Gene Therapy and Emerging Molecular Therapies

Advances in understanding molecular mechanisms of disease and manipulating genetic material, protein receptors, and antibodies provide new approaches for treatment of human diseases. Emerging therapeutic reagents include not only novel antibody and protein therapies, but also nucleic acid-based molecules, including complete genes, deoxyribonucleic acid (DNA), complementary DNA (cDNA), ribonucleic acid (RNA), and oligonucleotides. Together with cell therapies and targeted approaches for protein and nucleic acid delivery, these reagents and strategies for administration are conceptualized within the broad borders of gene therapy and the emerging field of molecular medicine.

Gene therapy was originally conceived as a treatment for monogenic (Mendelian) disease by complementation of a mutant gene with a normal (wild type) gene. However, gene therapy also includes treatment of acquired human disease by delivery of DNA encoding a therapeutic protein, or introducing a fragment of nucleic acid to interrupt the messenger RNA (mRNA) of a pathogenic protein. A key component of the use of nucleic acids is the spectrum of strategies used to focus therapies on specific organs, deliver genes to specific cells, or both. Targeting is achieved through genetically engineered viruses, receptor-ligand interactions, and antibodies. Therefore these gene-based reagents and the vehicles used to deliver them represent a novel form of “gene as drug.” This chapter presents a discussion of these molecular therapies.

Although the Food and Drug Administration (FDA) has not approved any product for human gene therapy to date, nearly 1000 clinical trials worldwide have been completed or are in progress using different genes and transfer strategies. The primary goal is to determine the safety of gene transfer and to detect evidence of gene transfer and expression. Thus far, only a handful of trials have resulted in significant therapeutic effects attributable to gene transfer. However, the rapid rate of improvement in gene transfer vectors and a greater understanding of the pharmacology and toxicity of gene transfer suggest that this will improve rapidly. Gene therapy has several potential advantages over drug therapy in that delivery of a functional gene: (1) can replace a mutant gene that results in disease, (2) can result in continuous production of a therapeutic protein with a short $t_{1/2}$ that would otherwise require frequent dosing, (3) can be targeted to a specific site or cell type to avoid potentially toxic systemic therapy, and (4) can improve patient compliance.

### Principles of Gene Transfer

The phases of nucleic acid delivery, gene expression, action of the newly produced protein, and consideration of adverse effects of gene delivery vectors and gene products are analogous to issues in conventional drug therapy. Development of molecular therapy using nucleic acid begins with the identification and cloning of a gene. The choice of DNA sequence to be transferred is typically a cDNA sequence containing its entire protein coding sequence but may include introns, nuclear localization, or protein secretion signals. In addition, DNA must also contain transcriptional regulatory sequences, a transcription start site, and an RNA polyadenylation sequence to transcribe and stabilize mRNA. These DNA sequences are linked into a single unit that is inserted (subcloned) into a circularized piece of DNA called a plasmid. Plasmids contain genetic sequences that allow replication within bacteria so that large quantities of DNA can be produced and purified. The plasmid containing the therapeutic DNA can be delivered to target cells using one of the many vehicles (“vectors”) for gene transfer. Intracellular transfer of most full-length human genes and associated regulatory elements (that may be more than 100 kilobases; kb) is theoretically optimal but limited by current technology.

Basic concepts of gene transfer and gene expression are similar, regardless of the vehicle used to carry genetic material to the target cell (Fig. 5-1). After administration

### Abbreviations

<table>
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<th>Abbreviation</th>
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<tr>
<td>ADA</td>
<td>Adenosine deaminase</td>
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<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
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<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
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<tr>
<td>CFTR</td>
<td>Cystic fibrosis transmembrane conductance regulator</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<td>HS-TK</td>
<td>Herpes simplex virus thymidine kinase</td>
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<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
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<tr>
<td>RISC</td>
<td>RNA-induced silencing complex</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<tr>
<td>RNAi</td>
<td>RNA interference</td>
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<tr>
<td>siRNA</td>
<td>Short interfering RNA</td>
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49
of the DNA-vector, the vehicle carrying DNA enters the cell by passing through the cell membrane or by active uptake via a specific receptor. The DNA is taken into the nucleus, where it can be processed. The host cell supplies enzymes necessary for transcription of the DNA into messenger RNA (mRNA) and translation of the mRNA into protein within the cytoplasm. The protein functions intracellularly or extracellularly to replace a hereditary deficient or defective protein, or to provide a therapeutic function. The protein may: (1) function intracellularly, such as adenosine deaminase (ADA) used to correct the mutation in lymphocytes responsible for one form of the severe combined immunodeficiency disease (SCID) syndrome; (2) replace a cell membrane protein, such as the cystic fibrosis (CF) transmembrane conductance regulator chloride channel (CFTR) mutant in CF; or (3) introduce a secreted protein, such as factor VIII, which is deficient in hemophilia. Successful gene transfer (also called transfection) and expression are evaluated by measuring RNA, protein, and, importantly, function in the target cell. In contrast to traditional pharmacological approaches, the goal of this therapy is alteration of the genotype of the cell, rather than alteration of the functional phenotype only.

Nucleic acids can also be delivered into the cell to interrupt specific mRNA translation and subsequent protein production. The two general classes of nucleic acids, using independent mechanisms to silence genes through binding and triggering destruction of targeted mRNA, are antisense and RNA interference (RNAi) (Fig. 5-2).

Antisense oligonucleotides are 12 to 28 nucleotide, single-strand sequences that are chemically modified to enhance their t1/2. Binding of these oligonucleotides to a complementary mRNA sequence results in cleavage of the targeted mRNA by endogenous RNaseH, an endoribonuclease that specifically recognizes RNA-DNA heteroduplexes. The cleaved mRNA and oligonucleotide are degraded, and protein translation is reduced. However, a high abundance of oligonucleotides is required for efficient antisense silencing. Larger oligonucleotides can also be engineered as a ribozyme to bind and directly cleave mRNA, and then repeat this process without self degradation. More than 50 different antisense oligonucleotides and ribozymes are in clinical trials, primarily directed toward oncogenic genes and infectious virus RNAs.

A recently developed mRNA silencing system is RNAi that takes advantage of endogenous RNA regulatory pathways (see Fig. 5-2). Large, double-stranded RNA sequences designed to target endogenous mRNA enter the cell and are cut into short interfering RNA (siRNA) by the dicer enzyme. Alternatively, synthetic siRNA may be delivered to the cell directly. In either case, the double-strand siRNA molecules are bound by a group of proteins called the RNA-induced silencing complex (RISC). The RISC proteins activate unwinding the siRNA to single-strand RNA that binds a specific mRNA molecule. The RISC cuts the...
mRNA in the region paired with the antisense siRNA sequence, and the cleaved mRNA is degraded to prevent protein translation. Clinical trials are in development using RNAi.

**PRINCIPLES OF CLINICAL GENE THERAPY**

**Prerequisites for Human Gene Therapy**

Application of gene transfer principles to gene therapy requires several critical considerations (Box 5-1). First, a candidate disease for gene therapy must be selected. Typically, this is a disease not successfully treated by currently available therapies. Second, the genetic basis of the disease must be determined by identifying the gene that encodes for a mutant protein or by locating the mutant gene using classical genetic studies. Third, the pathophysiology of the disease must be known so that the cellular site of normal and abnormal gene expression can be ascertained to target the therapy. A corollary is that the magnitude and duration of exogenous gene expression likely to ameliorate the disease should be estimated. Fourth, tools for detection of gene expression must be in hand, including methods for detection of RNA, protein, and protein function. Fifth, preclinical in vitro and in vivo systems for testing efficacy of gene transfer must be developed. This usually mandates that an animal model of disease be available. With few exceptions, these steps are developed in the laboratory before a strategy for clinical gene therapy is considered. Finally, pharmaceutical-grade nucleic acid or virus vector must be produced free of biological and chemical contaminants.

**Principles of Clinical Gene Therapy**

The basic concepts and principles of gene delivery also guide the development of strategies for gene therapy. However, no single approach can be used for all diseases, and many variables must be considered when designing a therapeutic strategy. The biological basis and clinical features dictate the strategic variables used for the appropriate therapeutic outcome.

Gene therapy as currently conceived is gene addition therapy, whereby exogenous DNA delivered to a cell complements a mutant DNA. Gene repair therapy, whereby the mutant gene is directly corrected using techniques of homologous recombination, is technically feasible and would be more definitive. Unfortunately, current methods of homologous recombination result in very low efficiency of gene repair.

**DNA and RNA Targeting and Delivery**

How therapeutic DNA is transferred to a specific target cell depends on the biology of the target cell and the vector. Targeting mechanisms and different features of these approaches can be used in combination. The procedure of gene transfer can take place in cells removed from the body (ex vivo), then reintroduced into the patient, or by direct delivery of DNA or RNA to the patient (in vivo).

**Ex Vivo Delivery**

Cells genetically altered by ex vivo gene transfer must be capable of removal, survival outside the body, and reimplantation. Examples of ex vivo cells targeted for gene transfer include lymphocytes, hepatocytes, tumor cells, myocytes, fibroblasts, and bone marrow cells. One valuable strategy of ex vivo transfer is to isolate bone marrow hematopoietic stem cells for gene transfer (CD34+ cells). These stem cells have a long life and the potential to pass on the transferred gene to progeny. An alternative approach to gene targeting, particularly feasible for secreted proteins having a systemic effect, such as a clotting factor (for hemophilia) or insulin (for diabetes), is to use human cells as depots for gene product delivery. In this strategy cells such as autologous skin fibroblasts, muscle cells, or bone marrow cells can be transfected with a selected DNA ex vivo and subsequently produce the desired therapeutic-specific protein. The cells can then be implanted (e.g., subcutaneously or intramuscularly) and function as “protein factories,” secreting a gene product into the circulation. This approach is used for gene therapy of hemophilia by transfection of autologous skin fibroblasts that were reinjected. A variation in anticancer gene therapy trials is the use of a patient’s own tumor cells modified to secrete a cytokine (e.g., interleukin-4), implanted subcutaneously, in an attempt to enhance a systemic immune response.

**In Vivo Delivery**

Often, in vivo gene delivery is the only feasible strategy. Examples of in vivo targets for gene therapy include brain, lung, liver, muscle, blood vessels, and tumors. Intravenous injection of DNA and oligonucleotides can result in broad distribution to multiple tissues, with concentrations often highest in the liver and kidney. Thus, for efficient in vivo delivery, gene vectors are often directed to a cell population through sophisticated interventional techniques often used in clinical medicine. For example, in vivo transfer to a specific organ may be achieved through catheterization of that organ, by surgical approaches, or by fiberoptic-guided methods. Therefore specificity of cell targeting to achieve cell-specific gene expression also depends on the technical aspects of the in vivo delivery system.
General Principles

Bone Marrow Cells as Vehicles for Organ Repair and Gene Therapy

Autologous bone marrow cells can function as vehicles for gene delivery and have also been demonstrated to traffic to injured organs and acquire the phenotype of an injured organ cell. With the use of animal models, bone marrow cells have been harvested, genetically modified by gene addition, reinjected into the donor, and observed to express the transgene (inserted gene) in cells of various organs. This suggests the possibility that stem cells can be used for expression of a normal gene in an organ or cell previously thought to be genetically modified only by in vivo therapy. For example, experimental models have suggested that stem cells trafficking to the lung can transdifferentiate or proliferate to generate epithelial cells expressing a gene inserted in the stem cell.

Receptor- and Antibody-Mediated Targeting

Cellular targeting can also be accomplished through receptor-mediated gene transfer. In these delivery systems, therapeutic DNA is linked to a ligand specific for a cell surface receptor or to an antibody (or antibody fragment) directed toward a specific cell surface protein. These strategies facilitate internalization of DNA through receptor-mediated endocytosis or other pathways. Alternatively, gene expression may be targeted by providing a cell type–specific promoter gene sequence driving the delivered DNA. This strategy uses transcriptional regulatory sequences to permit gene expression only in cells containing appropriate transactivating factors that bind the promoter.

Quantification of Gene Expression

The pharmacokinetics of gene therapy can be determined by the magnitude and duration of gene expression. After delivery of DNA, gene expression must be quantified by determining the amount of DNA reaching the target cell, the amounts of RNA and protein produced, and the functionality of the protein produced. The magnitude and duration of gene expression depend on the disease to be treated. To treat some diseases, it may be necessary to produce a minimal amount of functional protein but in a large number of cells. Alternatively, to treat other diseases, larger amounts of protein must be secreted to reach a large number of cells within an organ or systemically. The magnitude of expression is typically determined by the t1/2 of the protein, therapeutic goal, efficacy of gene transfer, and potency of the gene promoter used to direct gene expression.

Duration of gene expression varies with the type of vector used. Most nonviral and viral systems result in only transient (days to months) expression. This may be desirable for gene therapy not associated with hereditary diseases, such as cancer or infectious diseases. Alternatively, gene transfer may be repeated, potentially in a titratable fashion, so that benefits of transient expression are achieved. Persistent gene expression is usually associated with integration of the transferred gene into host cell genome and is possible only with a few viral-based gene transfer systems. Long-term expression (>1 year) has been achieved with current gene transfer systems using ex vivo transfer of hematopoietic cells by a retrovirus vector. Regardless of the system used, the life span of the cell targeted for gene transfer is also an important factor in duration of gene expression. The viability of cells targeted with DNA using viral vectors may be decreased due to host destruction as a response to a foreign invader, resulting in only transient gene expression.

Evaluation of Toxicity of the Gene Therapy System

Toxicity of gene therapy may result from some of the many components of the gene therapy system (e.g., the DNA, the transcribed protein, or the viral/nonviral vehicle). Assessment of balance between safety and efficacy is similar to that applied to standard drug use. Gene transfer vectors using viral genomes may produce several additional proteins, induce a host immune response, have oncogenic properties, or expose caregivers or family members to shed virus. Immunological response to gene transfer vectors (especially virus-based systems) has been a critical factor in causing toxicity and limiting the duration of gene expression. Gene product toxicity is an additional issue, even if the gene is normally expressed in healthy humans. This may occur because, after gene transfer, expression is often much higher than normal endogenous levels and concentrated within a localized population of cells, perturbing normal homeostasis. For example, transfer of the CF gene is potentially harmful, inducing high expression of the CFTR protein and overexpressing many copies of a chloride channel in cells having carefully balanced salt and H2O channel expression. The integration of foreign DNA in a sensitive site of the genome has also been shown to induce oncogenesis in animals and human trials. Like all experimental therapies, patients treated with gene therapy may be willing to tolerate adverse effects, if diseases are not treatable by currently available therapies.

Ethical Issues

The scientific goal of current gene therapy is directed at introducing genes into somatic cells only and not into germ cells containing inherited genetic material. Although it is technically possible to transfer DNA through the germ line (often done in experimental animals), application of these technologies to humans has profound social and ethical implications. Ethical considerations include: (1) the choice of disease; (2) attributes to be altered, for example, genetic defects that result in aberrant behaviors; and (3) cosmetic concerns. Human gene therapy trials are tightly regulated and reviewed at local institutions by Biosafety Committees and Human Investigation Review Boards and require informed consent from participants. As with any biological agent administered to humans, newly developed gene therapy vectors must be approved for use by the FDA.

VEHICLES FOR GENE TRANSFER

DNA and vector-based methods used to introduce DNA or RNA into mammalian cells and the advantages and
disadvantages of different systems are listed in Table 5-1. Therapeutic DNA transfected into cells by nonviral means is subcloned into a plasmid so that large quantities of plasmid DNA can be produced and purified. Plasmids can carry large pieces of DNA (more than 20 kb), thereby accommodating proteins with large coding sequences. Traditional methods for in vitro gene transfer are purified plasmid DNA delivered to cell lines by microinjection, coprecipitation of DNA with calcium phosphate, and transient electrical current to enhance permeability for DNA entry (electroporation). Although these techniques are often satisfactory experimentally, they generally result in DNA transfer to less than 1% of primary culture cells, are difficult to use in vivo, and have limited therapeutic use. More efficient vehicles have been developed for gene transfer, making in vivo gene delivery possible. Vehicles for DNA delivery include several plasmid- and virus-based vector systems. Plasmid-based vectors include plasmids mixed with liposomes and plasmids linked to ligand/receptor complexes, antibodies, or nanoparticles. Viral vectors are designed to use specific receptors and entry functions specific to particular cell types, using the host genome for transcription and translation. Viral vectors rarely use the wild-type virus but rather a genetically engineered virus that minimizes cytotoxicity and replication but retains the ability to enter and express a specific gene within the cell. The most widely studied vehicles for gene transfer are: (1) genetically engineered viruses that carry nucleic acid into cells; (2) liposomes mixed with DNA; and (3) DNA transferred alone by direct injection (“naked DNA”). The viral vectors commonly used in clinical trials include mouse Maloney retroviruses, adenoviruses, adeno-associated viruses, lentiviruses, herpes simplex virus, and vaccinia virus.

### TABLE 5–1 Comparison of Commonly Used Vectors for Gene Transfer

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<thead>
<tr>
<th>Vehicle</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td><strong>Nonviral</strong></td>
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<tr>
<td>Naked DNA</td>
<td>Ease of production</td>
<td>Low efficiency</td>
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<tr>
<td>Liposome-DNA</td>
<td>Ease of production</td>
<td>Transient expression</td>
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<tr>
<td><strong>Viral</strong></td>
<td></td>
<td></td>
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<tr>
<td>Retrovirus</td>
<td>Ease of production</td>
<td>Transfer to dividing cells only</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Low immune reaction</td>
<td>Host immune reaction</td>
</tr>
<tr>
<td>Adeno-associated</td>
<td>Prolonged expression</td>
<td>Difficult production</td>
</tr>
<tr>
<td></td>
<td>Stable expression</td>
<td>DNA transfer size limited</td>
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<tr>
<td></td>
<td>Efficient DNA transfer</td>
<td>Random DNA integration</td>
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<tr>
<td></td>
<td>Transfer to nontargeting cells</td>
<td>Transient expression DNA transfer size</td>
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### Plasmid-Based Vehicles

#### Plasmid DNA

Plasmid DNA alone enters cells but with low efficiency. However, delivery of DNA under pressure (using a “gene gun”) has been shown to transfect muscle cells. This has the advantage of simplicity and essentially no toxicity. DNA has been condensed and linked to ligands to form molecular conjugates, permitting cell entry by receptor-mediated endocytosis. Efficiency of DNA transfection is good in vitro but poor in vivo. The chemical binding of DNA to nanoparticles may enhance gene delivery, though clinical trials have not yet been developed.

### Liposomes

Liposomes are lipid molecular aggregates that bind to DNA, antisense oligonucleotides, or siRNA to facilitate cell entry (Fig. 5-3, A). Cell entry occurs by fusion with the cell membrane or by endocytosis. Liposome formulations have been developed containing monolayers, bilayers, or multilayers and possess charged (e.g., cationic lipids) or neutral surfaces. Transfection efficiency is variable, and the specific lipid type must be matched to that of the target cell to maximize gene transfer. Nucleic acid-lipid complexes have been combined with selected antibodies or receptor-specific ligands to further enhance cell targeting.

Advantages of DNA-liposome complexes are that large DNA sequences in plasmids can be used and that large-scale production and purification is simple. Toxicity of liposomes in vivo is less problematic, because proteins are not transferred. However, gene transfer using liposomes is relatively inefficient compared with virus-based vectors. Therefore large amounts of DNA-lipid complexes may be required, potentially increasing toxicity. Liposomes have been used successfully for gene transfer in humans, and improvements in lipid composition to enhance transfection efficiency and decrease toxicity are in progress.

### Virus-Based Vehicles

#### Retrovirus

Recombinant retrovirus vectors are among the most widely used gene transfer vehicles. They have the advantage of integrating a therapeutic gene into the target cell genome. Production of a retrovirus vector that can carry nonviral (therapeutic) genes and is not capable of replication is a two-step process similar to that used to produce vectors from many different viruses (Fig. 5-3, B). First, a cell line containing the genes necessary for creating viral envelopes and viral replication must be created by transferring the retrovirus genes GAG, POL, and ENV to a cell. This “packaging” cell line does not contain the psi (ψ) sequence necessary for inserting the genes into the envelope and hence produces empty retrovirus “packages” that do not contain the therapeutic gene. Second, the packaging cell line is modified to contain other retrovirus sequences with a therapeutic gene (up to 9 kb in length) and a ψ
encapsulation sequence, permitting the therapeutic gene (but not the GAG, POL, and ENV genes) to be inserted into the retrovirus envelope. This “producer” cell line creates and secretes viral particles containing the therapeutic gene that can enter a host cell, but, in the absence of GAG and POL, cannot replicate to make new virus. Thereby a replication-incompetent retrovirus is produced, collected from the cell media, and used in vitro or in vivo to deliver a gene to a target. After entering the target cell, integration of the therapeutic gene into the host genome is required for expression. Such integration is advantageous, if a sustained therapeutic effect is desired, but can only occur in dividing cells. Therefore this technique is particularly useful for ex vivo therapies. Retroviruses integrate DNA into the host genome randomly, potentially resulting in interruption of host DNA (insertional mutagenesis) or a silencing of transferred DNA expression.

**Adenovirus**

Gene transfer vectors derived from adenoviruses have the major advantage of high-efficiency delivery to nondividing cells and high virus production, making them attractive for in vivo gene delivery. Adenovirus is a double-stranded DNA virus whose genome consists of early genes (E1 to E4), which code for regulatory proteins necessary for replication, and late genes (L1 to L5), which code for structural proteins. To produce an adenovirus vector for gene transfer (Fig. 5-3, C), the immediate early gene E1, responsible for replication but not infection, is deleted and replaced with the therapeutic gene (up to 7 kb). The E1 deficient therapeutic adenovirus grows only in cells expressing the E1 gene (serving much the same function as the retrovirus producer cells), generating adenoviruses used for gene transfer. Adenovirus infection occurs through a defined receptor and functions within the nucleus without integration into the host cell genome. Expression of the therapeutic DNA transferred by adenovirus vectors is transient (often <1 month). Although adenovirus vectors are highly efficient for transfer of genes to cells in vivo, some limitations prevent their more extensive use. Expression of viral proteins in infected cells can trigger a cellular immune response that results in adverse clinical symptoms, precluding long-term expression of the transferred gene and repeat administration. Because adenovirus results in transient expression, after initial immune sensitization, repeat dosing may result in even briefer expression as a result of immune destruction of vector-containing cells. Newer adenovirus vectors developed by removal of genes known to contribute to an immune response are currently under evaluation.

**Other Virus-Based Vectors**

Adeno-associated and herpes simplex virus-based vectors have been approved for human use. Adeno-associated virus is a human single-stranded DNA parovirus that integrates DNA into target cell genomes of cells not actively dividing. The adeno-associated virus vector system is similar to the retrovirus vector system, relying on a packaging cell line but also requiring wild-type adenovirus...
as a “helper” to complete viral production in vitro. Disadvantages of the adenovirus-associated vector system include a minimal size of the therapeutic gene that can be carried (<4.5 kb) and a low titer of virus particles produced. Lentivirus can be used to infect nonrepli- 
cating cells and can be produced in high titer. Herpes simplex-derived vec-
tors can be used to enhance gene delivery to neurons.

**CLINICAL GENE THERAPY STUDIES**

The first gene therapy clinical trial began in 1990, and since that time numerous trials using various strategies have been attempted for the treatment of hereditary and acquired 
diseases. Some of these studies have been halted by the FDA 
because of toxicity, others are currently underway, and there are many other protocols in development. Although 
initial gene therapy studies focused on known genetic dis-
eases, cancer, and infectious disease, more recent studies have included multifactorial disorders including vascular 
disease and, most recently, Parkinson’s disease.

**Gene Therapy for Cancer**

Cancer cells are the most extensively evaluated target for 
gene therapy, because many malignancies are unrespon-
sive to conventional therapy and rapidly fatal. In contrast 
to hereditary diseases, cancer-related gene therapy is not 
exclusively directed toward correction of genetic muta-
tions but also uses gene delivery to target a therapeutic 
biological agent to the cancer cell. Several highly creative 
therapeutic approaches developed for gene therapy for 
cancer include: (1) addition of a wild-type tumor suppressor 
gene to complement a mutant tumor suppressor gene; 
(2) antisense RNA strategies to “turn off” expression of an 
oncogene; (3) transfer of a gene to enhance immunogenic-
ity of the tumor by expression of an immunomodulating 
gene or cytokine gene in the tumor; (4) transfer of a gene 
coding for a “prodrug” to the tumor, leading to tumor-
specific cell killing by production of a toxic metabolite; 
(5) inhibition of tumor angiogenesis; and (6) chemoprotec-
tive genes transferred to save patients’ hematopoietic cells 
from chemotherapy-induced toxicity.

**Gene Therapy for Infectious Disease**

Chronic infectious diseases with persistent virus expres-
sion, including human immunodeficiency virus (HIV), 
hepatitis B, and hepatitis C, represent targets for nucleic 
acid-based therapies to block virus production or enhance 
immune responses. Many approaches have been used for 
gene therapy of HIV infection such as enhancing the 
immune response to HIV and providing gene products 
that suppress virus replication. One approach is to use a 
retrovirus to transfer the HIV gp160 envelope protein gene 
as a vaccine to enhance virus-specific immune responses 
after injection into muscle. Vaccine-type gene transfer 
trials may be less successful in individuals who are already 
immunologically impaired by HIV infection. Another 
approach to decrease HIV replication is to modify CD4+ 
T cells ex vivo to express proteins that interfere with the 
function of the HIV TAT or REV transcription factors. 
These protocols depend on persistent gene expression 
and long-term survival of genetically modified HIV-
infected cells infused into the patient. Several hundred 
individuals have been involved in these trials, and the pro-
tocols appear to be safe; however, sufficient data are not 
yet available to judge clinical efficacy.

**Multifactorial Diseases**

Many diseases can be amenable to molecular therapies by 
identification of therapeutics. Vascular diseases caused by 
thrombosis and atherosclerosis have been studied follow-
ing the delivery of genes coding for angiogenic growth 
factors. Inflammatory diseases, such as inflammatory 
bowel disease, arthritis, asthma, and skin diseases, have 
been studied as candidates for delivery of genes that 
encode anti-inflammatory or immunomodulatory cyto-
kines. In some trials antisense oligonucleotides designed 
to silence expression of pro-inflammatory cytokines are 
under investigation.

The use of gene-based therapies for neurological disor-
ders is a relatively new development. However, prelimi-
nary studies using an adeno-associated virus to deliver 
the enzyme (glutamic acid decarboxylase) responsible for 
the synthesis of the neurotransmitter GABA have been 
very favorable. During the first year of the study, improve-
ments in motor function were observed without any evi-
dence of adverse events, immunological alterations, or 
infections.

**New Horizons**

Continued progress in the understanding of molecular 
mechanisms of disease will lead to the development of 
novel genetic-based therapies. Broad application of in 
vivo gene transfer for the treatment of human inherited 
or acquired diseases will require development of new viral 
or nonviral systems or a substantial improvement of exist-
ing systems. Critical issues being evaluated are immuno-
logical responses of gene transfer vectors, regulation of 
gene expression, and persistence of expression. High-
efficiency approaches for repair of mutation, rather than 
addition to mutant genes, using homologous recombin-
ation is also being actively pursued. Tailoring therapies to 
humans with specific genetic mutations or polymorphisms 
is a related area for future investigation. The application of 
basic principles of drug therapy continues to guide evalua-
tion of novel gene therapy strategies.

**FURTHER READING**


Ratko TA, Cummings JP, Blebea J, Matuszewski KA. Clinical gene 
Because this field is rapidly emerging, for the most up-to-date 
information on gene therapy clinical trials worldwide, see 
The Journal of Gene Medicine Clinical Trial web site at: 
http://www.wiley.co.uk/genetherapy/clinical. 
Information on gene therapy clinical trials in the United States is at: 
SELF-ASSESSMENT QUESTIONS

1. The most common current strategy for human gene therapy of monogenic (Mendelian) disease is to:
   A. Repair the mutant gene in the cell.
   B. Repair the mutant RNA in the cell.
   C. Repair the mutant protein in the cell.
   D. Add a nonmutant gene to the cell to complement the mutant gene.
   E. Add nonmutant RNA to the cell to interrupt the mutant gene expression.

2. Current concepts for successful human gene therapy dictate that:
   A. After gene transfer, expression of the transferred gene must be undetectable.
   B. After gene transfer, immune response to the vector should be monitored.
   C. After successful gene transfer, life-threatening toxic effects are expected to occur.
   D. Gene transfer to germ cells is essential.
   E. Gene transfer to somatic cells is not important.

3. Vectors for gene transfer in clinical trials include the following except:
   A. Recombinant plasmids.
   B. Recombinant plasmids mixed with phospholipids.
   C. Genetically engineered murine leukemia virus.
   D. Genetically engineered human adenovirus.
   E. Genetically engineered adeno-associated virus.

4. Mechanisms of gene silencing using mRNA antisense oligonucleotides for gene therapy include:
   A. Oligonucleotide binding to mutant DNA to block the function of DNA.
   B. Degradation of mutant proteins.
   C. Complementary pairing of the oligonucleotide and mRNA.
   D. Permanent blockade of targeted gene expression.
   E. Production of new mRNA sequence by a virus vector.