea use tumor-infiltrating lymphocytes (TILs), tumor cells, or fibroblasts to express cytokine genes. Initially, it was felt that TILs had a propensity to traffic specifically to tumor cells and, therefore, that expression of cytokines by TILs might avoid the toxicity associated with systemically administered cytokines. Thus, the expression of cytokine genes, such as tumor necrosis factor (TNF), by adoptively transferred gene-transduced TILs could possibly be concentrated at the tumor site. Subsequently, however, this was disproved by a study that showed that tumors did not selectively take up or retain TILs marked with the neo^r gene (*20*).

Another approach is to use autologous tumor cells transduced with a cytokine gene as a vaccine (*9,11-13,15*). In many instances, however, tumor cells will not be available from patients, and even if available, the transduced cells may not express the cytokines. An alternative approach is to inject fibroblasts that have been engineered to express the cytokine gene. However, this approach also has its disadvantages: In one study, antitumor immunity was not induced by IL-2-expressing mammary stromal fibroblasts (*16*).

Yet another approach is to make tumor cells more immunogenic. T cells recognize protein antigens after they are degraded into peptides that bind to histocompatibility complex molecules and are then transported to the tumor cell surface. Tumor cells, however, may be defective in their expression of class I or class II histocompatibility molecules, thus leading to defects in antigen presentation. Additional costimulatory molecules, such as B7-1 and B7-2, may be needed for effective induction of the efferent arm of the immune response to tumor antigens, but tumor cells may be defective in the expression of such molecules (*21*). If so, then restoration of costimulatory gene expression might enhance tumor cell immunogenicity (*22*). One substantial difficulty with this approach is the heterogeneity and unpredictability of loss of costimulatory molecules in human cancers. For example, one study of prostate cancer showed that cells vary in their loss of histocompatibility and transporter molecules (*21*). This approach may therefore require replacement of multiple genes within the tumor cell to elicit an effective immune response. It is also possible that, despite activation of the efferent arm of the immune response to tumor antigens, ineffective transport mechanisms may result in an antigen density too low to be recognized by the cytotoxic effector cells.

Another immunologic approach involves the use of vectors that express tumor-rejection antigens. Recently, immunodominant epitopes on human melanoma cells have been identified that are recognized by TILs and are associated with tumor regression (*23*). However, as with the other approaches, there are potential difficulties. The immune response to an immunogen is clonal, but in tumors, antigen expression is heterogeneous (*24*). Tumors also produce factors that suppress the immune response (*25*). Moreover, as discussed above, cancer cells possess a variety of defects in the machinery of antigen presentation. Thus, it is not clear that an immune response elicited by cells possessing the appropriate genes for antigen presentation will elicit an immune response effective against cells expressing the antigen but defective in components of antigen presentation. The identification of tumor rejection antigens from a variety of cancers and of the critical components of afferent and efferent limbs of the immune response that are defective in cancer patients are important topics for future studies.

A survey was conducted of principal investigators for cancer gene therapy protocols to obtain current information on protocols completed or in progress. Protocols were identified from existing comprehensive listings (*26-29*). Principal investigators personally communicated the results of their studies with full knowledge that the results would be summarized in this review. Following completion of the survey, the results were resubmitted to the principal investigators for verification of accuracy. The results were tabulated as of June 19, 1996 (Appendix Table 1). Published clinical trial reports are cited.

Sixty protocols worldwide had been designed to augment the immune response against cancers by gene therapy (i.e., by vaccine or direct cytokine or costimulatory molecule gene transduction) (Appendix Table 1). A total of 376 patients have been entered in these ongoing protocols. Major tumor regressions (defined as either complete responses or partial responses) have been observed in 15 of 237 patients with sufficient information to evaluate responses.

Drug-Sensitivity Genes

Selective transduction of tumor cells with a gene whose product can convert a relatively nontoxic prodrug administered systemically to a toxic metabolite in the cancer cell was one of the first strategies proposed for the use of recombinant DNA constructs in cancer patients. In the first protocol to be approved using this strategy, brain tumors are transfected with a retroviral vector expressing the herpes simplex virus thymidine kinase (HSV-TK) gene. Systemic gancyclovir that enters the tumor cell is metabolized to cytotoxic gancyclovir triphosphates by cells expressing HSV-TK (*30*). A potential advantage of this technique is selective uptake of the vector and expression by proliferating cells, presumably the tumor cells. Studies (*31-33*) in a rat glioma model showed that marked tumor regression occurred when only a small fraction of tumor cells were transfected with the retroviral HSV-TK. This cytotoxic effect of transduced on nontransduced cells has been termed the ''bystander effect.'' There are several mechanisms that appear to mediate bystander

effects and these may act concurrently. They include transfer of toxic metabolic products of gancyclovir through gap junctions, phagocytosis of apoptotic vesicles of dead tumor cells by live tumor cells that mediate apoptosis, and induction of an immune response against the tumor. Retrovirus- and adenovirus-mediated transfer of the cytosine deaminase gene, which confers cellular sensitivity to 5-fluorocytosine, to tumor cells has been reported (*34*,*35*).

As of June 1996, 21 protocols using this strategy had been proposed (Appendix Table 1). A total of 104 patients have been entered in these ongoing protocols. Major tumor regressions have been observed in eight of 62 patients, with sufficient information to evaluate responses.

Drug-Resistance Genes

The transfer of genes into normal cells to augment existing cancer treatments is also under investigation. Current protocols are attempting to enhance marrow protection during chemotherapy by transducing the multiple-drug resistance gene (MDR1) into normal bone marrow or blood-derived stem cells (*36*,*37*). The MDR1 gene produces P-glycoprotein, which functions as a cellular efflux pump and may be responsible for the resistance of some tumor cells to various hydrophobic cytotoxic drugs. Insertion of the MDR1 gene into normal marrow stem cells produces a population of cells that can be selected for resistance to a systemically administered chemotherapeutic agent (*38*,*39*). A potential advantage of this approach is that it may permit higher doses of chemotherapy to be given with less toxicity and more efficacy. Retrovirus-mediated expression of the DNA repair protein O-methylguanidine-DNA-methyltransferase protected mouse primary hematopoietic cells from nitrosourea-induced toxicity, and marrow from transgenic mice expressing methotrexate-resistant dihydrofolate reductase was protective against methotrexate toxicity in recipient syngeneic mice (*40*-*42*).

Approved protocols for MDR1 include protocols for the treatment of patients with breast or ovarian cancer who are receiving paclitaxel (Taxol). There are, however, some potential problems with this strategy: higher doses of chemotherapy may not translate into higher response rates, nonhematologic toxic effects may be dose limiting, and cancer cells in the marrow may be transduced with the drug-resistance gene. As of June 1996, eight protocols using this strategy had been proposed and are being tested in clinical trials (Appendix Table 1). Insufficient information is available, however, to evaluate the therapeutic efficacy.

Tumor Suppressor Gene Replacement and Oncogene Inactivation

The identification of specific genes that contribute to the development of cancer presents an opportunity to use these genes and their products as prevention and treatment targets. The genes that are implicated in carcinogenesis include dominant oncogenes and tumor suppressor genes (*43*,*44*). Proto-oncogenes (normal homologues of oncogenes) participate in critical cell functions, including signal transduction and transcription, but only a single mutant allele is required for the malignant transformation of a cell. Primary modifications in the dominant oncogenes that confer gain of transforming function include point mutations, amplifications, translocations, and rearrangements. Tumor suppressor genes, which regulate gene transcription and cell proliferation, undergo homozygous loss of function, either by mutation, deletion, or a combination of these. It is possible that modification of the expression of dominant and tumor suppressor oncogenes may influence certain characteristics of cells that contribute to the malignant phenotype. Thus, gene replacement could mediate induction of tumor cell death by direct killing (e.g., apoptosis) or a bystander effect, induction of tumor cell dormancy, or prevention of malignant progression of premalignant cells.

Oncogenes. The ras family of oncogenes is among the most common activated oncogenes found in human cancer and is therefore a potential target for oncogene-inactivation strategies. The ras (i.e., H-ras, N-ras, and K-ras) genes each encode an oncoprotein that is located on the inner surface of the plasma membrane, that has guanosine triphosphatase (GTPase) activity, and that may participate in signal transduction. These genes are activated by point nucleotide mutations that alter the amino acid sequence of their protein product, p21 (*45*). Antisense technology involving introduction into the cell of a gene construct that has a base sequence complementary to the RNA sequence targeted for inhibition has been used to study the effects of eliminating expression of a mutant K-ras oncogene in human lung cancer cells (*46*). The antisense and sense sequences bind by Watson–Crick base pairing. Protein synthesis may be inhibited at the level of messenger RNA (mRNA) splicing, transport, or translation (*47*). Transduction with either an antisense K-ras complementary DNA (cDNA) plasmid or retroviral construct selectively blocked the production of mutant K-ras mRNA and reduced the growth rate of human lung cancers in vitro and in vivo in nu/nu mice (*46*,*48*,*49*). Alternative methods of reducing or blocking the expression of oncogenes involve the use of antisense oligonucleotides (that bring about the degradation of oncogene-encoding mRNA), ribozymes (that directly cleave oncogene mRNA), and intracellular single-chain antibodies (*50*-*55*).

Tumor suppressor genes. The inactivation of certain genes may contribute to tumor growth. In one scenario, both copies of the gene must be eliminated or inactivated to eradicate the growth-suppressive function of the gene (*43*,*56*,*57*). Theoretically then, replacement of a functioning copy of the tumor suppressor gene in cells with homozygous loss of function could restore normal growth and proliferation pathways. Mutations in the p53 (also called TP53) gene are common in a wide spectrum of tumors (*57*). The p53 gene encodes a 393-amino acid phosphoprotein that can form complexes with viral proteins, such as large-T antigen and E1B, both of which functionally inactivate the p53 protein. Missense mutations that occur in the p53 gene are essential for its transforming ability. The p53 protein also appears to be multifunctional because it has major domains that interact with other proteins, that allow p53 oligomers to form, that bind DNA in a sequence-specific manner, and that transactivate the expression of certain genes. Thus, abnormalities in one or more of these functions could contribute to abrogation of the tumor suppressor function of p53 (*58*). Since the wild-type p53 (wt-p53) protein may either suppress the expression of genes that contribute to uncontrolled cell growth and proliferation or

activate genes that suppress uncontrolled cell growth, the absence or inactivation of wt-p53 may contribute to transformation. The restoration of wt-p53 expression in cells with mutant or deleted p53 is sufficient to cause apoptosis or growth arrest, despite multiple genetic abnormalities present in the cell (*59,60*).

Restoration of wt-p53 gene expression using a retroviral p53 expression vector suppressed growth in H358a (deleted p53) and H322a (mutated p53) human lung cancer cell lines but had no effect in another transduced human lung cancer cell line, H460a, which has an endogenous wild-type p53 gene (*61*). In mixing experiments, retroviral wt-p53-transduced cells could reduce the growth rate of nontransduced cells in human lung cancer, indicating a bystander effect. In another study (*62*), the direct administration of a retroviral wt-p53 expression vector (LNp53B) in an orthotopic human lung cancer model led to the suppression of tumor growth.

An adenovirus expression vector was also developed for delivery of wild-type human p53 cDNA (Ad-p53) to cells. This p53-expression vector induced apoptosis in cancer cells with mutated or deleted p53 but only minimally affected growth of cells containing wt-p53 (*63*). The vector inhibited tumorigenicity in the mouse model of orthotopic human lung cancer (*49*). Moreover, similar p53–adenovirus vector constructs can inhibit growth of rat gliomas, human head and neck cancers, and human colon cancers in nu/nu mice and can mediate p53 gene expression in bladder and liver cancers (*64-71*). The products of other tumor suppressor genes, such as p16 and a truncated retinoblastoma gene (Rb), have been found to suppress tumor growth in animal models (*72,73*). The evaluation of gene therapy combinations involving use of tumor suppressor genes and constructs that inactivate oncogenes is an important area for future research.

The presence of a wt-p53 gene may be necessary for induction of apoptosis by some chemotherapeutic agents (*74,75*). One study (*76*) examined whether sequential administration of Ad-p53 and cisplatin (CDDP) could induce synergistic tumor regression in vivo. After 3 days of direct intratumoral injection of Ad-p53, p53-deleted human lung tumors grown as subcutaneous xenographs in nu/nu mice showed a modest slowing of growth; tumors injected with an adenovirus vector containing the p53 transgene regressed if CDDP was administered intraperitoneally for 3 days. CDDP alone had no effect on tumor growth in this model system. Moreover, in tumors treated directly with the combination, extensive areas of apoptosis were visualized by use of the terminal deoxynucleotidyl transferase biotin deoxyuridine triphosphate nick end-labeling method (that detects 3′-hydroxyl ends of DNA fragments). In contrast, tumors treated with either CDDP or Ad-p53 alone showed no apoptosis.

Clinical applications. The use of viral vectors to replace defective tumor suppressor genes or inactive oncogenes in tumors is thus supported by the following observations: 1) Viral gene transfer is more efficient in cancer cells than expected from studies of normal organ gene transfer (*61,77*); 2) viral vectors spread readily through three-dimensional cancer cell matrices (*78*); 3) transduced cells mediate bystander killing of nontransduced cells (*61*); and 4) correction of a single genetic lesion is sufficient to mediate potentially clinically significant tumor regressions (*61,77*). There are limitations with this therapeutic approach. At present, the administration of viral vectors to patients is limited to delivery to local and regional tumors since current vectors are not approved for systemic administration. Immune responses to existing vectors may, however, limit repetitive administrations. Improvements in vector transduction efficiency are needed to increase the percentage of tumor cells that take up the vector.

As of June 1996, 13 protocols using this strategy had been proposed (Appendix Table 1). A total of 78 patients have been entered in these clinical trials. Major tumor regressions have been observed in six of 26 patients with sufficient information to evaluate responses.

Critical Areas for Future Development

Despite the proliferation of clinical protocols using gene therapy strategies, there are many aspects of gene transfer that are less than ideal. One of the most important areas for future research is vector design. The vector is critical for gene delivery and expression, but existing vectors have limitations (Table 1). The remainder of this review will focus on specific topics related to vector development.

Viral Vectors

Retrovirus. The majority of approved gene-transfer protocols use retroviral vectors. For cancer applications, the propensity of the retrovirus to integrate into dividing cells appears to be an advantage. For ex vivo applications, the retrovirus has a sufficiently high transduction efficiency. To date, this has been the most useful vector for achieving stable integration of foreign DNA into the target cell. However, there are several potential limitations for retroviruses (*5,79*). First, their capacity is limited to about 8 kilobases (kb). Second, serum complement can inactivate retroviruses, although a recent study (*80*) showed that retroviruses produced by producer cells of human origin are resistant to complement inactivation. Third, the currently achievable titers (10^7) are low compared with what will be needed for the treatment of large tumors. Fourth, large-scale production is difficult, with the most substantial problem being the appearance of replication-competent retroviruses. This last problem has been reduced by using packaging cell lines that split the packaging signals between two plasmids (*81*). Fifth, the host range of some retroviruses may be limited. However, the technique of pseudotyping may overcome this limitation because it involves the use of packaging cell lines in which envelop proteins having the desired host range are substituted for the endogenous retroviral protein (*82,83*). For example, amphotropic Moloney leukemia virus infects hepatocytes poorly; using a packaging cell line that provides a Sendai virus surface-F glycoprotein conferred infectivity to hepatocytes (*84*).

Adenovirus. The limitations of recombinant retroviral vectors have led to the need for more versatile vectors for the successful application of gene therapy. Attention has focused on viral vectors based on the Ad5 since this double-stranded DNA virus is capable of high-level transduction of many cell types, regardless of the mitotic status of the cell (*85*). First-generation recombinant adenoviruses have been generated by homologous recombination into the E1 or E3 regions of the viral genome, resulting in a dependence on the embryonic kidney cell line 293

Table 1. Vectors for gene delivery into cells

Vector, size	Advantages	Disadvantages
Retrovirus, 10 kilobase (kb)	Integration Requirement of cell division for transduction	Low transduction efficiency Packaging cell line required No targeting Replication competence Insert size, 9-12 kb Requirement of cell division for transduction
Adenovirus, 35 kb	High transduction efficiency Infection of many cell types Infection does not require cell division	No integration Packaging cell line required Safety/toxicity/immunogenicity Replication competence No targeting Insert size, 4-5 kb
Adeno-associated virus, 5 kb	Integration? No viral genes Infection does not require cell division	No targeting Packaging cell line required Safety Insert size, 5 kb
Herpes simplex virus, 152 kb	Neuronal tropism Large insert size, 40-50 kb Latency expression	No targeting Packaging cell line Toxicity
Vaccinia virus, 187 kb	Large insert size, 25 kb	Immunogenicity Toxicity Safety Efficiency? No targeting
Avipox virus, 260 kb	Infection does not require cell division Large insert size, >4 kb	Immunogenicity Toxicity Safety Efficiency? No targeting
Baculovirus, 80-230 kb	Expression of protein at high levels Liver-directed gene transfer?	Immunogenicity Toxicity Safety Efficiency? No targeting
Liposomes	Completely synthetic No limitation on size and type of nucleic acid	No targeting Inefficiency
Mechanical administration	No limitation on size of nucleic acid	No targeting Possible requirement for surgical procedure Inefficiency
Protein/DNA complex	No limitation on size and type of nucleic acid Cell-specific targeting	No integration Safety/toxicity Inefficiency in vivo Immunogenicity

as a helper cell that provides the E1 gene product in *trans* missing from the recombinant virus. The E1 region must be intact for viral replication so that the E1-deleted virus cannot replicate outside the packaging cell line. This vector can be produced at substantially higher viral titers ($>10^{11}$) than retrovirus with little manipulation (*86*). Moreover, studies (*85*,*87*) have now shown that minimal amounts of the virus can generate high-level transduction of cells with efficient levels of gene expression in most tissues except hematopoietic cells. So far, the application of recombinant adenoviral vectors to gene therapy has primarily been in the treatment of benign disorders, such as cystic fibrosis (*88*), but recently, the focus has broadened to encompass cancer applications (*89*). Some protocols propose using this vector to deliver drug-sensitivity genes, such as the HSV-TK gene, to kill cancer cells in either the brain or the liver, while others propose using it to deliver the gene for the tumor suppressor p53 into lung, head and neck, or liver cancer cells after direct tumor injection.

Although adenovirus has shown much use in gene therapy, this vector is not without problems. The presence of intact viral genes in the recombinant virus have resulted in low levels of viral gene expression, which leads to cellular toxicity and cell death (*90*). As a result, the length of gene expression is limited, and the virus may not be able to be administered to previously treated recipients because of an immune response to viral antigens. For example, specific lymphocyte-mediated cytotoxicity to E1a-deleted adenovirus-infected mouse hepatocytes has been observed (*91*). Second-generation recombinant adenoviruses have been developed by taking advantage of temperature-sensitive mutations in the E2a gene to bring about minimal viral

late gene expression, to blunt the host immune response against viral proteins, and to increase the duration of therapeutic gene expression (*90,92*). Another approach to this problem is to use an adenovirus deleted of all sequences except the internal terminal repeats and contiguous packaging sequences (*93*). Humoral immune responses of the immunoglobulin A type have been reduced by the administration of IL-12, which inhibits the T_H2 subset of T-helper cells that are necessary for initiation of antibody production (*94*). Other groups have used immunosuppressive agents, such as cyclosporin, to mediate a transient attenuation of the immune response in previously treated recipients, allowing for subsequent gene delivery and expression (*95,96*). And finally, since adenoviruses have 47 different serotypes, some investigators have proposed that distinct serotypes be sequentially administered to patients in an attempt to circumvent the neutralizing antibody response (*97*).

Adeno-associated virus, herpesvirus, poxvirus, vaccinia virus, and baculovirus. Work in the field of virology has also identified a large number of viruses that have unique characteristics useful for their application in gene therapy. Adeno-associated virus, a small, linear single-stranded DNA virus, can be generated in which most of the viral genome has been replaced with DNA encoding a potentially therapeutic gene. As a result, this virus has less chance of generating an immune response. A preliminary study (*98*) has shown that this virus is capable of infecting both dividing and nondividing cells as well as hematopoietic cells. This virus has also been applied in gene therapy protocols for cystic fibrosis. However, this vector also has its problems, which primarily result from the removal of the viral genome. The wild-type virus has the ability to integrate at a specific location in chromosome 19; however, this activity seems to be lost in the recombinant virus (*99*). Also, the generation of recombinant virus results in low viral titers (10^4), partly because of the inefficient process of producing the virus. Production requires the use of a helper virus, such as adenovirus, to provide the missing viral genes, and, as a result, contamination of preparations with helper virus can frequently occur. The inability to develop a high-titer-producing packaging cell line continues to be a limiting factor for the efficient use of this system.

The herpes simplex virus, a large, double-stranded DNA virus, has received attention because of its ability to establish latent infection in the brain. As a result, it has been used in some initial applications to deliver therapeutic genes to neurons and could potentially be used to deliver therapeutic genes to some forms of brain cancer (*100*). However, the characteristics of this virus have yet to be fully determined because of the large size of the genome (150 kb). Vaccinia viruses, poxviruses, and baculoviruses have also received recent attention for use in the delivery of genes for therapeutic purposes. Recombinant forms of the vaccinia virus can accommodate large inserts and are generated by homologous recombination. Thus far, this vector has been used to deliver ILs, such as human IL-1β and the costimulatory molecules B7-1 and B7-2, for either in vitro or ex vivo applications (*101,102*). Avipox virus vectors can infect and express recombinant proteins in human cells without viral replication, allowing this vector to be used for vaccination against disease (*103*). Baculovirus, an insect virus system, has been used primarily for protein expression but is now being considered for applications in gene therapy because of its ability to express proteins at high levels (*104*). The further improvement of present viruses as well as the use of new viral vectors will likely expand the applicability and efficacy of gene therapy.

Nonviral Vectors and Naked DNA

One of the more promising areas of vector development has been that of nonviral vectors. These vectors are designed to deliver therapeutic genes to cells without the aid of an intact virus. This group of vectors primarily consists of liposomes, molecular conjugates, and naked DNA delivered by mechanical methods. Liposomes, when combined with DNA of any size, form a lipid–DNA complex that is capable of delivery to many cell types (*105*). The wide range of lipid molecules available (most notably, *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methylsulfate [DOTAP] and *N*-[1-(2,3-)dioleyloxy)propyl]-n,n,n-trimethylammonium-chloride [DOTMA]) and the ease with which the complexes can be generated have resulted in clinical protocols in which the use of liposomes (i.e., the technique of lipofection) for the delivery of therapeutic genes in cancer gene therapy has been proposed (*91*). However, this system lacks the ability to target to specific cell types and mediates gene transduction mainly at the site of administration. Other forms of lipids, such as glycolipids, can be used to target specific organs, such as the liver, but the presence of the lipid component in the complex can result in nonspecific uptake by the reticuloendothelial system, causing a loss of targeting specificity (*108*).

The emerging need for targeted gene delivery to a specific cell type has resulted in the development of molecular conjugates, which consist of protein or synthetic ligands to which a nucleic acid- or DNA-binding agent has been attached for the specific targeting of nucleic acids (i.e., plasmid DNA) to cells (*109-111*). Once the DNA is coupled to the molecular conjugate, a protein–DNA complex results. This gene delivery system, originally developed by Wu and Wu (*109*), has been shown to be capable of targeted delivery to many cell types through the use of different ligands (*109*). This system can also deliver any type or size of nucleic acid and has resulted in highly specific and efficient gene delivery in vitro (*112,113*). Problems such as the need for a nonviral endosomal lysis agent and short duration of gene expression have limited its use in vivo (*114*). However, this system does provide the basis for the generation of ''synthetic viruses'' capable of efficient gene delivery without the detrimental effects of intact viruses.

The simplest of delivery systems for gene therapy is the delivery of DNA without the use of a virus or synthetic vector. This has been accomplished by using mechanical methods of delivery, such as direct injection of DNA into tissue or by high-velocity bombardment of tissues with DNA attached to gold particles. The injection of naked DNA into muscle has led to DNA delivery and expression in vivo (*115,116*). Recently, this technology has been applied to cancer for the generation of cancer vaccines, and the related studies have resulted in at least three clinical protocols for the generation of antitumor immunity against colon cancer and melanoma (*89*). However, this method of delivery is limited only to cells near the injection site acquiring the DNA, with no tissue targeting. The delivery of DNA by particle bombardment can generate gene expression in the liver and in tumors, but it also suffers from a lack of targeting, the

inability to transduce a large number of cells, and the need for a surgical procedure to allow access to the tissue (*115,116*). A universal gene delivery system has yet to be identified, but the further optimization of each of these vectors should result in each having a unique application.

Vector Targeting and Specificity

One of the more important aspects of gene therapy continues to be the specificity of therapeutic gene expression. This is being addressed at several levels, namely, vector targeting, tissue-specific promoters, route of delivery, and modulation of immune receptors. Viral vectors, which normally cannot target specific cells, do have some specificity that results from tissue tropisms. Adenovirus, which normally infects the lung epithelium, can also mediate high-level transduction of the liver parenchyma (*85*). Retroviral vectors have no specific tissue tropism; however, the genes delivered by such vectors are integrated and expressed only in dividing cells, owing to their ability to integrate into the host cell genome during DNA replication (*117*). Also, the herpes simplex virus, which is capable of infecting many cell types, can efficiently infect and mediate prolonged expression in neuronal cells (*100*).

Two research groups (*118,119*) have shown that viral vectors can be targeted to specific cell types after attachment of ligands to the viral capsid either chemically or with antibodies. A third group (*120*) has shown that the retroviral envelope gene can be manipulated to express chimeric protein that consists of the envelope with a cell-specific ligand. This work is still evolving and potentially provides the capability of developing targeted retroviral vectors. It may also be possible to alter binding motifs of the adenovirus coat proteins to enhance specific binding (*97*).

Nonviral vector targeting has been accomplished primarily through the use of molecular conjugates and protein/DNA complexes (*107-109*). Several ligands have been used for cell-specific gene delivery, such as the vitamin folate to promote delivery into cells that overexpress the folate receptor (e.g., ovarian carcinoma cells) (*121*) and the glycoprotein asialoorosomucoid (ASOR), which has been used to mediate specific uptake by the liver parenchyma. The malaria circumsporozoite protein has been used for the liver-specific delivery of genes under conditions in which ASOR receptor expression on hepatocytes is low, such as in cirrhosis, diabetes, and hepatocellular carcinoma (*122*). Recently, the overexpression of receptors for epidermal growth factor (EGF) on cancer cells has allowed for specific uptake of EGF/DNA complexes by lung cancer cells (*123*). Another approach involved use of the human papillomavirus (HPV) capsid to partially identify the HPV receptor by using the capsid as a ligand for the attachment and delivery of a reporter gene to cells (*124*). Since the targeting of these complexes is done through a receptor that is internalized by receptor-mediated endocytosis, fusion of the endosome with the lysosome results in degradation of the DNA attached to the molecular conjugate (*109*). Although agents have been identified that increase gene expression through endosomal lysis (i.e., adenovirus), a truly efficient nonviral endosomal lysis has yet to be identified.

The next level of specificity can be generated by using tissue or cell-specific promoters. The cytomegalovirus promoter and enhancer has been identified to be active primarily in rapidly dividing cells, since the enhancer is activated by transacting factors present in the nucleus (*125*). Adding to this is the fact that this promoter is the strongest identified thus far and as a result, these characteristics have made it a good choice for use in cancer gene therapy (*126*). The promoter for the carcinoembryonic antigen (CEA) gene has been incorporated in vectors and it has shown that cell-specific expression of the resulting CEA-expression vector constructs in tumors cells, such as those of pancreatic carcinoma, can be achieved (*127*). The regulatory sequences of the human surfactant protein A gene have been used to generate cell-specific expression in non-small-cell lung cancers that express this protein (*128*). Also, melanoma cell-specific expression of β-galactosidase has been generated by using as little as 769 base pairs of the 5′-flanking sequences of the tyrosinase gene (*129*). A stress-inducible glucose-related protein (grp) promoter for grp78 that is responsive to hypoxic conditions has been shown to mediate high-level reporter gene expression in a mouse fibrosarcoma model (*130*). Nevertheless, the identification of a truly universal cancer-specific promoter has been difficult because of the heterogeneity of cancer types.

The route of delivery can also aid in specificity. The direct injection into tumor of retroviral producer cells, e.g., by stereotactic administration to gliomas in the brain and bronchoscopic administration to lung tumor, allows for infection of cells in the area injected and has formed the basis for several clinical protocols (*89*). Systemic, intravenous administration of vectors, while not providing tissue specificity, provides a simple means of administration (*105,106*). Peritumoral injection, which limits the administration to the neighboring tumor, and compartmental administration, such as intraperitoneal injection, have both shown efficacy in preclinical models (*131,132*). An alternative to these in vivo approaches is the ex vivo approach, which was the first approach used for gene therapy. In this approach, cells are removed from the patient, transfected by the delivery system, and then replaced into the patient. This procedure provides the greatest degree of specificity and is now being used to treat melanoma and hematologic disorders (*133,134*).

Cytotoxic lymphocytes or TILs could be targeted to tumors by the induction of specific immune receptors mediated by gene transfer. Already, the T-cell receptor recognizing the MART-1 melanoma antigen has been cloned and expressed in Jurkat cells (*135*). The expression of T-cell receptors that recognize tumor antigens could then be used to redirect TILs or other cytotoxic lymphocytes to the tumor. Single-chain antibodies to a renal cell carcinoma antigen have also been expressed in anti-CD3-activated human peripheral blood lymphocytes and have been shown to lyse tumor cells specifically (*136*).

Gene Integration and Duration of Expression

The length of therapeutic gene expression is an important determinant of the effectiveness of the therapy. Vectors such as retrovirus and possibly adeno-associated virus have the ability to integrate into the host genome, resulting in long-term expression that may also be associated with genotoxicity. As for cancer gene therapy, long-term expression may apply more to the expression of antisense genes. However, therapeutic genes, such as those for tumor suppressors or drug sensitivity, may only require short-term expression to kill cells. Short-term gene expression has been seen with adenoviral vectors, since the linear viral ge-

nome remains episomal and therefore is susceptible to degradation (*85,137*). This also occurs with nonviral vectors, since the nucleic acid lacks maintenance sequences and becomes degraded. However, the nonviral systems, by virtue of their ability to provide the necessary signals, may provide the best potential for the further development of gene constructs that mediate episomal maintenance, replication, or integration into the host genome.

Regulation of Transcription: Inducible Promoters and Temperature-Sensitive Mutants

The control of gene expression at the transcriptional level may be the most difficult goal to achieve. The strengths of various promoters can be easily determined; however, more critical to expression may be the regulation of the promoter. Moreover, the heterogeneity of tumor types will make the generalization of regulation difficult. Thus, the ability to simply induce rather than regulate the expression of therapeutic genes may be much more practical at present. Early growth response gene-related promoter sequences that increase gene expression following exposure to radiation have been identified (*138*). As a result, therapeutic gene expression induced by irradiation has been shown to result in cell-specific killing. Another way to control gene expression is to use temperature-sensitive mutants; for example, it has been shown that viral gene expression can be ablated with the use of temperature-sensitive mutant sequences in the viral genes (*92*).

Interactions With Other Cancer Therapies

Surgery, radiation therapy, and chemotherapy are currently the primary methods for cancer treatment. Gene therapies may interact in synergistic or additive ways with them. For instance, Fujiwara et al. (*76*) have shown that replacing the p53 gene in p53-deficient cancer cell lines enhances the sensitivity of these cells to CDDP and results in greater tumor cell killing. Recently, Son and Huang (*139*) have shown that treating CDDP-resistant tumor cells with CDDP increases the sensitivity of these cells to transduction by DNA-carrying liposomes. Also, Chen et al. (*140*) have shown that HSV-TK and IL expression can be combined to mediate improved tumor killing. As a whole, these studies indicate that improved methods for treating cancer that combine conventional cancer treatments and gene therapy can be developed and applied for a greater therapeutic effect.

Vector Immunogenicity and Toxicity

To date, no major toxic effects associated with the use of retrovirus vectors for gene transfer in humans have been reported. Longer term toxic effects are theoretically possible, as discussed above, but these have not yet been observed. The use of adenovirus vectors, however, has highlighted two problems: immune responses to the vector that reduce transgene expression on subsequent administrations (discussed above) and toxic effects related to expression of viral proteins.

Toxic effects in a patient have been reported after administration of an adenovirus vector expressing the cystic fibrosis transmembrane conductance regulator (CFTR) gene into the right lower lobe bronchus (*141*). The patient developed headache, fatigue, fever, tachycardia, and hypotension, but the specific contributions of the expression of adenovirus proteins or the CFTR gene product to the observed syndrome is not known. Adenovirus proteins can mediate inflammatory responses in lung tissue that are abrogated to some extent by the presence of the adenovirus E3 protein, which reduces histocompatibility antigen expression (*142-144*).

Also, minimal toxicity of an adenovirus vector expressing the wt-p53 gene has been reported in human bronchial epithelial cells and in a mouse model (*145*). Growth of the human bronchial epithelial cells was not altered, and localized peribronchiolar and perivascular infiltrates were observed following intratracheal injection of 10^9 and 10^{10} plaque-forming units. These problems can likely be overcome, however, by modifying vector structure to eliminate expression of endogenous viral genes, improving vector targeting, and using immunomodulators to reduce the immune response against the vector.

Summary and Conclusions

Conceptually, it is possible to extend the technique of gene therapy to cancer prevention, that is, to use it to halt the progression of premalignant lesions to invasive cancer. For example, premalignant lesions, such as bronchial dysplasia or Barrett's epithelium, have tumor suppressor gene mutations that may be amenable to gene therapy (*146,147*). In any case, preventing the development of invasive cancers is clearly preferable to treating them once established.

So far, the application of gene therapy agents in phase I trials has been limited to patients with advanced incurable cancer. However, once the safety and efficacy of these agents have been demonstrated, trials in patients with earlier stages of disease should be undertaken. Indeed, there is a potential role for these agents in the treatment of patients with limited invasive cancer. Since local recurrence or persistence of local disease is still a major problem for many cancers, such as those of the lung, head and neck, and pancreas, intralesional injections or adjuvant use of gene-based agents to prevent local recurrence after surgery could be considered. Sites of limited metastatic disease could also be injected with these agents percutaneously. Moreover, if these agents are efficacious, their lack of toxicity may provide a sufficiently high therapeutic index to allow their use as an adjuvant to surgery in patients with earlier stages of cancer or as a preventative for second primary cancers in individuals with genetic abnormalities in premalignant lesions. The high titers achievable with adenovirus vectors suggest that they could be used systemically, and vector targeting by expression of receptor ligands in the viral capsid is also possible. Aerosolized delivery to the respiratory epithelium of such agents encapsulated in liposomes has also been reported (*148*).

Although the first clinical protocols in gene therapy began just 5 years ago, progress has been rapid, and important observations have emerged from ongoing clinical trials. In general, retroviral vectors appear to be safe vehicles for gene transfer. Neither short- nor long-term toxicity has yet been associated with these vectors. Gene transfer and expression into cancer cells in vivo are possible, both with viral and nonviral vectors. Tumor regression has been observed by several investigators for immunotherapy/cytokine, drug sensitivity, and tumor suppressor/antisense protocols. Despite these successes, however, inter-

preting response rates in phase I trials is difficult. Taken at face value, these response rates appear comparable to those achieved with many single-agent chemotherapy protocols in comparable advanced-stage patients. Yet, most cancer gene therapy protocols have used vectors to augment existing therapeutic approaches, such as immunotherapy and chemotherapy. Thus, the limitations in response rates may be more a function of the inherent limitations of these existing approaches rather than limitations of gene therapy. Consequently, it is imperative to avoid unrealistic expectations for this emerging approach. Since failure to meet unrealistic expectations in patients with highly advanced disease may discourage further development, reaction to negative trials should be kept in perspective. Indeed, the publication of negative results should be encouraged since this can identify problems in vector and clinical trial design and can point the way to better studies in the future. It would be expected that the response rate to gene-based agents given as single modalities would be as low as those for single-agent chemotherapy. However, gene-based treatments combined with other types of treatment could be very effective. The lack of toxicity of gene-based therapies will allow them to be tried with other therapeutics so that additive and synergistic effects can be evaluated.

The fundamental knowledge of the molecular genetics of cancer and the mechanisms underlying carcinogenesis is expanding rapidly. Consequently, the design and testing of therapeutic strategies targeted to the fundamental processes that have gone awry in the cancer cell may allow the development of novel cancer treatment and prevention strategies. Moreover, improvements in vector design that increase the efficiency of expression, that increase the precision of targeting, and that reduce toxicity should also improve response rates. Although much research needs to be done, the possibility of specific gene targeting with a high therapeutic index makes this area of gene therapy a promising one for future investigations.

Appendix Table 1. Worldwide clinical trials for cancer gene therapy*,†

Principal investigator	Protocol title	Gene transduction	Target	Vector name	Delivery vehicle	No. of patients entered	Evidence of gene expression‡	No. and type of response§	Adverse reactions‖
				Immunotherapy/cytokine					
Berchuck A, Duke University, Durham NC	IL-2 gene-modified tumor cells in patients with metastatic ovarian cancer	Ex vivo	Metastatic ovarian cancer cells	IL-2	Lipid	0	NA	NA	NA
Black KL, UCLA, School of Medicine, Los Angeles	Injection of glioblastoma patients with TGF-β_2 antisense and IL-2 gene-modified autologous tumor cells	Ex vivo	Autologous glioblastoma cells	IL-2/TGF-β2	Retrovirus	0	NA	NA	NA
Bozik ME, Univ. of Pittsburgh Cancer Institute, PA	Gene therapy of malignant gliomas: IL-4 gene-modified autologous tumor cells	Ex vivo	Malignant glial cells	DFG-HIL4-neo	Retrovirus	0	NA	NA	NA
Brenner M, St. Jude Children's Research Hospital, Memphis, TN	Cytokine gene-modified autologous neuroblastoma cells for treatment of relapse/refractory neuroblastoma	Ex vivo	Neuroblasts	G1NaCvIL2 or AD-IL2	Retrovirus adenovirus	14	Yes	1 CR; 1 PR; 6 SDs	None
Cascinelli N, Sylvester Cancer Center/Univ. of Miami Hospital, FL	Immunization of metastatic melanoma patients with IL-4-transduced, allogeneic melanoma cells	Ex vivo	Human melanoma cell line	Lh48SN	Retrovirus	6	Yes	1 MR	Erythema and induration
Cascinelli N, Sylvester Cancer Center/Univ. of Miami Hospital, FL	Immunization of metastatic melanoma patients with IL-2 gene-transduced, allogeneic melanoma cells	Ex vivo	Human melanoma cell line	Lh2SN	Retrovirus	6	NA	2 MRs	Erythema, fever
Chang AE, Univ. of Michigan Medical Center, Ann Arbor	Immunotherapy for cancer by direct gene transfer into tumors	In vivo	Melanoma cells	HLA-B7, β2-microglobulin	Lipid	10	Yes	NA	None
Chang AE, Univ. of Michigan Medical Center, Ann Arbor	Phase II study of immunotherapy of metastatic cancer by direct gene transfer	In vivo	Cancer cells	HLA-B7, β2-microglobulin	Lipid	0	NA	NA	NA
Chang AE, Univ. of Michigan Medical Center, Ann Arbor	Activated lymph node cells primed with autologous tumor cells transduced with GM-CSF gene	Ex vivo	Tumor cells	MFG-S-GM-CSF	Retrovirus	2	Yes	1 PR	None
Chen AP, NCI, National Naval Medical Center, Bethesda, MD	Recombinant vaccinia virus expressing PSA vaccine in patients with adenocarcinoma of the prostate	In vivo	Prostate cancer cells	PSA	Vaccinia	0	NA	NA	NA
Cole DJ, Medical Univ. of South Carolina, Charleston	CEA vaccinia virus vaccine	In vivo	Fibroblasts	rV-CEA	Vaccinia	0	NA	NA	NA

Appendix Table 1—continued. Worldwide clinical trials for cancer gene therapy*,†

Principal investigator	Protocol title	Gene transduction	Target	Vector name	Delivery vehicle	No. of patients entered	Evidence of gene expression‡	No. and type of response§	Adverse reactions‖
Conry RM, University of Alabama, Birmingham	Polynucleotide immunization to human CEA in patients with metastatic colorectal cancer	In vivo	Myocytes	pGT63	Plasmid	0	NA	NA	NA
Das Gupta T, Univ. of Illinois at Chicago	Allogeneic melanoma cells transduced with retroviral vector expressing IL-2	Ex vivo	UTSO-H-MEL2 melanoma cells	pZipNeoSVIL-2	Retrovirus	0	NA	NA	NA
Dranoff G, Dana-Farber Cancer Institute, Boston, MA	Vaccination with autologous-irradiated melanoma cells producing human GM-CSF	Ex vivo	Melanoma cells	MFG-GM-CSF	Retrovirus	10	Yes	NA	None
Economou J, UCLA School of Medicine, Los Angeles	Vaccination with autologous-irradiated melanoma cells producing IL-2	Ex vivo	Melanoma cells	IL-2	Retrovirus	0	NA	NA	NA
Economou J, UCLA School of Medicine, Los Angeles	Vaccination with autologous-irradiated melanoma cells producing IL-7	Ex vivo	Melanoma cells	IL-7/HyTK	Retrovirus	4	NA	NA	NA
Figlin RA, UCLA Medical Center, Los Angeles	Immunotherapy of metastatic cancer by direct gene transfer	In vivo	Renal cell carcinoma cells	Allovectin-7	Lipid	8	NA	NA	NA
Figlin RA, UCLA Medical Center, Los Angeles	HLA-B7 as an immunotherapeutic agent in renal cancer with IL-2 therapy	In vivo	Renal cell carcinoma cells	Allovectin-7	Lipid	6	NA	3 SDs	NA
Fox BA, Providence Portland Medical Center, OR	Adoptive cellular therapy of cancer combining direct HA-B7/β_2 microglobulin gene transfer with autologous tumor vaccination for generation of vacccine-primed anti-CD3 activated lymphocytes	In vivo	Irradiated autologous tumor cells	HA-B7/Z2 microglobulin	Plasmid	6	Yes	NA	NA
Gansbacher B, Memorial Sloan-Kettering Cancer Center, New York	Immunization with HLA-A2-matched allogeneic melanoma cells that secrete IL-2 in patients with metastatic melanoma	Ex vivo	Irradiated HLA-A2-matched allogeneic tumor cells	NAPAD/IL-2	Retrovirus	12	NA	None	Erythema, induration, pruritus, pain
Gansbacher B, Memorial Sloan-Kettering Cancer Center, New York	Immunization with IL-2-secreting allogeneic HLA-A2-matched irradiated renal cell carcinoma cells in patients with advanced renal cell carcinoma	Ex vivo	Renal cell carcinoma cells	NAPAD/IL-2	Retrovirus	12	NA	None	Erythema, induration, pruritus, pain
Gluckman JL, Univ. of Cincinnati, OH	Allovectin-7 in the treatment of squamous cell carcinoma of the head and neck	In vivo	Squamous cell carcinoma	Allovectin-7	Lipid	3	NA	1 PR; 2 MRs	None
Gore M, Royal Marsden Hospital, London, U.K.	Treatment of metastatic malignant melanoma with melanoma cells genetically engineered to secrete IL-2	Ex vivo	Melanoma cells	MFGs-IL-2	Retrovirus	6	NA	NA	NA
Harris AL, Churchill Hospital, Oxford, U.K.	Cancer therapy for metastatic melanoma	In vivo	Melanoma cells	pTyrIL-2/pTyr β-Gal	Plasmid	7	Yes	None	None
Hersh E, Arizona Cancer Center, Tucson	Study of gene transfer of IL-2 gene	In vivo	Tumor cells	Leuvectin	Lipid	24	Yes	NA	NA
Hersh E, Arizona Cancer Center, Tucson	Study of gene transfer of HLA-B7 gene	In vivo	Tumor cells	Allovectin-7	Lipid	14	Yes	1 CR	NA
Hwu P, National Institutes of Health, Bethesda, MD	Treatment of patients with advanced epithelial ovarian cancer using anti-CD3-stimulated peripheral blood lymphocytes transduced with chimeric T-cell receptor gene	Ex vivo	PBLs	MFG-Movy	Retrovirus	0	NA	NA	NA
Ilan J, Case Western Reserve Univ., Cleveland, OH	Episome-based antisense cDNA transcription of IGF-I for brain tumors	Ex vivo	Glioblastoma cells	pAntilGF-I	Plasmid	0	NA	NA	NA

Appendix Table 1—continued. Worldwide clinical trials for cancer gene therapy*,†

Principal investigator	Protocol title	Gene transduction	Target	Vector name	Delivery vehicle	No. of patients entered	Evidence of gene expression‡	No. and type of response§	Adverse reactions‖
Lindemann A, Medizinische Universitätsklinik, Freiburg, Federal Republic of Germany	Vaccination study with B7.1 + IL-2 gene-transfected allogeneic cell lines in renal cell carcinoma	Ex vivo	Renal cell carcinoma cell lines	B7.1/IL-2	Lipid	0	NA	NA	NA
Lindemann A, Medizinische Universitätsklinik, Freiburg, Federal Republic of Germany	Evaluation of vaccine preparations in melanoma patients	Ex vivo	Allogeneic fibroblasts + NATC	IL-2 or GM-CSF	Lipid	0	NA	NA	NA
Link CJ, Human Gene Therapy Research Inst., Des Moines, IA	Adoptive immunotherapy for leukemia: donor lymphocytes transduced with HSV-TK for remission induction	Ex vivo	Lymphocytes	LTK0SN	Retrovirus	0	NA	NA	NA
Lotze MT, Univ. of Pittsburgh, School of Medicine, PA	IL-4 gene-modified antitumor vaccines	Ex vivo	Irradiated autologous fibroblasts + NATC	G1IL4SvNa	Retrovirus	18	Yes	3 MRs; 1 SD	NA
Lotze MT, Univ. of Pittsburgh, School of Medicine, PA	IL-12 gene therapy with genetically engineered autologous fibroblasts	Ex vivo	Autologous fibroblasts	TFG-hIL-12-neo	Retrovirus	12	Yes	3 PRs	NA
Lyerly HK, Duke Univ., Durham, NC	Autologous human IL-2 lipofection gene-modified tumor cells in patients with refractory or recurrent metastatic breast cancer	In vivo	Metastatic breast cancer cells	IL-2	Lipid	0	NA	NA	NA
Marshall JL, Georgetown Univ., Washington, DC	Study of recombinant ALVAC virus that expresses CEA in patients with advanced cancers	In vivo	Autologous muscle cells	CEA	Pox virus	13	NA	NA	NA
Mertelsmann R, Medizinische Universitätsklinik, Freiburg, Federal Republic of Germany	T-cell-mediated immunotherapy by cytokine gene transfer in patients with malignant tumors	Ex vivo	Irradiated autologous fibroblasts + NATC	IL-2	Lipid	15	No	None	None
Nabel GJ (*49*), Univ. of Michigan Medical Center, Ann Arbor	Immunotherapy of cancer by in vivo gene transfer into tumors	In vivo	Melanoma cells	HLA-B7, β_2-microglobulin	Lipid	5	Yes	1 PR	None
Osanto S, Academisch Ziekenhuis Leiden, The Netherlands	Immunization with IL-2-transfected melanoma cells for patients with metastatic melanoma	Ex vivo	Melanoma cells	IL-2	NA	0	NA	NA	NA
Paulson DF, Duke Univ. Medical Center, Durham, NC	Autologous IL-2-gene-modified tumor cells for locally advanced or metastatic prostate cancer	Ex vivo	Prostate cancer cells	pMP6A/IL-2	Lipid	0	NA	NA	NA
Podack E, Antoni Van Leeuwenhoek Hospital, Amsterdam, The Netherlands	Small-cell lung tumor cells transduced with a vector expressing IL-2	Ex vivo	Small-cell lung cancer cells	IL-2	Lipid	0	NA	NA	NA
Rankin EM, Antoni Van Leeuwenhoek Hospital, Amsterdam, The Netherlands	Vaccination with autologous GM-CSF-transduced and irradiated tumor cells in patients with advanced melanoma	Ex vivo	Melanoma cells	MFGH-S	Retrovirus	30	Yes	6 SDs (6+ mo)	Fever
Rosenberg SA, National Cancer Institute, Bethesda, MD	Gene therapy of patients with advanced cancer using TILs transduced with gene coding for TNF	In vitro	TILs	TNF-neo	Retrovirus	12	Yes	1 PR	Elevation of LFTS
Rosenberg SA, National Cancer Institute, Bethesda, MD	Immunization of cancer patients using autologous cancer cells modified by insertion of the gene for IL-2	In vitro	Autologous tumor cells	G1NaCV12	Retrovirus	2	NA	NA	NA

Appendix Table 1—continued. Worldwide clinical trials for cancer gene therapy*,†

Principal investigator	Protocol title	Gene transduction	Target	Vector name	Delivery vehicle	No. of patients entered	Evidence of gene expression‡	No. and type of response§	Adverse reactions‖
Rosenberg SA, National Cancer Institute, Bethesda, MD	Immunization with autologous melanoma tumor cells transduced with the gene for TNF	In vitro	Autologous tumor cells	TNF	Retrovirus	3	NA	NA	NA
Rosenberg SA, National Cancer Institute, Bethesda, MD	Patients immunized with recombinant adenovirus containing the gene for the MART-1 tumor antigen	In vivo	Melanoma cells	MART-1	Adenovirus	33	NA	NA	NA
Rosenberg SA, National Cancer Institute, Bethesda, MD	Recombinant adenovirus containing the gene for the gp100 melanoma tumor antigen	In vitro	Melanoma cells	gp100	Adenovirus	7	NA	NA	NA
Rosenblatt J, Univ. of California, Los Angeles, CA	Interferon gamma gene-transduced tumor cells in patients with neuroblastoma	Ex vivo	LAN-6/ CHLA-138	Da/Huy(v)	Retrovirus	1	NA	NA	NA
Rubin J, Mayo Clinic, Rochester, MN	Study of immunotherapy of advanced colorectal carcinoma by direct gene transfer into hepatic metastases	In vivo	Colorectal carcinoma cells	HLA-B7	Lipid	0	NA	NA	NA
Schmidt-Wolf I, Institut Fuer Molekularbiologie, Berlin, Federal Republic of Germany	IL-7 gene therapy for lymphoma	Ex vivo	Lymphoma cells	IL-7	Plasmid	0	NA	NA	NA
Seigler HF, Duke University Medical Center, Durham, NC	Human interferon gamma-transduced autologous tumor cells for disseminated malignant melanoma	Ex vivo	Melanoma cells	N2	Retrovirus	20	Yes	2 CRs; 1 PR	None
Silver H, BC Cancer Center, Vancouver, BC, Canada	Immunotherapy by direct gene transfer	In vivo	Melanoma/ renal/ lymphoma cells	VCL-1005-201	Lipid	5	NA	NA	NA
Silver H, BC Cancer Center, Vancouver, BC, Canada	Intralesional transfection with plasmid HLA-B7 in melanoma	In vivo	Melanoma cells	VCL-1005	Lipid	7	Yes	NA	NA
Simons J, Johns Hopkins Oncology Center, Baltimore, MD	Phase I study of nonreplicating autologous tumor cell injections using cells prepared with or without GM-CSF gene transduction in patients with metastatic renal cell carcinoma	Ex vivo	Renal cell carcinoma cells	MFG	Retrovirus	18	NA	1 PR	None
Sobol RE (*150*), San Diego Regional Cancer Center, CA	Injection of a glioblastoma patient with autologous tumor cells and irradiated fibroblasts genetically modified to secrete IL-2	Ex vivo	Autologous tumor cells and fibroblasts	G1NCvi2 and DC/AD/R/IL-2	Retrovirus	1	NA	1 PR	Peritumor edema
Sobol RE, San Diego Regional Cancer Center, CA	Injection of colon carcinoma patients with autologous-irradiated tumor cells and irradiated fibroblasts genetically modified to secrete IL-2	Ex vivo	Autologous fibroblasts	LSXSN-tIL2	Retrovirus	6	NA	1 SD (3+ mo)	Fatigue
Sznol M, National Institutes of Health, Frederick, MD	Trial of B7-transfected lethally irradiated allogeneic melanoma cell lines to induce cell-mediated immunity against tumor-associated antigens	Ex vivo	Irradiated allogeneic melanoma cells	CMV-B7	Lipid	0	NA	NA	NA
Vogelzang NJ, Univ. of Chicago Medical Center, IL	Immunotherapy of metastatic renal cell carcinoma by direct gene transfer: phase II study in renal, colon, breast	In vivo	Renal cancer cells	Allovectin-7 (HLA-B7)	Lipid	14	Yes	None	Injection pain
Vogelzang NJ, Univ. of Chicago Medical Center, IL	Immunotherapy of metastatic cancer by direct gene transfer	In vivo	Cancer cells	Allovectin-7	Lipid	4	Yes	None	NA
Yee C, Univ. of Washington, Seattle	Adoptive immunotherapy using autologous CD8+ tyrosinase-specific T cells for metastatic melanoma	Ex vivo	Tyrosinase-specific T cells	HyTK	Retrovirus	0	NA	NA	NA

Appendix Table 1—continued. Worldwide clinical trials for cancer gene therapy*,†

Principal investigator	Protocol title	Gene transduction	Target	Vector name	Delivery vehicle	No. of patients entered	Evidence of geneexpression‡	No. and type of response§	Adverse reactions‖
			Drug sensitivity						
Albelda SM, Univ. of Pennsylvania Medical Center, Philadelphia	Gene therapy for malignant mesothelioma with HSV-TK	In vivo	Malignant mesothelioma cells	H5.01ORSVTK	Adenovirus	10	Yes	None	Fever, abnormal liver function
Crystal RG, Cornell Medical Center, New York, NY	Administration of replication-deficient adenovirus vector containing the *Escherichia coli* cytosine deaminase gene to metastatic colon carcinoma of the liver with 5-fluorocytosine	In vivo	Liver cells	AdCVcD.10	Adenovirus	1	NA	NA	None
Curiel D, Univ. of Alabama, Birmingham	Adenovirus intraperitoneal HSV-TK for ovarian and extra ovarian cancer patients	In vivo	Ovarian cancer cells	AdTK	Adenovirus	0	NA	NA	NA
Eck SL, Univ. of Pennsylvania, Philadelphia	Recombinant adenovirus for the treatment of CNS cancer	In vivo	Glioblastoma/ astrocytoma cells	H5.01ORSVTK	Adenovirus	2	NA	None	None
Fetell MR, Columbia-Presbyterian Medical Center, New York, NY	Stereotactic injection of HSV-TK vector producer cells for treatment of recurrent malignant glioma	In vivo	Glioma cells	G1TK1SvNa.7	Retrovirus	2	NA	None	NA
Finocchiaro G, Inst. Nazionale Neurologico C. Besta, Milan, Italy	Gene therapy of glioblastoma with HSV-TK	In vivo	Glioblastoma cells	HSV-TK	Retrovirus	0	NA	NA	NA
Freeman SM, Tulane Univ. Medical Center, New Orleans, LA	Treatment of ovarian cancer with a modified HSV-TK cancer vaccine	Ex vivo	PA-1 ovarian tumor cells	STK	Retrovirus	14	NA	2 CRs	Fever, abdominal pain, nausea
Freeman SM, Tulane University Medical Center, New Orleans, LA	Vaccination with HER-2/neu-expressing tumor cells and HSV-TK gene-modified cells	Ex vivo	PA-1 ovarian/ MDA breast cancer cells	STK/B7	Retrovirus	0	NA	NA	NA
Grossman RG, Baylor College of Medicine, Houston, TX	HSV-TK for central nervous system tumors	In vivo	Brain tumor cells	Adv. RSV-tk	Adenovirus	0	NA	NA	NA
Izquierdo M, Universidad Autonoma de Madrid, Spain	Gene therapy of glioblastoma with HSV-TK	In vivo	Glioblastoma cells	p tk zip neo	Retrovirus	9	No	1 PR; 1 MR	Fever
Klatzmann D, Hôpital Pitié Salpétrière, Paris, France	Gene therapy for metastatic melanoma with HSV-TK	In vivo	Melanoma cells	pM TK	Retrovirus	7	Yes	NA	None
Klatzmann D, Hôpital Pitié Salpétrière, Paris, France	Gene therapy for glioblastoma with HSV-TK	In vivo	Glioblastoma cells	pM-TK	Retrovirus	13	NA	NA	None
Kun LE, St. Jude Children's Research Hospital, Memphis, TN	Stereotactic injection of HSV-TK-producer cells for progressive or recurrent primary supratentorial pediatric brain tumors	In vivo	Neoplastic glial cells	G1TksvNa.7	Retrovirus	2	NA	1 MR	Increased local edema
Link CJ, Human Gene Therapy Research Institute, Des Moines, IA	HSV-TK treatment of refractory or recurrent ovarian cancer	In vivo	Ovarian carcinoma cells	LTKOSN	Retrovirus	0	NA	NA	NA
Mariani L, Neurochirugische Klinik Inselspital, Bern, Switzerland	Gene therapy for glioblastoma with HSV-TK	In vivo	Glioblastoma cells	G1TK1svNa.7	Retrovirus	6	NA	NA	None
Mulder NH, Academisch Ziekenhuis Groningen, The Netherlands	Gene therapy for glioblastoma with HSV-TK	In vivo	Glioblastoma cells	G1TK1svNa.7	Retrovirus	3	No	1 MR	Seizures, abducens paresis, confusion

Appendix Table 1—continued. Worldwide clinical trials for cancer gene therapy*,†

Principal investigator	Protocol title	Gene transduction	Target	Vector name	Delivery vehicle	No. of patients entered	Evidence of gene expression‡	No. and type of response§	Adverse reactions‖
Munshi NC, Univ. of Arkansas Medical Sciences, Little Rock	TK-transduced donor leukocyte infusions for patients with relapsed or persistent multiple myeloma after bone marrow transplant	Ex vivo	Lymphocytes	G1TK1svNa.7	Retrovirus	1	NA	NA	None
Oldfield EH, National Institutes of Health, NINDS, Bethesda, MD	Gene therapy of brain tumors with HSV-TK	In vivo	Malignant glial tumors	G1Tk1svNa	Retrovirus	20	Yes	2 CRs; 3 PRs	Intratumoral hemorrhage
Raffel C, Mayo Clinic, Rochester, MN	Gene therapy for treatment of recurrent pediatric malignant astrocytomas with in vivo tumor transduction with the HSV-TK gene	In vivo	Astrocytoma cells	HSV-TK	Retrovirus	0	NA	NA	NA
Van Gilder JC, Univ. of Iowa Hospital, Iowa City	Gene therapy for glioblastoma with HSV-TK	In vivo	Glioblastoma cells	HSV-TK	Retrovirus	14	NA	NA	NA
Yla-Herttuala S, Univ. of Kopio, Finland	Gene therapy for glioma with HSV-TK	In vivo	Glioma cells	retrovec-TK	Retrovirus	0	NA	NA	NA
			Drug resistance						
Cowan K, National Institutes of Health, Bethesda, MD	Retroviral mediated transfer of MDR-1 into hematopoietic stem cells during transplantation after chemotherapy for metastatic breast cancer	Ex vivo	Hematopoietic stem cells	G1MD	Retrovirus	3	Yes	NA	None
Cowan K, National Institutes of Health, Bethesda, MD	Antimetabolite induction followed by high-dose single alkylating agent consolidation, and retroviral transduction of the MDR-1 and NEO-R genes into peripheral blood progenitor cells	Ex vivo	Hematopoietic stem cells	G1MD	Retrovirus	3	NA	NA	NA
Deisseroth AB, Yale University, New Haven, CT	Use of retrovirus to introduce chemotherapy resistance sequences into normal hematopoietic cells for chemoprotection during therapy for breast cancer	Ex vivo	Hematopoietic cells	MDR-1	Retrovirus	10	NA	NA	NA
Deisseroth AB, Hammersmith Hospitals NHS Trust, London, U.K.	Use of retrovirus to introduce retroviral chemotherapy-resistance sequences into normal hematopoietic stem cells for chemoprotection during therapy for ovarian cancer	Ex vivo	Hematopoietic cells	MDR-1	Retrovirus	10	NA	NA	NA
Hesdorffer C, Columbia Univ., New York, NY	MDR gene transfer in patients with advanced cancer	Ex vivo	CD34	AM12	Retrovirus	4	No	None	None
Mickioch C, Univ. Hospital Rotterdam, The Netherlands	Autologous reinfusion of hematopoietic precursor cells genetically modified by retroviral gene transfer of the multidrug-resistance gene in patients with metastatic, refractory bladder carcinoma	Ex vivo	Hematopoietic stem cells	pICSA	Retrovirus	0	NA	NA	NA
Sonneveld P, Univ. Hospital Rotterdam, The Netherlands	Autologous reinfusion of hematopoietic stem cells derived from bone marrow and blood, genetically modified by retroviral gene transfer of the multidrug-resistance gene in patients with relapsed or primary refractory high-risk non-Hodgkin's lymphoma	Ex vivo	Hematopoietic stem cells	pIGSA	Retrovirus	0	NA	NA	NA

Appendix Table 1—continued. Worldwide clinical trials for cancer gene therapy*,†

Principal investigator	Protocol title	Gene transduction	Target	Vector name	Delivery vehicle	No. of patients entered	Evidence of gene expression‡	No. and type of response§	Adverse reactions‖
Stoter G, Univ. Hospital Rotterdam, The Netherlands	Reinfusion of autologous bone marrow genetically modified by retroviral gene transfer of the multidrug-resistance gene in patients with metastatic breast cancer refractory to first-line chemotherapy	Ex vivo	Hematopoietic stem cells	pIGSA	Retrovirus	0	NA	NA	NA
			Tumor suppressor/antisense						
Bishop M, Univ. of Nebraska Medical Center, Omaha	Antisense p53 for ex vivo treatment of autologous peripheral blood stem cells with OL (1) in patients with acute myelogenous leukemia	Ex vivo	Myelogenous leukemia cells	p53 antisense	Oligonucleotide	0	NA	NA	NA
Clayman GL, M. D. Anderson Cancer Center, Houston, TX	Modification of tumor suppressor gene expression in head and neck squamous cell carcinoma with an adenovirus expressing wild-type p53	In vivo	Squamous cell carcinoma of head and neck	Ad5CMV-p53	Adenovirus	17	NA	NA	None
Habib N (*80*), Hammersmith Hospitals NHS Trust, London, U.K.	p53 DNA injection in colorectal liver metastases	In vivo	Colorectal liver metastases	pC53/SN3	Plasmid	6	Yes	None	Fever
Habib NA (*151*), Hammersmith Hospitals NHS Trust, London, U.K.	p53 DNA injection in hepatocellular carcinoma	In vivo	Hepato-cellular carcinoma cells	pC53/SN3	Plasmid	8	Yes	1 CR; 2 PRs; 1 MR	Fever
Holt J, Vanderbilt Univ. Medical School, Nashville, TN	Retroviral antisense c-fos RNA for metastatic breast cancer	In vivo	Breast cancer cells in effusions	XM6:antifos	Retrovirus	1	No	None	None
Holt J, National Institutes of Health, Bethesda, MD	BRCA1 retroviral gene therapy for ovarian cancer	In vivo	Ovarian cancer cells	LXN-BRCA1	Retrovirus	2	No	None	None
Hortobagyi GN, M. D. Anderson Cancer Center, Houston, TX	E1A gene therapy for patients with metastatic breast or epithelial ovarian cancer that overexpresses HER-2/neu	In vivo	Breast cancer	E1A gene	Lipid	0	NA	NA	NA
Luger S, Hospital of the Univ. of Pennsylvania, Philadelphia	Autologous bone marrow transplantation using c-myb antisense oligodeoxynucleotide-treated bone marrow in CML in chronic or accelerated phase	Ex vivo	Leukemic cells in bone marrow	Antisense c-myb	Oligonucleotide	8	NA	NA	NA
Luger S, Hospital of the Univ. of Pennsylvania, Philadelphia	Infusional c-myb antisense oligodeoxynucleotide in chronic myelogenous leukemia and acute leukemia	Ex vivo	Leukemic cells	Antisense c-myb	Oligonucleotide	20	NA	NA	NA
Roth JA, M. D. Anderson Cancer Center, Houston, TX	Modification of tumor suppressor gene expression and induction of apoptosis in NSCLC with adenovirus vector expressing wild-type p53 and cisplatin	In vivo	Lung cancer cells	Ad5CMV-p53	Adenovirus	7	Yes	NA	None
Roth JA (*152*), M. D. Anderson Cancer Center, Houston, TX	Modification of oncogene and tumor suppressor gene expression in NSCLC	In vivo	Lung cancer cells	ITRp53/ ITRASKRAS	Retrovirus	9	Yes	1 CR; 2 PRs; 3 SDs	None
Steiner M, Univ. of Tennessee, Memphis	Treatment of advanced prostate cancer by in vivo transduction with prostate-targeted retroviral vectors expressing antisense c-myc RNA	In vivo	Prostate cancer cells	XM6	Retrovirus	0	NA	NA	NA

Appendix Table 1—continued. Worldwide clinical trials for cancer gene therapy*,†

Principal investigator	Protocol title	Gene transduction	Target	Vector name	Delivery vehicle	No. of patients entered	Evidence of gene expression‡	No. and type of response§	Adverse reactions‖
Venook A, Univ. of California, San Francisco	Adenovirus expressing p53 via hepatic artery infusion for primary and metastatic liver tumors	In vivo	Primary and metastatic liver cancers	rAD/p53	Adenovirus	0	NA	NA	NA

*Sources: June 1995 Recombinant DNA Advisory Committee (RAC) Data Management Report; The RAC & Worldwide Gene Therapy Report, TMC Development, Paris, France; Herrmann E, Clinical application of gene transfer, J Mol Med 1996;74:213-221; and Internet Book of Cancer Gene Therapy, Sobol RE, Scanlan KJ, eds. Appleton and Lange, Stamford, CT, 1995. Survey forms were sent to all cancer gene-therapy protocol principal investigators with results tabulated and verified by each investigator as of June 19, 1996.

†IL-2 = interleukin 2; TGF-β2 = transforming growth factor-β2; GM-CSF = granulocyte–macrophage colony-stimulating factor; NA = not available; CR = complete response; PR = partial response; SD = stable disease; MR = minor response; NCI = National Cancer Institute; PSA = prostate-specific antigen; CEA = carcinoembryonic antigen; c-DNA = complementary DNA; IGF = insulin-like growth factor; PBLs = peripheral blood lymphocytes; NATC = nontransfected autologous tumor cells; TILs = tumor-infiltrating lymphocytes; TNF = tumor necrosis factor; LFTS = liver function tests; CNS = central nervous system; MDA = MDA-MB-231 cell line; CML = chronic myelogenous leukemia; NSCLC = non-small-cell lung carcinoma; HSV-TK = herpes simplex virus thymidine kinase; MDR = multidrug resistance; NEO-R = neomycin-resistance; and UATC = untransduced autologous tumor cells.

‡In vivo or in target cell.

§All principal investigators were requested to use the following response criteria: a complete response (CR) was defined as the disappearance of all clinical evidence of tumor in the treated area for local treatment or for all lesions for systemic treatment without the appearance of new lesions for a period of at least 4 weeks. Patients evaluable for a less-than-complete response were those having a bidimensionally measurable tumor. Partial response (PR) was defined as a 50% or greater reduction in the sum of the products of the diameters of the measurable disease including the treated lesion for local treatment or all lesions for systemic therapy. A minor response (MR) was defined as a 25% to less than 50% reduction in the sum of the products of the diameters of the measurable lesion. Patients were designated as having progressive disease if they showed a 25% or greater increase in the size of their disease or if they developed unequivocal new lesions during treatment and as having stable disease (SD) if they had any tumor change that did not meet the criteria described above.

‖Related to gene transfer.

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Notes

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