Gene Therapy Review

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After completing this article, the reader should be able to:
- Understand basic gene therapy methodologies.
- Describe current modes of therapeutic delivery.
- List key diseases that are targeted using gene therapy.
- Identify the role of medical imaging in gene therapy research and clinical application.
- Discuss current and future challenges and ethical issues associated with gene therapy.

Medical and health sciences have experienced pronounced advances in recent decades. Genetic research has progressed, and now scientists can attempt to modify human genes for therapeutic purposes. By modifying defects in genes that manifest into serious diseases, physicians can, in effect, treat or cure the disease. This particular branch of applied genetic research is known as gene therapy, and although still in its infancy after nearly 3 decades, the discipline is quickly gaining momentum in the medical sciences.

The concept of gene therapy evolved from confirmation that certain diseases are caused when an individual inherits a single gene that is functionally defective. Further, researchers realized that diseases could be managed or potentially cured by the insertion and expression of an operational copy of the mutant or deleted gene. An operational copy of a gene is one that has had its deformities and defects corrected or repaired for use in therapy.

This idea of gene therapy was conceptualized in the early 1970s but was not applied until the 1990s. With knowledge now available about the human genome and the discovery of genes responsible for medical conditions, along with development of gene transfer vector and delivery systems, physicians can target particular genes and pathways that play key roles in how the human body functions.

The Human Genome Project, a large-scale international research program that started in 1990, was designed to document and understand sequences that make up human DNA. Information learned from mapping the human genome supported many goals in molecular medicine, including improved understanding and management of cancer and rare diseases. The field of gene therapy therefore is rapidly expanding; however, the biology and strategy of gene therapy is complex and diverse.

In one sense, the goal of gene therapy is to modify a gene or genetic pathway to prevent or reduce effects of disease. In addition to modifying a gene or pathway, approaches can include:
- Replacing a mutated gene with a functional copy of the gene.
- Inactivating a mutated gene that is functioning improperly or introducing an entirely new gene to help fight a disease.
- Repairing an abnormal gene through selective reverse mutation.
- Regulating the degree to which a gene is turned on or off.

Of the more than 14,000 known diseases, more than 10,000 are known to be monogenic, or controlled by a single gene. Each disease varies in how rare and serious it manifests in humans, and each year the number of known genetic diseases increases. Generally, 10% of people will, at some point in their lives, have a type of genetic mutation, activated by environmental, dietary, or lifestyle factors that cause a disease.

Gene therapy research and clinical trials are ongoing in the United States and around the world. More than half of the research has occurred in the past decade and most clinical trials (approximately 90%) are concentrated in just 10 countries. The United States and Europe account for 90% of all clinical trials worldwide, the majority of which target cancer-related disease. Many of these trials still are in the early clinical phases. Multiple conditions and diseases have been targeted for gene therapy, many of which are listed in Table 1.

Commercial development of gene therapy is quickly gaining momentum. Hundreds of millions of dollars have been raised by biotechnology companies toward gene therapy. In fall 2013, the Children’s Hospital of Philadelphia invested $50 million in a new biotechnology start-up that aims to be the nation’s first commercial provider of gene therapy.

Table 1

<table>
<thead>
<tr>
<th>Conditions and Diseases Targeted for Gene Therapy</th>
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<tbody>
<tr>
<td><strong>Monogenic disorders</strong></td>
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<tr>
<td>Cystic fibrosis, severe combined immunodeficiency, α-1 antitrypsin deficiency, hemophilia A and B, Hurler syndrome, Hunter syndrome, Huntington chorea, Duchenne muscular dystrophy, Becker muscular dystrophy, canavan disease, chronic granulomatous disease, sickle cell disease, familial hypercholesterolemia, Gaucher disease, Fanconi anemia, purine nucleoside phosphorylase deficiency, ornithine transcarbamylase deficiency, leukocyte adherence deficiency, gyrate atrophy, Fabry disease, familial amyotrophic lateral sclerosis, junctional epidermolysis bullosa, Wiskott-Aldrich syndrome, lipoprotein lipase deficiency, late infantile neuroaxonal lipofuscinosis, retinal disease, and mucopolysaccharidosis</td>
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<tr>
<td><strong>Cancers</strong></td>
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<tr>
<td>Breast cancer, ovarian cancer, cervical cancer, glioblastoma, leptomeningeal carcinomatosis, glioma, astrocytoma, neuroblastoma, colon cancer, colorectal cancer, liver metastases, posthepatitis liver cancer, pancreatic cancer, prostate cancer, renal cancer, melanoma, nasopharyngeal carcinoma, adenocarcinoma, mesothelioma, leukemia, lymphoma, sarcoma, and multiple myeloma</td>
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<tr>
<td><strong>Infectious diseases</strong></td>
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<td><strong>Cardiovascular disease</strong></td>
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<tr>
<td>Peripheral vascular disease, intermittent claudication, critical limb ischemia, myocardial ischemia, coronary artery stenosis, stable and unstable angina, venous ulcers, diabetes-derived vascular complications, pulmonary hypertension, and heart failure</td>
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<tr>
<td><strong>Neurological conditions</strong></td>
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<tr>
<td>Alzheimer disease, carpal tunnel syndrome, cubital tunnel syndrome, diabetic neuropathy, epilepsy, multiple sclerosis, myasthenia gravis, Parkinson disease, and peripheral neuropathy</td>
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<tr>
<td><strong>Ocular diseases</strong></td>
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<tr>
<td>Diabetic macular edema, Leber congenital amaurosis, glaucoma, retinitis pigmentosa, superficial corneal opacity, wet age-related macular degeneration, Stargardt disease, Usher syndrome 1B, and age-related macular degeneration</td>
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<tr>
<td><strong>Other diseases and conditions</strong></td>
</tr>
<tr>
<td>Inflammatory bowel disease, rheumatoid arthritis, chronic renal disease, fractures, erectile dysfunction, anemia of end-stage renal disease, parotid salivary hypofunction, type I diabetes, detrusor overactivity, Down syndrome, Rett syndrome, and graft vs host disease</td>
</tr>
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therapy company in Cambridge, Massachusetts, raised more than $100 million in an initial public offering. Recently, another company agreed to develop and market a potential gene therapy to treat hemophilia B that was in a phase 1/2 trial at a cost of nearly $25 million.8

However, completion of various clinical trials provides no guarantee that all or even many of the currently funded technologies will become accepted or widely used. Only about 5% of trials to date are in later (phase 3 and 4) clinical stages.7 In 2012, however, the European Union approved alipogene tiparvovec after years of research.11 The therapy, which targets the genetic disorder lipoprotein lipase deficiency, has provided a significant boost to the gene therapy field. This came well after the first U.S. Food and Drug Administration (FDA)—approved gene therapy trial in 1990 for adenosine deaminase deficiency, an inherited disorder that compromises an individual’s immune system and causes severe combined immunodeficiency.2

Neither alipogene tiparvovec nor any other gene therapy has been approved by the FDA to date. Worldwide, recombinant adenovirus-p53 was the first gene therapy product approved. The adenovirus vector carrying the p53 gene as a treatment for cancer was first approved in China in 2003 for clinical use. There have been claims that large numbers of patients already have been treated.11 In the United States, several gene therapies in late-phase testing or near late-phase testing are contending to be the first FDA-approved therapy. Currently, 8 to 10 clinical trials are nearing final stages of testing.11 These late-phase trials include therapies targeting genes involved in various cancers, cardiovascular disease, and blindness.

Radiologic technology has played a continual role in gene therapy administration and will remain essential. Molecular imaging, a specialized branch of radiology, is consequently emerging. The radiologic sciences are becoming increasingly complex in equipment and methodology. Imaging professionals are expected to master new terms and concepts related to gene therapy delivery to targeted cells and to monitoring cellular function.

**Gene Therapy Fundamentals**

**Genes**

Gene therapy typically involves substituting correctly functioning genes for defective ones. In terms of basic biology, genes are “a locatable region of genomic sequence, corresponding to a unit of inheritance, which is associated with regulatory regions, transcribed regions, and/or other functional sequence regions.”14,15 In simpler terms, genes are the physical and functional units of heredity. Humans are estimated to have between 20,000 and 25,000 genes.16

Genes are made up of DNA, long twisting ladders of paired nucleotides (see Figure 1). Four nucleotides serve as the chemical building blocks of DNA: adenine (A), guanine (G), cytosine (C), and thymine (T). The nucleotides are arranged in pairs, with adenine linked to thymine and guanine coupled with cytosine.16,17

![Figure 1. Double helix shape of DNA showing the 4 nucleotides. Image courtesy of The National Human Genome Research Institute.](image-url)
The combination and sequence of these nucleotides provide the instructions for manufacturing the thousands of proteins needed by the human body. A small percentage of genes (about 1%) are alleles. Their unique base pair coding helps account for the traits passed from parents to offspring. Genes vary in size from a few hundred nucleotide pairs to more than 2 million base pairs, and there are approximately 3 billion base pairs in the human genome.\textsuperscript{16,17}

Genes become functional through a chain of biochemical events. A gene’s DNA sequence, or code, is arranged in a string of nucleotide triplets known as codons. For example, the string CATATCATT would be arranged CAT ATC ATT. The codons specify, or code for, any one of the 20 different amino acids used to manufacture proteins.

To make a protein, the coded DNA information is copied, or transcribed, into messenger ribonucleic acid (mRNA), a single-stranded molecule. The mRNA then travels from the nucleus to the cytoplasm of the cell (see \textbf{Figure 2}). Once in the cytoplasm, tiny units known as ribosomes read the mRNA code and use the information to transform amino acids into proteins (see \textbf{Figure 3}).\textsuperscript{18,19}

Proteins are monitored and regulated by a number of mechanisms such as repressor proteins that prevent excessive production and activator proteins that stimulate production. In addition, any change or modification to genetic DNA can affect the structure and quantity of proteins produced by the body.\textsuperscript{20,21}

In principle, a faulty gene can create a malfunctioning metabolic pathway that can manifest as a disease. If the building blocks of DNA are altered or mutated in such a way that the genetic blueprint is changed, the resulting protein could be altered. Most mutations are neutral or silent, only affecting regions of DNA that have no known function.\textsuperscript{19}
In some cases, a mutation is beneficial and can improve the function of an important protein enzyme.\textsuperscript{22} However, in a hereditary disease, a mutation in the DNA code can cause a vital protein to malfunction or cease functioning; occasionally the mutation is so acute that the protein is not synthesized at all. Because some proteins are more crucial to a cell’s normal function, the severity of a disease often reflects the importance of the protein.

**Categories**

Gene therapy generally has been classified into 2 categories: somatic cell (nongerm/nonstem cell) therapy and reproductive cell, or “germ-line,” therapy. The cells targeted for somatic cell therapy are corrective only for the specific patient receiving treatment. The genetic therapy or responding traits and outcomes are not inherited.\textsuperscript{23} Germ-line therapy is based on the Weismann theory of heredity, which states that all inheritable characteristics are transmitted by the reproductive cells (eg, ova or sperm, also called a gamete). Therefore, any therapeutic alteration to the germ cells of the patient will be passed on to his or her descendants.\textsuperscript{23,24}

**Approaches**

Typically, there are 2 main approaches to gene therapy. One approach, the in vivo approach, is the introduction of a gene into a vector that is administered directly into the patient via injection or intravenously. This vector transfers the therapeutic gene into the target tissue to produce the therapeutic protein. The second approach, the ex vivo approach, involves the transfer of vectors carrying the therapeutic gene into cultured cells that have been extracted from the patient. The genetically engineered cells are reintroduced into the patient, where they express the necessary therapeutic protein. In vitro procedures normally occur outside the body and often use artificial media such as test tubes in laboratory conditions.

Clinicians must consider several key aspects when designing a gene therapy approach, including:
- Choice of therapeutic gene.
- Route of administration.
- Choice of an animal model in which to test the approach.

For monogenic diseases, such as most muscular dystrophies caused by the dysfunction of a single gene or lack of a single protein, choosing a gene for therapy is made simpler. However, multifactorial diseases such as diabetes, cancer, spina bifida, Alzheimer disease, and congenital heart defects have more complex origins. Interactions of more than one gene, associated environmental causes, or both, lead to the disease processes. In these instances, choosing a therapeutic gene is more challenging.

In addition, clinicians must focus on finding the right regulatory sequences that determine exactly when and where on the gene encoding of a therapeutic protein takes place. These regulatory sequences are called promoters and are positioned in front of the therapeutic gene to be expressed. Some promoters direct gene expression to specific cell types such as cardiomyocytes (heart cells) and hepatocytes (liver cells). Other promoters, known as ubiquitous promoters, allow gene expression simultaneously to multiple tissues.

**Gene Delivery**

Gene therapy often requires delivery of a gene into a target cell’s chromosome. Treatment success depends largely on the agent or vector available to deliver therapeutic genes. A vector is a vehicle that packages the therapeutic gene of interest. Vectors have several functions, including protecting the gene from degradation, facilitating entry into target cells, and securing stable gene transcription upon arrival in the nucleus.\textsuperscript{25} Ideally, a vector should be efficient in gene transfer and be safe. Safe transfer means that the vector introduces zero to minimal risk of infection or immunogenicity (immune response). In addition, a safe vector causes no mutation in the host cell or patient-to-patient transmission of a virus or other pathogen.\textsuperscript{25,26}

In recent years, a number of vectors have been researched or developed, each with unique characteristics. No universal vector currently exists, so when choosing a vector, researchers must weigh factors such as which target therapy to use and whether short-term or chronic treatment is necessary. More than 40 variants of vectors have been evaluated or implemented in gene therapy clinical trials as of spring 2014. These vectors fall into the 2 main categories of viral or nonviral...
methods. These have included *Escherichia coli*, the *Vaccinia virus*, *Herpes simplex virus*, *Vibrio cholerae*, and plasmid DNA. Of the nearly 2100 clinical trials to date, most taking place within the past decade, the most prominently used vectors for gene therapy delivery include adenoviruses, retroviruses, and naked plasmid DNA; together these vectors account for approximately 60% of all vectors tested in trials (see Figure 4).7,27

**Viral Vectors**

Viruses can carry genes into cells. Generally speaking, viruses are infectious agents that have evolved highly efficient methods of transferring genetic material into host cells. Viruses adapt extremely well to host organisms. Gene therapy takes advantage of this attribute of viruses to introduce therapeutic genes into target cells.

Although viral structure varies, all viruses share some general characteristics. All viruses have a region for containing genetic material called a **capsid**, which is made of proteins and glycoproteins. Viruses vary in genetic material, either DNA or RNA, and, in some cases, supplementary enzymes. Classification of viruses is based on characteristics of the virus’ genetic makeup. Particular viruses also have a membrane that surrounds their capsid and allows penetration into host cells through membrane fusion.

A viral vector works through the innate ability of a virus to enter a cell and deliver genetic material to the cell’s nucleus. Typically, through genetic manipulation, most existing viral genes are replaced by the therapeutic gene, turning the virus into a vector that cannot cause disease. Common types of viral vectors used are retroviral, lentiviral, adenoviral, and adeno-associated vectors. These viruses vary in efficiency of gene transfer to the cells they recognize and infect, along with their ability to alter a target cell’s DNA permanently or temporarily. As a result, genetic researchers rely on different vectors, depending on the characteristics of each and the requirements of the associated clinical study.

**Lentiviruses and Retroviruses**

Lentiviral and retroviral vectors share common characteristics and similar structures based on an RNA genome because they stem from the same taxonomic family, Retroviridae.4 Lentiviruses are enveloped and spherical and possess 2 copies of a single-stranded RNA genome measuring approximately 80 to 100 nanometers in diameter. Some lentiviruses are the human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), and feline immunodeficiency virus (FIV). These viruses are known for having gene integration properties that make them highly suitable for use in gene therapy.28

Retrovirus virions (the complete virus particle) contain a protein capsid that is lipid encapsulated and ranges in diameter from 80 to 130 nm. The genome for the virus (3.5-10 kilobases, or kb) has 2 copies of single-stranded RNA and contains a complete set of genetic instructions. The genome is encased within the capsid, along with integrase (a retroviral enzyme enabling integration of the virus’ DNA into the target cell) and reverse transcriptase proteins.29

The RNA genome from a lentiviral or retroviral vector is altered to contain a therapeutic gene. When the vector infects a target cell, the therapeutic gene is...
reverse transcribed to DNA in the target cell’s cytoplasm through a reverse transcriptase enzyme carried by the vector. Once transcribed, the DNA with the therapeutic gene enters the nucleus of the cell, where it integrates into the genome of the target cell. In simple terms, this means the viral vector copies its genetic material by inserting the material into the target cell’s DNA.

Lentiviral vectors are highly successful in crossing the nuclear membrane of the target cell and permanently changing the cell. This advantage potentially increases the efficacy and longevity of therapeutic treatment. When therapeutic genes are integrated into a target cell’s genome, new cells are created through mitosis. In mitosis, or nuclear division, the daughter cells are genetically identical to the parent cell. Daughter cells from a target cell infected by a lentiviral vector also contain the therapeutic gene, which allows for the stable and long-term expression of the therapeutic gene.

All types of retroviruses efficiently integrate into the target cell’s genome, potentially allowing persistent gene transfer. A disadvantage associated with most lentiviral vectors is that the largest genome that can be inserted is 5 kb, whereas some classes of retroviruses have cloning capacities between 7 and 10 kb. However, retroviruses generally are difficult to grow in large quantities.

The main difference between the lentiviral and retroviral vectors is that lentiviruses can infect both quiescent (nondividing) and mitotically active cells, whereas retroviruses can infect target cells only during division. As a result, retroviral vectors only can be used for ex vivo gene therapy methodologies. Lentiviral vectors can be used in both ex vivo and in vivo gene therapy methodologies. Lentiviral vectors can infect a broader variety of cell types and sustain gene delivery through stable vector integration into the target cell’s genome. In addition, the lentiviral vector’s viral envelope protein can interact with a broad number of receptors at the target cell surface, a characteristic called viral tropism.

The first clinical trial for lentiviral gene therapy was in 2006 and involved treatment of the cerebral form of X-chromosomal linked adrenoleukodystrophy, a demyelinating disease of the central nervous system. Since then, lentiviral vectors have been used in approximately 4% of clinical trials. Conversely, as of 2013, retroviral vectors had been used in approximately 20% of the nearly 2000 trials conducted around the world, representing the second-most-often-used therapeutic vehicles after adenoviral vectors.

Adenoviruses

Adenoviral vectors are derived from adenoviruses and contain a double-stranded DNA genome measuring about 36 kb. More than 55 different variations or serotypes have been identified to date and have been classified according to their characteristics and differences in capsid structure and receptor use.

Following cell contact, the vector enters the target cell via endosomes, a type of coated vesicle, or membranous pouch inside the target cell. This process is called endocytosis. After release from the endosome, the adenoviral DNA enters the target cell’s nucleus where it remains in an extra-chromosomal form. Adenoviral vectors have high transfection efficiency, meaning they are effective at adding DNA to target cells. Adenoviral vectors are similar to lentiviral vectors in that they also can infect both actively dividing and quiescent cells.

An advantage of adenoviral delivery of therapeutic genes over lentiviral delivery is the intrinsic characteristic of adenovirus DNA not to integrate into the target cell. This characteristic avoids the addition of extraneous DNA bases and the possibility of inducing mutation. Adenoviral vectors also are more easily manipulated genetically, so the virus cannot continue to replicate. These replication-deficient vectors ultimately reduce unwanted adverse effects. However, producing adenoviral particles takes longer than for other viruses. It takes several weeks to produce an adenoviral virus vector vs several days to produce retroviruses.

Initially, there were problems associated with adenovirus vectors. Only a portion of the adenovirus genes were removed, and target cells proved to be capable of recognizing the remaining viral proteins and destroying the newly introduced foreign genes, or transduced, therapeutic genes shortly after infection. This resulted in short-term expression of the therapeutic gene. However, use of vectors in which all of the viral genes have been removed has made long-term expression of the therapeutic gene possible with adenoviruses.
adenovirus production schemes have been developed that allow for scaling up production of adenoviruses, facilitating their use in human clinical trials.

**Adeno-Associated Viruses**

Adeno-associated (Parvoviridae family) vectors are derived from particularly small (approximately 20 nm), replication defective, nonenveloped viruses. The adeno-associated virus genome consists of a single-stranded DNA, which is about 4.7 kb long. Adeno-associated viruses are among the most promising viral vectors for human gene therapy and are becoming progressively common as a vector for clinical use. With approximately 12 serotypes, most have a variety of tropism, meaning they can respond or orient to different types of tissue. Adeno-associated viruses can infect several types of cells, ranging from skeletal muscle cells to vascular muscle cells.

As with lentiviral vectors, adeno-associated viral vectors can infect both dividing and quiescent cells. The virus persists outside the chromosome and has the ability to integrate with stability at a site-specific point within the genome. Adeno-associated viruses integrate with stability at a specific point within the human genome. After entry into the target cell’s nucleus, the vector follows 1 of 2 distinct, interchangeable pathways of its life cycle: the lytic or lysogenic. The lytic pathway develops in target cells infected with a helper virus, such as adenovirus or herpes simplex virus. Once the adeno-associated virus attaches to and infects the target cell, it makes copies. In the lysogenic pathway, the virus, in absence of a helper virus, becomes established in a target cell and transmits DNA directly from the virus genome into a region of human chromosome 19.

Adeno-associated viruses have been shown to have low immunogenicity and have no known roles in disease. These vectors have demonstrated efficiency for gene transfer to the retina and also have been implemented as vectors in gene therapy for lipoprotein lipase deficiency. Adeno-associated vector’s cloning capacity is relatively limited because the virus has only a 4.7 kb genome. New technologies might allow for expanding vector capacity in the future. Further research into the use of adeno-associated viruses likely will continue because of the vector’s promise as a gene therapy delivery method.

**Risks of Using Viral Vectors**

Viruses typically can infect more than one type of cell; therefore, viral vectors can potentially infect healthy cells along with target cells. Once recognized by the body, or if the vaccine or therapy fails, effective use of a viral vector in the patient a second time proves difficult. The same viral vector most likely cannot be used in the patient for a different future vaccine or gene therapy. Furthermore, some patients may have preexisting immunity to a viral vector, ultimately rendering the gene therapy ineffective.

Viral vectors that require cells to be actively dividing for transduction (the insertion of genetic material) have drawbacks because some cells are highly resistant to infection and transduction by retroviruses. Transferred genes also can become overexpressed and produce too much of the necessary protein, which can lead to harmful effects such as inflammation or an immune reaction. In addition, the integrase enzyme has the innate ability to insert genetic material in arbitrary spots within the target cell’s genome. If genetic material is inserted in the wrong location, insertional mutagenesis can occur. This has been demonstrated in clinical trials for X-linked severe combined immunodeficiency patients in which hematopoietic (blood) stem cells were transduced and led to development of T-cell leukemia in a few patients. The induced mutation problem can be addressed by inserting sequences to control site integration and using engineered DNA-binding proteins that create a double-stranded break in DNA at user-specified locations.

**Nonviral Methods**

Nonviral gene vectors have been routinely used in gene therapy, partly because of concerns regarding the safety of viral vectors. Nonviral gene therapy can be performed by administering plasmid DNA that encodes a transferred gene, or transgene, locally or systemically (eg, injection into arteries). This procedure yields expression of a therapeutic protein to amend a disease state. Clinical applications of nonviral gene delivery have included peripheral arterial occlusive disease, arthritis, and cancer. Using plasmid vectors is one of many approaches that can be used to insert therapeutic genes at preselected
target sites. Most often found in bacteria and archaea (single-celled microbes) plasmids are small circular double-stranded DNA molecules. In nature, plasmids transmit genes that support an organism’s survival, such as antibiotic resistance. Plasmids frequently can be transmitted between bacteria via nonreproductive horizontal gene transfer allowing exchange of genetic material with neighboring bacteria. The plasmids contain a gene for acquiring antibiotic resistance, and after gene transfer, the gene can then be used by the bacteria.

Natural and artificial plasmids are essential tools in genetics and biotechnology labs, where the molecules are routinely used for the genetic cloning of small DNA fragments and genes and for mass production of various proteins. Many plasmids are commercially available for such uses.

Plasmid DNA can be injected alone and yield therapeutic gene expression. This was first discovered by intramuscular injection using a reporter gene used as a marker to verify and measure gene expression, resulting in the marking of muscle cells at the site of injection. Reporter genes also are called marker genes. Recent late-stage clinical applications have taken advantage of this type of plasmid-based gene delivery. Methodologies have been applied to peripheral vascular disease by expressing a therapeutic gene that encodes for angiogenic growth factors, basic fibroblast growth factor, or hepatocyte growth factor.

The development of genetic vaccines is another active area of plasmid DNA therapy. Genetic vaccines using plasmid DNA alone are being developed for pandemic flu, HIV, and hepatitis C. Full implementation has included reduced and limited transgenic transcription through their use. Once the plasmid vector DNA enters the target cell’s nucleus, efficient transcription of the DNA must occur for effective cell division and transgene expression. Therapeutic DNA introduced into target cells must remain functional to obtain a permanent cure. Further, the rapidly dividing nature of various cells might necessitate patients undergoing multiple sessions of gene therapy.

In addition, a patient’s immune system is stimulated when a foreign object, including a gene therapy vector, is introduced. The immune system can reduce therapeutic effectiveness, especially for recurring therapy. The protein must be purified before the vector is used, usually with a commercially available preparation that strips it of DNA. Antigens can be problematic with regard to the yield and purity, often giving rise to antibody responses to the impurity. There is also a limit to the size of inserts (30-40 kb pairs). Proteins have isoelectric points, and molecular charges might cause electrostatic repulsion of therapeutic DNA at the cell surface. Use of cationic liposomes to enclose the therapeutic DNA can improve endocytosis.

Clinical Targets

Initially, gene therapy was established to cure patients with hereditary diseases caused by single-gene defects, or monogenic disorders (dominant, recessive, or x-linked). Examples of monogenic disorders include cystic fibrosis, muscular dystrophy, and hemophilia. For many years, gene therapy has been considered most successful when applied to diseases caused by defects on a single gene or the absence of a single gene. At present, however, much gene therapy research and development is focused on diseases caused by polygenic and noninherited diseases. Some of these diseases include hepatitis C, retinitis pigmentosa, and various types of cancer and cardiovascular diseases. In recent years, the focus has shifted from the conceptual stage through technology development and laboratory research to present clinical translational trials.

Cancer

Early gene therapy clinical trials targeted severe diseases with minimal therapeutic options and associated substantial morbidity and mortality, such as severe combined immunodeficiency with bone marrow transplant. To date, more than 60% of trials worldwide are focused on cancer with formidable results. For instance, in 2012, a sizable percentage (> 70%) of patients treated for multiple myeloma, a cancer related to plasma cells, showed signs of remission after being treated with genetically engineered T-cells to target specific proteins NY-ESO-1 and LAGE-1 that exist in cancerous myeloma cells.

Current gene therapy approaches for cancer are less hindered by complications. Many current approaches are designed to elicit increased tumor cell immunogenicity
or to enhance cell death by replacing a gene. In addition, many efforts for cancer therapy do not require sustained and closely regulated gene expression.74 Most gene therapy trials have used adenoviral and retroviral vectors to deliver therapeutic genes to target sites. Adeno-associated viral-based vectors also are proving to be suitable for cancer gene therapy.

Ongoing research involves the application of gene-directed enzyme prodrug therapy in addition to, or as a replacement for, standard chemotherapy for cancer treatment. Essentially, “suicide” genes are directed at cancer cells to make tumors more susceptible to radiation therapy and chemotherapy. These directed genes cause an inactive drug, or prodrug, to become toxic only when the prodrug is metabolized by proteins produced by the directed genes injected into the tumor. For example, a directed suicide gene encodes an enzyme that converts a nontoxic prodrug such as ganciclovir into highly toxic metabolites.

The herpes simplex virus type 1 thymidine kinase (HSVtk) gene is the driving agent that makes transformed cells sensitive to the antiviral medication ganciclovir. Gene-directed enzyme prodrug therapy introduces viral or bacterial genes into tumor cells and has successfully been used in several in vitro and in vivo studies.75–78 Use of the prodrug therapy with the HSVtk gene has been thoroughly investigated.79–82 Induction of HSVtk into tumor cells makes cells sensitive to various drug regimens.83,84 The therapy’s potential has been demonstrated in animal studies, often with results that include complete eradication of the tumor.85–87

Gene therapy offers potential for targeted destruction of tumor cells in patients and can be used to delineate new targets or investigate the role of specific genes in carcinogenesis or cancer progression. In 2013, highly positive results were shown in subjects who had acute lymphoblastic leukemia, a cancer of the blood and bone marrow, and were treated with genetically modified T-cells that attacked cells with CD19 genes on their surface.88

Glioblastoma multiforme is highly invasive and the most common brain tumor occurring in adults.89 The gene therapy approach most often used for treating glioblastoma multiforme relies on various adenoviral and retroviral vectors, as well as nonviral vectors. The viral and nonviral vectors are genetically modified to express genes for an enzyme that transfigures a prodrug targeted at the tumor sites and ultimately destroys tumor cells. Several enzyme-prodrug systems have been evaluated in phase 1, 2, and 3 trials,90 with the HSVtk gene as the most extensively investigated.

To date, only modest successes have been recorded.91,92 Unfortunately, anatomical and physiological aspects associated with the brain reduce transduction efficiency and effect overall vector targeting and delivery. Researchers continue to review limitations and potential strategies.90,93,94

Cardiovascular Disease

Targeted gene therapy also is being investigated for the management of cardiovascular disease. The use of gene therapy is currently being studied for several cardiac diseases, including heart failure and coronary artery disease. Heart failure is one of the leading causes of morbidity and mortality in the United States.95 Several preclinical and clinical studies have shown some efficacy in administering genes that upregulate enzymes, notably a transporting gene involved in myocardial contraction and relaxation to treat advanced heart failure.96–101

Jessup et al conducted a study on calcium upregulation using gene therapy in patients with heart failure. Low levels of calcium uptake have been identified in striated heart muscle cells of patients with heart failure. Further, the deficient levels have been associated with low expression and activity of an enzyme that triggers reloading of calcium while the sarcoplasmic reticulum is at rest, the sarcoplasmic reticulum adenosine triphosphatase, calcium 2+ ion. In phase 1 and 2 clinical trials investigators restored levels of the associated enzyme in patients who had advanced heart failure using gene transfer via an adeno-associated viral vector. Patients who received intracoronary administration of the gene therapy had significant improvement in functional capacity and symptoms from heart failure.96

Coronary artery disease is another leading cause of mortality in the United States99 and is currently the most common cause of death in the world.102 Alternative options to conventional pharmacologics and revascularization methodologies are being developed. One option...
is therapeutic angiogenesis, which involves administering certain genes for angiogenic growth factors to augment collateral vessel development and increase new blood cell formation.

Recent clinical studies have investigated a number of angiogenic growth factors, including fibroblastic growth factor, vascular endothelial growth factor, and hepatocyte and platelet-derived growth factors. However, angiogenesis is a highly complex mechanism requiring the organization of multiple growth factors acting on receptors to stimulate and sustain growth, thereby complicating therapeutic applications. Moreover, positive and encouraging effects of gene therapy on initiating heart impulse activity have been shown in several arrhythmia investigations. Selective expression of certain genes producing ion-stimulated enzymes could ultimately be used to initiate and regulate heartbeats, thereby establishing genetic alternatives to electrical pacemakers.

**Vision**

Gene therapy offers great promise for treating vision loss from a variety of causes. Significant advances in understanding disease processes of human retinopathy and the development of efficient retinal gene transfer using adeno-associated vectors or lentivirus-based vectors have led to proof-of-concept studies of gene replacement therapy. The potential of gene therapy has been demonstrated in the treatment of degenerative diseases such as retinitis pigmentosa, which is characterized by a slow, progressive loss of photoreceptors and caused by defects specific to the retinal epithelium, a pigmented cell layer just outside the neurosensory retina.

In 2011, the first gene therapy clinical trial was initiated for Stargardt disease, a form of juvenile macular degeneration that affects 30,000 to 50,000 people in the United States alone. The trials are designed to work by delivering healthy copies of the protein coding gene \(ABCA4\) to cells in the retina. Another example of progress is the reconstitution of vision following adeno-associated viral gene therapy in patients with Leber congenital amaurosis, a hereditary disorder that leads to retinal dysfunction and visual impairment at an early age. To date, 19 genes have been identified with mutations that lead to the disease.

In 2007, positive effects were reported concerning subretinal delivery of recombinant adeno-associated viral vectors carrying the \(RPE65\) gene for inherited retinal disease. Several modes of therapy are currently in varying stages of development, including a human clinical trial for gene replacement for the retinal pigment epithelial gene \(RPE65\) Leber congenital amaurosis mutation. A 2009 phase 1 clinical trial reported substantial restoration of vision to patients, 50% of whom improved enough to be declassified as legally blind. More recently, stable reversal of congenital blindness was demonstrated using injections of the adeno-associated viral vector with the \(RPE65\) gene.

**Noninherited Diseases**

Many genetic syndromes are not inherited. A form of Down syndrome called Trisomy 21 is one of the most common noninherited genetic syndromes. The syndrome occurs in approximately 1 in every 700 births. Early research has shown that insertion of the X-inactive nonprotein coding gene \(XIST\) can silence the extra copy of chromosome 21 that causes Down syndrome. In in vitro experiments, chromosome 21, the extra chromosome in cells of individuals with Down syndrome, has been removed via a type of genetic modification that uses stem cells to naturally remove the third copy.

Parkinson disease is another example of a disease in which not all cases are directly inherited; only a fraction (15%-25%) of people with Parkinson disease report having a relative with the disease. A gene therapy product using adeno-associated viral vectors carrying neurturin (\(NTN\)), a gene encoding for a neurotrophic growth factor, was well tolerated and appeared to reduce symptoms in about 40% of subjects with advanced Parkinson disease in a 2006 phase 1 study. Neurturin is the protein responsible for the survival and function of neurons. Instead of simply treating symptoms of Parkinson disease, helping to preserve neurons could halt degeneration associated with the disease.

Studies by Muramatsu et al and During et al using adeno-associated viral vectors have shown promise in treating Rett syndrome, a serious brain disorder caused by mutations in the protein-coding \(MECP2\) gene. Fewer
than 1% of cases are inherited and the disorder affects mostly young girls. In the Muramatsu study, the vectors carried genes encoding for the aromatic L-amino acid decarboxylase, which is required for synthesis of the neurotransmitters dopamine and serotonin, and in the During study, vectors carried genes encoding for glutamic acid decarboxylase, an enzyme involved in producing neurotransmitters.\(^\text{127,128}\)

Participants in the Muramatsu study showed signs of improvement after 2 years, while those in the During study improved after 3 years of treatment. For many years, Rett syndrome was widely regarded as incurable. Recently, using an animal (mouse) model, it was demonstrated that Rett syndrome could be reversed by restoring the function of the \(\text{MECP2}\) gene,\(^\text{129}\) providing some hope for future management.

Recent reports have been made of promising and long-term results in canines chemically induced to have diabetes that were given single shots of an adeno-associated viral vector. The vector carried genes for insulin and glucokinase in skeletal muscle, making it possible for the dogs’ bodies to produce insulin and glucokinase.\(^\text{130,131}\)

**Medical Imaging in Gene Therapy**

Gene therapy offers great promise for various diseases and conditions and for managing several cancer types. Medical imaging plays a key role in implementing and advancing strategies for successful delivery and function of desired genes. These strategies comprise both traditional and new molecular imaging technologies using reporter probes. In medical imaging, reporter probes are radiopharmaceuticals that help detect or identify disease biomarkers.\(^\text{132}\)

Imaging techniques used in gene therapy have included ultrasonography, magnetic resonance (MR) imaging, computed tomography (CT), positron emission tomography (PET), and single-photon emission computed tomography (SPECT), and optical imaging. Each imaging modality offers unique advantages and limitations related to sensitivity or spatial resolution. For example, MR imaging offers superior contrast for soft tissue and high spatial resolution for anatomical information, yet has lower sensitivity for detecting reporter probes compared with PET scanning.\(^\text{133}\)

**Monitoring Gene Expression**

An objective in gene therapy is to ensure as rapidly as possible that the administered therapeutic gene is being expressed in the target cells. Most current in vivo molecular imaging strategies use a reporter gene with a complementary reporter probe. By coupling a reporter, or marker, gene with a therapeutic gene, molecular imaging techniques in particular can be used to evaluate gene expression. The marker gene might serve no therapeutic role but only act in reporting gene expression, providing an indirect measure of the location and magnitude of the expression of the therapeutic gene.

Reporter genes and probes have been developed mostly for PET and MR imaging. MR imaging relies on measuring magnetization of nuclei (eg, carbon 13, fluorine 19, sodium 23, and phosphorous 31) subjected to radiofrequency radiation, which offers several strategies for use of markers with MR imaging. These strategies include enzyme-based markers using enzyme-catalyzed chemical modification that exploit various enzymes based on contrast agents with covalent bonds. A covalent bond refers to sharing of electrons between compounds. Other strategies include bond cleavage, or splitting of bonds, and iron-based strategies in which protein accumulation of iron can act as a contrast agent.

Depending on the protein structure and oxidation state, iron can act as a paramagnetic reactive metal. Manipulation of iron concentration yields detectable contrast and has been implemented for monitoring therapeutic gene expression in which an engineered transferrin receptor\(^\text{134}\) was expressed as part of a vector that carried several genes, among them a prodrug therapy gene.\(^\text{135}\)

Chemical exchange saturation transfer (CEST) can be monitored using a variety of compounds (eg, organic, organometallic, or both) with suitable chemical exchange rates and resonance frequencies. The technique works by chemical exchange between protons in solutes such as contrast agents with those in water.\(^\text{136,137}\) An advantage of using MR reporter genes is that the specific signal can be recorded for both soft-tissue anatomy and functional tissue information, providing data on gene expression along with anatomic and functional information.\(^\text{138,139}\)
PET is one of the most exacting approaches to imaging for detection and quantification of picomolar amounts of radiolabeled materials in vivo. A further advantage of PET is its accuracy regardless of the depth of the tissue of interest. Reporter probes labeled with positron-emitting isotopes, such as fludeoxyglucose F 18, oxygen O 15, and copper Cu 64, are taken up by target cells, binding to specific receptors. The receptors are phosphorylated, which means that a chemical reaction or transfer occurs in a phosphate group that is catalyzed by a substrate-specific enzyme that can be detected on PET images.

A number of PET reporter genes have been found suitable for imaging vector-mediated gene delivery and expression in both preclinical and clinical situations. The findings have been based on the radiolabeled substrates that interact with specific transgenic proteins. Much like MR-based markers, these reporter genes enable noninvasive analysis of the location, level, and kinetics of transgenic activity. The PET methodology has optimum characteristics, however, in terms of sensitivity and quantification of in vivo gene expression. In addition, increased availability of and familiarity with PET equipment has helped define the modality as an applicable method for analyzing gene therapy in patients.

As with vectors for gene delivery, reporter genes and probes should have certain characteristics, such as inability to induce immune responses, accumulation of probes only at the site of reporter gene expression, stability and lack of cytotoxicity, and the ability to fit into selected vectors. In addition, no single combination of marker genes and probes is suitable for all imaging applications.

A few strategies are used to facilitate imaging of the reporter gene and probe using PET or SPECT. Intracellular or enzyme-based strategies involve phosphorylation of a radiotracer substrate by the imaging reporter enzyme, in which cells expressing the imaging reporter gene ensnare the probe. For example, the enzymatic activity of HSV1-tk has been used widely for in vivo imaging of gene expression via various radioisotope-labeled substrates. Surface-based (receptor-based) strategies involve the delivery of membrane receptors such as a dopamine-2 receptor or somatostatin receptor to target cells that bind specifically to radiolabeled tracers such as F18 (fluoroethyl) spiperone or radiolabeled somatostatin analogues. The technique results in the trapping of the probe on or in cells expressing the gene.

Transporter-based strategies using radiopharmaceuticals with half-lives averaging about 110 minutes are time-sensitive approaches that deliver membrane transporters to target cells that facilitate uptake of the reporter probe. The major advantages of surface-expressed receptors are satisfactory kinetics, or chemical changes, and the fact that synthetic reporters can be manufactured to recognize probes already approved for use by the FDA. Advantages of intracellular strategies include their simple design and lower immune responses compared with surface-based strategies. HSV1-tk (and variants of the gene) is the most frequently used reporter gene. The gene is imaged by both PET and SPECT. In addition, HSV1-tk conceptually can be used both as a therapeutic gene and a reporter gene.

**Gene Delivery**

A leading challenge in gene therapy is the release of a therapeutic gene to the precise desired location. Research in cancer gene therapy is focused on how to improve gene delivery to malignant tumors. Medical imaging can play a critical role when combined with medical biology and oncology in the delivery of suicide genes that can help destroy malignant cells. The targeted delivery of therapeutic genes helps limit potential adverse effects from drug toxicity. Vascular and interventional radiology techniques are particularly suited for marginally invasive and readily monitored gene delivery. Some medical imaging techniques in gene therapy delivery are already implemented or undergoing evaluation in clinical trials.

**Ultrasound**

Exposure of cells to ultrasound waves increases membrane permeability and assists with intentional introduction of nucleic acids or transfection into cells. The first applied demonstration of ultrasonography in gene transfer took place in 1987. Local administration of naked plasmid DNA results in a small amount of gene transfer to cells at the site of injection. Cells are known to spontaneously uptake nucleic acids. However, gene transfer efficiency can be
increased by applying electroporation or sonoporation. Electroporation is use of electrical pulses to permeate a cell membrane with tiny holes, and sonoporation develops temporary pores in the membrane using sound waves. Applying electrical or ultrasound energy in a series of pulses (lasting microseconds) allows for cell entry and results in increased gene expression.145-150

When oncologists use liposomes for gene therapy, focused ultrasound can implode the liposomes, prompting them to release their genetic contents. Liposomes are artificial vesicles made from lipids. The lipids encapsulate microbubbles generally filled with gas and contrast agents. Success of the therapy depends on effective delivery of the plasmid DNA and adequate acoustic energy. In addition, clinicians must select the most suitable plasmid to maximize delivery.151 The magnitude and duration of pulses must be optimized to maximize gene transfer and minimize damage to cell membranes. Several studies have shown that ultrasound can be used to enhance gene expression from liposomal transfection.152

Transfection rates have recently increased several orders of magnitude in in vitro studies using ultrasound exposure on various tissue cells.152-154 In addition, in vitro transfection rates using ultrasound are generally much higher than those recorded for in vivo studies.155-157 These methods result in increased uptake by cells and therefore an increase in gene expression. Still, nonviral vectors continue to offer lower levels of gene transfection and subsequent cellular expression than do viral vectors.

Computed Tomography

Imaging methods such as CT have been used for decades for gene therapy monitoring and for precise needle guidance for therapeutic gene delivery. Use of micro-CT imaging assists in evaluating healing responses of allografts. The gene therapy is delivered by adeno-associated viral vectors with the protein carrying the ALK2 gene.158 Micro-CT also has been used to evaluate articular fracture healing following mesenchymal stem cell-mediated delivery of a gene encoding for bone morphogenetic protein-2.159

In a laboratory study, investigators used micro-CT to evaluate progression of pulmonary fibrosis in mice followed by intertracheal administration of an adenoviral gene vector encoding for the transforming growth factor-β1 gene.160 In human trials, CT imaging has guided intratumoral injection of plasmid DNA in trials directed at treating melanoma.161 CT scanning also has been used to safely steer gene-therapy injections directly into the tumor when treating patients with metastatic kidney cancer.162 Several therapeutic agents have been developed that improve the use of interleukin-2, an approved treatment for metastatic kidney cancer.

In addition, in phase 1 and 2 clinical trials for treatment of pancreatic cancer, CT was used to evaluate liver lesions following administration of targeted gene therapy.163 Attempts also have been made to correlate CT images with gene expression, although the methods have been indirect, occurring by means of imaging patterns.164,165

Positron Emission Tomography

PET is a specialized nuclear medicine procedure used to examine various body tissues and to isolate molecular activity in the body to evaluate function and disease. PET scanning can detect chemical substances such as glucose (usually fludeoxyglucose), which is used naturally by organs or tissues during metabolic processes. Because of this feature, PET scans can display the metabolism of a particular organ or tissue to provide information about the physiology, anatomy, and biochemical properties of the organ or tissue. PET scans can identify the onset of a disease process before anatomical changes related to the disease can be seen on other structurally based imaging scans, such as CT and MR. PET has been used to evaluate conditions such as Alzheimer, Parkinson, and Huntington disease, and cardiac disease, and is a useful tool in evaluating cancer and cancer therapy.

In gene therapy, PET scanning is a noninvasive tool for diagnosing the pathology, biology, and safety of vectors. In addition, PET scanning is useful for imaging biochemical effects of gene therapy; quantitatively monitoring the site, scale, and continuity of gene expression; and to better understand vector biology and safety.

Conceptually, combined use of PET with reporter genes and probes could be used to noninvasively monitor all aspects of transgene and cell kinetics in virtually
all types of living mammals. Its usefulness has been shown in animal trials and in human patients. For example, Jacobs et al demonstrated that a combination of PET and a specific marker substrate (I-124-labeled 2'-fluoro-2'-deoxy-1β-D-arabinofuranosyl-5-iodo-uracil, or 124I-FIAU) could be used to monitor gene therapy response in a phase 1/2 clinical trial for recurrent glioblastoma in 5 patients.

PET scanning is not without limitations. Minimizing exposure to ionizing radiation and signal loss from leakage into nontarget signal cells are goals for improvement. In addition, radiotracers have relatively short half-lives, and PET scanning has limited ability to track signals longitudinally and long term. Combined CT and PET imaging has become more commonplace. The fusing of structural and functional imaging in a single instrument substantially increases the utility and applicability of imaging in gene therapy patient management. For example, PET-CT has been evaluated for use in gene delivery and for monitoring of gene expression using animal models.

**Magnetic Resonance**

MR imaging generates high-contrast and high-resolution 3-D images and assists in diagnostic evaluation of organ function and morphology. Recent efforts have focused on using MR technology and methodologies for monitoring gene therapy delivery, enhancing gene transfection and transduction, and for tracking gene expression.

Various markers have been evaluated for use with MR scanning to display transgene expression. Rehemtulla et al followed patients who had intratumoral injection of adenoviral vectors carrying therapeutic genes into gliomas. The investigators recorded anatomical and diffusion-weighted MR images every 2 or 3 days over a period of 4 to 6 weeks to determine therapeutic efficacy and spatial heterogeneity of cancer cell destruction.

MR spectroscopy has been used in laboratory studies involving mice to evaluating transgenic expression. MR also was used in test studies for therapeutic treatment of brain tumors in rats, documenting the disappearance of large tumors by detecting 5-bromodeoxyuridine–labeled progenitor (neural stem) cells after the injection. More recently, in a phase 2 trial, MR neuroimaging was used to monitor participants in which lentiviral vectors containing functional protein coding ARSA genes were delivered as therapy for metachromatic leukodystrophy, a neurodegenerative lysosomal storage disease caused by arylsulfatase A (ARSA) deficiency. Neurosurgeons at the University of California San Diego School of Medicine and Moores Cancer Center used real-time MR navigational technology to deliver genes carrying an investigational anticancer drug (Toca 511) directly to brain malignancies.

A new method of using MR/CEST imaging may be implemented to monitor the effectiveness of gene therapy for cancer. MR/CEST is considered an alternative to relaxivity-based contrast protocols that is better suited for molecular imaging. The technique involves detecting proton exchange when certain chemotherapy compounds such as glutamate are broken down. Unlike nuclear imaging, MR provides multiple image planes with no ionizing radiation. However, MR sensitivity is limited in some studies.

**Challenges and Ethics**

Gene therapy researchers must answer several questions to gain approval for gene therapy trials in humans. They must determine whether the disease being treated is a good candidate for gene therapy, and be certain that the gene they introduce will be correctly inserted and regulated so that it is clinically expressed in the patient. Researchers also must explain technical details of the DNA and the vector they will use. Even if these questions are answered, human gene therapy experiments can be delayed because of the technical aspects involved, risks to study participants and future patients, and the fear of human genetic engineering.

The future of gene therapy has been riddled with both overly optimistic claims and overly exaggerated statements of risk. Whether future research and application will remain constrained or open to wider possibilities remains to be seen. Ethical and moral issues implicit in gene therapy have drawn notice from several governmental and religious organizations. Debate regarding use of genetically engineered material in human subjects has been complex, with viewpoints from the fields of law, medicine, politics, biology, philosophy, and religion.
During the 1980s, when gene therapy was in its infancy, representatives of government agencies and government-appointed groups debated the topic extensively, resulting in a succession of guidelines that legitimized gene therapy clinical trials. The emergence of a scientific discipline of gene therapy in the 1990s stimulated mixed reviews within and outside of the scientific community, episodes of public excitement, and, subsequently, some ill-conceived and unsuccessful clinical trials.

Gene therapy manipulates cells in the human body. Therefore, its use is accompanied by several unique ethical and moral concerns. Gene therapy is initiated for patients who have serious diseases and conditions. However, clinicians, ethics committees, patients, and family members must draw lines between which traits constitute a unique disorder in an individual and which constitute advancement of common human traits. Further, society must address which governing body will oversee determining and enforcing these lines. Inevitably, issues regarding affordability and economic fairness will arise.

Current therapeutic research has focused on treating patients by targeting therapy to body cells such as blood cells. Somatic gene therapy cannot be passed on generationally. In the future, however, germ-line gene therapy could potentially target reproductive cells sparing a family’s future generations from a particular genetic disorder. On the other hand, the therapy could affect the development of a fetus in unexpected ways or have long-term adverse effects that are yet unknown.

Individuals who would be most affected by germ-line gene therapy are not yet born, so they cannot choose whether to have the treatment. Because of these ethical concerns, the U.S. government does not allow federal funds to be used for research on germ-line gene therapy in humans. The idea of germ-line gene therapy is quite controversial, and acceptance may take years or decades.

Despite the dramatic increase of gene therapy funding and clinical trials over the past decade, as well as recent successes, attitudes toward gene therapy vary. In a recent study, a sizable proportion of European Union opinions favored regulation, and European public opinion was mixed, with residents weighing the risk vs usefulness of gene therapy to society. People in several countries in the European Union knew little about the subject. In the United States, public opinion is generally favorable, though citizens have expressed concerns about eugenics and control of genetic information.

New Discoveries

New molecular methodologies and technologies can accomplish much good but could cause great harm. These issues arise whenever powerful new technologies are developed. Genetic sequencing, for example, has been established as a powerful diagnostic and prognostic tool, but it is not yet fully clear how useful sequencing will be for disease prevention or health promotion and the role of regulation and ethics in those uses of the technology. Current high-throughput sequencing technology is extremely powerful and has led to an increase in sequencing projects in laboratories around the world. The cost and accuracy of genome sequencing have improved dramatically. Genomics is no longer seen as the expensive venture that the initial $2.7 billion human genome mapping project cost in 2003.

If and when genome sequencing becomes a clinical mainstay, it is uncertain whether scientists and physicians will know enough about how genes code for health to make genomic data useful for preventing disease. In addition, physicians are unclear specifically what genetic findings they should reveal to patients, and whether the answer differs based on the patient’s age and health. Ownership of privacy of genetic data for research is questionable. Current gene therapy research is focused on correcting genetic flaws and curing life-threatening disease. Although regulations have been developed for conducting these types of studies, the techniques of gene therapy will likely become more refined and more widely available in the future. At that point, more complex issues should be addressed.

There is a distinction, of course, between reparation of genetic damage and efforts to make genetic “enhancements.” Some also have concerns that genetic therapy technologies could be abused to improve athletic performance (gene doping), even at risk to an athlete’s own health or the health of others. Moreover, decisions might need to be made regarding whether and when it is appropriate for society to intervene on
behalf of anyone who abuses or could be harmed by gene therapy or doping. Decisions about ethical issues, commercial interests, political choices, intellectual property, and patient rights might be necessary and some rules might need to be enforced, including in what circumstances it is morally acceptable to manipulate human genes. In 1990 the Ethical, Legal, and Social Implications research program was established to address basic and applied research on the ethical, legal, and social implications of genetic and genomic research. 188

It has been argued that correcting a gene’s function is merely a further extension of medicine. As our understanding of the human body has increased, we can now envisage treating patients at the genetic level. In effect, somatic gene therapy does not represent a major departure from established medical practice. Instead of injecting a necessary, yet deficient, protein into a patient, the gene that normally would regulate the body’s production of that protein could have its normal function restored. These viewpoints have been expressed with regard to the ethics of somatic gene therapy. 188

Current laws in the United States and United Kingdom prohibit early embryonic and germ-line gene therapy, which is in part because of our current state of genetic knowledge. It is possible that enough information might never be obtained. Concerns about cost and debates regarding the benefit of eradicating genetic diseases vs the justification of making genetic decisions on behalf of future generations are potentially irrevocable decisions.

**Regulation**

Regulation of gene therapy is intended to assess potential risks and to ensure a measure of safety. Although gene therapy holds much promise, a few serious adverse events have occurred in its use, including death of a patient from an inflammatory reaction to use of an adenovirus-based vector in 1991, 189 development of leukemialike symptoms following successful gene therapy, 190 and death from disseminated histoplasmosis following a gene therapy trial. After a subsequent investigation of the trial participant’s death from histoplasmosis, however, it was concluded that the gene therapy treatment was not the cause of death. 191 With these examples in mind, investigators must consider how safe a therapeutic approach must be before it is ethical to try it on humans, and whether the review process is sufficient to determine clinical safety.

In the United Kingdom, genetic research and its applications in gene therapy are controlled by organizations such as the national Gene Therapy Advisory Committee and regional and local ethics committees for hospitals, universities, and research institutes. 192 In the European Union, the European Medicines Agency is responsible for the conduct of clinical trials and vigilance of pharmaceutical development activities. 193 Numerous steps must be followed for approval.
In the United States, gene therapy is regulated by a myriad of governmental departments and organizations. Since 1976, the Department of Health and Human Services has overseen most gene therapy activities. The Department of Health and Human Services has supervision of clinical trials, and the National Institutes of Health, which has considerable oversight of research for drug development, testing, and safety, is under the auspices of the Department of Health and Human Services.

Also within the Department of Health and Human Services are the Office for Human Research Protections and the FDA. The FDA is the essential agency for protecting the health of U.S. citizens by ensuring the safety of drugs, medical devices, and biological products before their commercial use. All research involving human subjects must undergo review and approval from an institutional review board and additional requirements made by the National Institutes of Health must be met in addition to those specified in the Code of Federal Regulations. Furthermore, the Center for Biologics Evaluation and Research, a center within the FDA that was created by the Federal Food, Drug, and Cosmetic Act and the Public Health Service Act, regulates cellular therapy products, human gene therapy products, and certain devices related to cell and gene therapy.

A manufacturer considering developing and selling a gene therapy product must first inform the FDA. Before implementing human trials, the manufacturer must obtain special permission, called an investigational new drug application, from the FDA. Manufacturers must meet rigorous FDA requirements for safety, purity, and potency from the FDA and additional agencies such as the National Institutes of Health before marketing a new product.

The process for obtaining an investigational drug license is more difficult for gene therapy products than for standard chemical or biological drugs for several reasons. The distinctive nature of the products and the fact that early trials using gene therapy have resulted in some unexpected outcomes have led to extra caution on the part of regulators. Other concerns involve questions regarding drug dosage and toxicity, drug persistence in the patient, and ensuring quality in manufacturing and preservation of the therapeutic products.

**Conclusion**

Gene therapy is a complicated subject of research, and questions remain to be answered. Nonetheless, the field of gene therapy has been marked by astounding progress despite early pitfalls. Medical researchers are developing more accurate and proficient methods of diagnosing and managing several diseases and disorders, and these advancements will only become more impressive. (See the Box for resources about genomics and gene therapy.) Recent developments in gene therapies and the ability to pharmacologically target and administer these therapies to infected cells suggest that new curative treatments will soon prove applicable to a broader range of diseases and patients.

In the future, gene therapy will provide an exciting new therapeutic option, particularly for disorders with no available treatment. It remains to be seen how near or distant this future lies or the potentially expanding role of medical imaging in gene therapy.

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Gene Therapy Review

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Read the preceding Directed Reading and choose the answer that is most correct based on the article.

1. The United States and ______ account for most gene therapy trials worldwide, and most trials focus on ______.
   a. Canada; HIV/AIDS
   b. Europe; cancer
   c. Europe; Parkinson disease
   d. Canada; noninherited diseases

2. The first European Union–approved gene therapy product was developed to treat:
   a. smallpox.
   b. Huntington disease.
   c. lipoprotein lipase deficiency.
   d. retinitis pigmentosa.

3. Which of the following diseases was first targeted for genetic therapeutic trials?
   a. AIDS
   b. adenosine deaminase deficiency
   c. Parkinson disease
   d. retinitis pigmentosa

4. Which country was first to approve a gene therapy product for clinical use?
   a. United States
   b. United Kingdom
   c. China
   d. Germany

5. The chemical building blocks of DNA comprise:
   a. amino acids.
   b. adenine, guanine, cytosine, and thymine.
   c. RNA and simple proteins.
   d. complex proteins.

6. A gene’s DNA sequence or code is arranged in a string of nucleotide triplets known as:
   a. codons.
   b. bases.
   c. micro-RNA.
   d. alleles.
7. Before translation into proteins, DNA normally is:
   a. modified into alleles.
   b. transcribed into vectors.
   c. transcribed into messenger RNA.
   d. amplified via polymerase chain reaction.

8. Which of the following statements is not true about germ-line theory?
   a. It is based on the Weismann theory of heredity.
   b. Therapeutic alteration of germ cells cannot be passed on to a descendant.
   c. Inheritable characteristics are passed on by reproductive cells.
   d. Reproductive cells include ova and sperm.

9. A gene delivery approach in which the therapeutic gene is administered directly into the patient via injection or intravenously is known as an ______ approach.
   a. in vitro
   b. ex vivo
   c. in vivo
   d. intra vivo

10. What are the 2 main categories of therapeutic gene delivery?
    a. somatic and plasmid
    b. viral and nonviral
    c. plasmid and germ line
    d. gene-gun and ultrasound

11. Approximately how many gene therapy trials have been approved to date?
    a. 250
    b. 200
    c. 2100
    d. 22 000

12. The following is not among the commonly used viral vectors for gene therapy.
    a. adenovirus
    b. adeno-associated virus
    c. amur virus
    d. retrovirus

13. Lentiviruses and retroviruses both have a ______ genome.
    a. single-stranded RNA
    b. single-stranded DNA
    c. double-stranded RNA
    d. double-stranded DNA

14. A main difference between retroviral and lentiviral vectors is the fact that lentiviral virus vectors can infect ______ cells.
    a. only mitotically active
    b. both mitotically active and quiescent
    c. only somatic
    d. both somatic and germ-line

15. The adeno-associated virus genome consists of a:
    a. single-stranded RNA.
    b. single-stranded DNA.
    c. double-stranded RNA.
    d. double-stranded DNA.

16. Which of the following statements is true regarding the lytic pathway of a viral vector life cycle?
    a. The lytic pathway is the only pathway a viral vector can follow once it enters a target cell’s nucleus.
    b. The virus transmits DNA directly into a region of chromosome 19.
    c. Once attached and infecting the target cell, the virus makes copies.
    d. The lytic pathway does not develop in cells infected with a helper virus.
17. Which of the following might be a risk associated with use of viral vectors?
   1. potential infection of healthy cells
   2. preexisting, inherent immunity
   3. low yield and purity

   a. 1 and 2
   b. 1 and 3
   c. 2 and 3
   d. 1, 2, and 3

18. A gene that is overexpressed can:
   a. insert DNA in the wrong location.
   b. produce too much of the necessary protein.
   c. produce no protein.
   d. produce the incorrect protein.

19. Plasmids are most often found in:
   a. cells of patients who have bleeding disorders.
   b. liver cells.
   c. somatic cells.
   d. archaea and bacteria.

20. Plasmids are natural and cannot be made artificially.
   a. true
   b. false

21. Which of the following factors are a complication associated with use of a nonviral vector?
   1. limited transgenic transcription
   2. limited size of gene insert
   3. electrostatic repulsion at cell surface

   a. 1 and 2
   b. 1 and 3
   c. 2 and 3
   d. 1, 2, and 3

22. Which of the following is an example of a monogenic disorder?
   a. hepatitis C
   b. spina bifida
   c. cystic fibrosis
   d. retinitis pigmentosa

23. Clinical trials have shown that gene therapy can restore vision in some patients who have a congenital mutation to the point that the patients are declassified as legally blind.
   a. true
   b. false

24. In vitro experiments have been successful in using stem cells to naturally remove the third copy of chromosome 21 in individuals who have:
   a. spina bifida.
   b. muscular dystrophy.
   c. hydrocephalus.
   d. Down syndrome.

25. A covalent bond refers to:
   a. sharing of electrons between compounds.
   b. sharing of proteins between cells.
   c. bond splitting.
   d. iron-based transfer.

26. Ideally, characteristics of reporter genes should include:
   1. a fit into the selected vectors.
   2. inability to induce immune response.
   3. stability and lack of cytotoxicity.

   a. 1 and 2
   b. 1 and 3
   c. 2 and 3
   d. 1, 2, and 3

continued on next page
27. Exposure of cells to ultrasound waves increases:
   a. resolution of viral vectors.
   b. viral vector tropism.
   c. membrane permeability.
   d. transcription.

28. According to the article, computed tomography (CT) imaging has been used in human trials to:
   1. evaluate progression of pulmonary fibrosis following administration of an adenoviral gene vector.
   2. guide intratumoral injection of plasmid DNA for treating melanoma.
   3. steer gene-therapy injections into tumors of patients with metastatic kidney cancer.
   a. 1 and 2
   b. 1 and 3
   c. 2 and 3
   d. 1, 2, and 3

29. In gene therapy, _______ is a noninvasive tool for diagnosing the pathology, biology, and safety of vectors.
   a. ultrasonography
   b. CT
   c. magnetic resonance imaging
   d. positron emission tomography

30. In the United States, a manufacturer considering developing and selling a gene therapy product must first inform the:
   b. U.S. Food and Drug Administration.
   c. National Institutes of Health.
   d. Institutional Review Board.