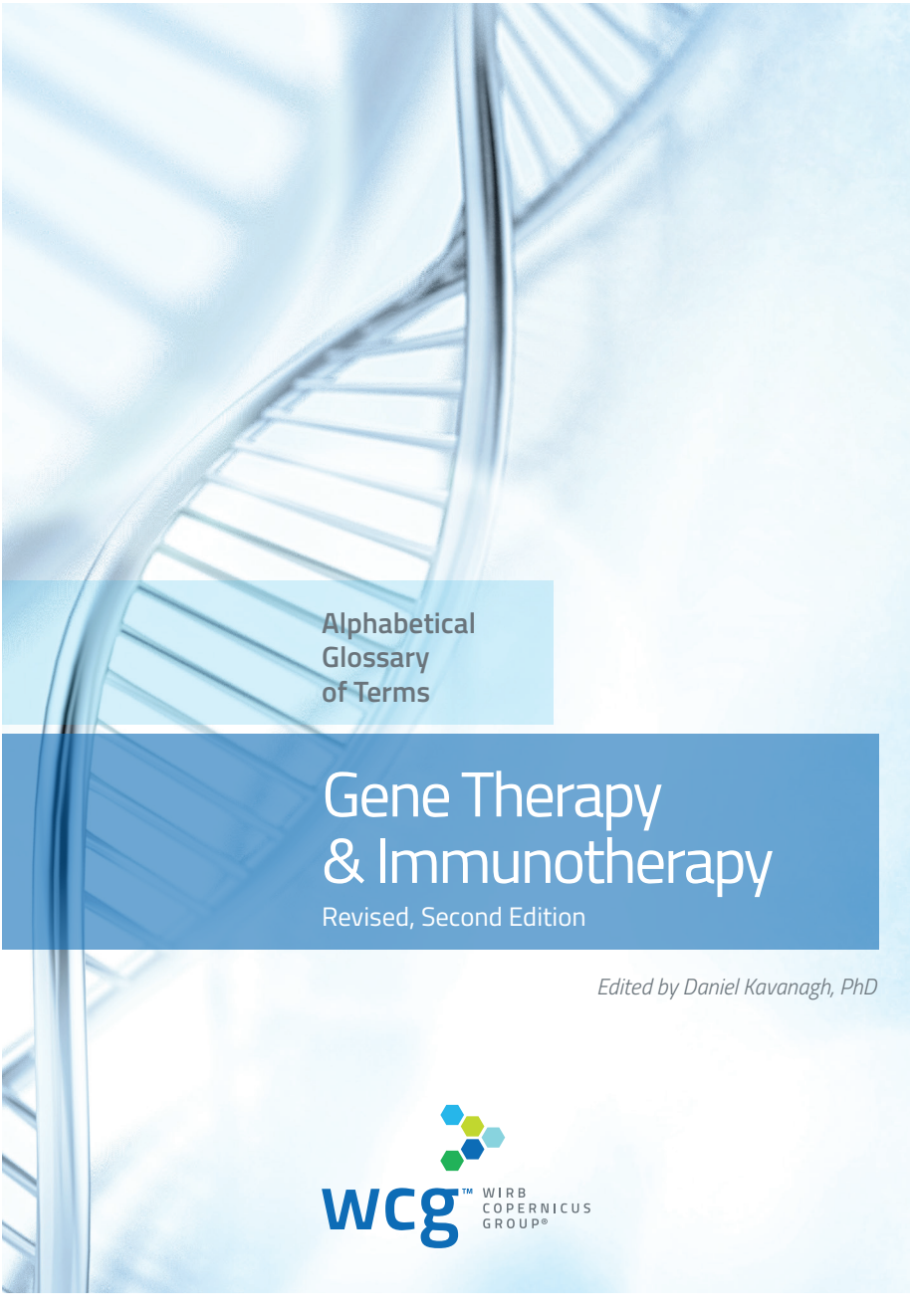

Alphabetical
Glossary
of Terms

Gene Therapy & Immunotherapy

Revised, Second Edition

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Introduction

The purpose of this glossary is to provide clarification of commonly used terms in gene therapy, gene editing, genetic-based precision medicine and immunotherapy. The terms in this glossary are listed alphabetically, with illustrations for key concepts. For more information on gene therapy and immunotherapy, read our whitepaper, **Gene Therapy and Genomic Editing: Understanding Basic Concepts**, available at www.wcgclinical.com/gene-therapy-genomic-editing-understanding-basic-concepts.

Allele: one of two or more DNA sequences occurring at a particular gene locus, or place on the chromosome.

Allogeneic ("Allo") Therapy: an approach to immunotherapy in which the therapy product is manufactured from donor cells of another person. (Compare with autologous).

Antigen: a protein target recognized by T cells or antibodies. "Self-antigens" are all of the normal protein structures found in healthy tissue; under normal conditions the immune system does not attack self-antigens. "Non-self-antigens" are protein structures originating in the environment or on infectious microbes; in the presence of "danger signals" the immune system will launch an attack against non-self-antigens. "Tumor antigens" are protein structures found on cancer cells that allow the immune system to distinguish cancer cells from healthy cells. Cancer immunotherapy in general is focused on promoting immune attack specifically against tumor antigens.

Antigen Receptor: a class of proteins, found on the surface of T cells or B cells, that recognizes specific protein targets (antigens), notably those found on tumors or microbes. The antigen receptor on T cells is called the T Cell Receptor (TCR). The antigen receptor on B cells is a special form of antibody. Engineered Chimeric Antigen Receptors can incorporate components of a TCR and antibodies, as well as functional portions of other proteins.

Autologous Therapy: an approach to immunotherapy in which the therapy product is manufactured using a patient's own cells as the source material. (Compare with allogeneic).

Autosomal Mutation: a mutation occurring among one of the 22 pairs of chromosomes that are not sex chromosomes.

Antibodies: defensive proteins produced by B cells in the human body that are carried by circulatory systems to attack germs and cancer cells. (See also monoclonal antibodies).

B Cell: a kind of lymphocyte that produces antibodies. If we imagine that the immune system is the body's military defense force, B cells are like bomber aircraft that attack targets from a distance.

BSL (Biosafety Level): the level of containment for research with recombinant and infectious materials, including physical containment based on facilities design and engineering controls, as well as special practices and training. Biosafety levels range from the lowest level of containment (BSL-1) to the highest level containment (BSL-4). Specific requirements for biosafety levels are specified in the *NIH Guidelines* (for research subject to the *NIH Guidelines*), while recommendations for biosafety levels are specified in the Centers for Disease Control and Prevention (CDC) publication *Biosafety in Microbiological and Biomedical Laboratories*. Biosafety levels for human gene transfer studies subject to the *NIH Guidelines* must be confirmed by the local Institutional Biosafety Committee, and are typically assigned as BSL-1 or BSL-2.

CAR (Chimeric Antigen Receptor)-T Cell Therapy: a cutting-edge form of immunotherapy using CD4 and CD8 T cells to kill tumors. "Chimeric" comes from the Greek "chimera" — a mythical animal made up of parts of naturally occurring animals (often a lion, a goat and a snake). Genetic engineers use the word chimeric to describe a protein artificially designed from parts of other proteins. A "chimeric antigen receptor" is an antigen receptor engineered to have functional properties of multiple, naturally occurring proteins, frequently B cell receptors (antibodies) and T cell receptors. When T cells are armed with a chimeric antigen receptor, they become CAR-T cells. CARs may be designed to recognize any of

several target proteins ("antigens") found on tumors. Some of the first CAR therapies to be tested in humans were directed against the CD19 B cell tumor antigen. CAR therapies targeting a diverse array of other tumor antigens are under development.

CD Number (Cluster of Differentiation Number): a number assigned to protein "markers" found on cells, especially cells of the immune system. CD numbers are useful for identifying specific types of immune cells. There are currently more than 300 CD designations assigned to unique proteins.

CD4 T Cell: a kind of lymphocyte that recognizes tumors and microbes via a T cell receptor. If we imagine that the immune system is the body's defense force, CD4 T cells are the "commanding officers." Also called "helper T cells," they usually do not attack targets directly, but they do detect danger and direct the immune response.

CD8 T Cells: another kind of lymphocyte that recognizes tumors and infected cells via a T cell receptor. If we imagine that the immune system is the body's defense force, CD8 T cells are some of the most important foot soldiers. Also called "killer T cells," they grab onto cancer cells or infected cells and destroy them.

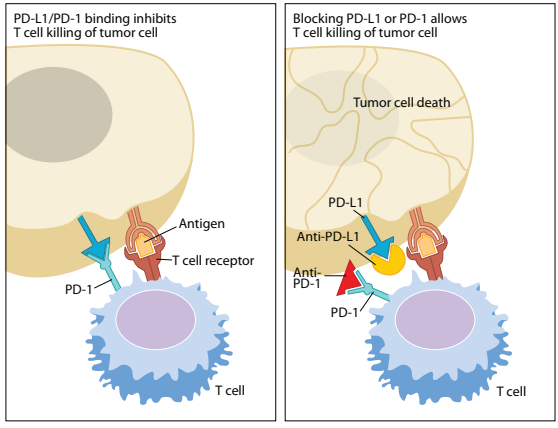
CD19: a protein expressed on the surface of B cells, and on the surface of some cancer cells. Several first-generation CAR-T cell treatments use CARs that recognize CD19 and thus direct immune attack against the cancer cells.

Checkpoint Inhibition: any of several therapeutic strategies that enhance the immune response by blocking immune "checkpoints". The term checkpoint refers to a variety of molecular switches that tend to decrease the immune response. Two of the most important checkpoint proteins targeted by current therapies are known as "PD-1" and "CTLA-4." Most checkpoint inhibitors in current use are

monoclonal antibodies, and many experimental gene transfer approaches are incorporating some form of checkpoint inhibition to maximize anti-tumor effects. Examples of commercial monoclonal antibodies targeting PD-1 include Opdivo™ and Keytruda™; examples targeting CTLA-4 include Yervoy™ and Tremelimumab™.

Checkpoint Inhibition

T cells sometimes express inhibitory molecules that act as "brakes" or "checkpoints" on the immune response--in the figure shown, the inhibitory molecule is PD-1. In a healthy person, checkpoints prevent immune hyperactivation, but cancer cells frequently express a "ligand" (in the figure shown PD-L1) that allows cancer to "put its foot on the brakes" of the immune response. *Checkpoint inhibitors* (in the figure, anti-PD-1, and anti-PD-L1) force cancer to "take its foot off the brakes" and allow the immune response to accelerate and destroy the tumor.



Chromosomes: thread-like structures located inside the nucleus of human cells containing almost the entire DNA of the cell. Human chromosomes are highly organized structures composed of DNA and proteins. Almost all human cells contain 23 matched pairs of chromosomes. The DNA content includes "coding" DNA (DNA encoding genes), and "noncoding" DNA (all of the DNA not encoding genes). The majority of human chromosomal DNA is noncoding.

CRISPRs (Clustered Regularly-Interspaced Short Palindromic Repeats): originally natural features of bacterial DNA that bacteria use to protect themselves from infection – CRISPR sequences in bacterial DNA provide signals that guide nuclease proteins such as CAS9 (CRISPR-Associated Protein 9) to cleanse genetic material that may be harmful to the bacterium. In modern biotechnology, CRISPRs are used as the basis for an extremely flexible and accurate form of gene editing.

CRISPR-CAS9: one of a number of naturally occurring systems that bacteria use to protect their own DNA and destroy viruses that infect them. Molecular biologists have adapted this system to allow gene editing of DNA in live human cells. Along with Zinc-Finger Nucleases and TALENs, CRISPR-CAS9 is being used to develop gene editing technology.

CRS (Cytokine Release Syndrome): an effect that occurs in some subjects receiving an immunotherapy such as CAR-T cell therapy. Cytokines released by CAR-T cells can promote tumor destruction, which is beneficial, but in excessive amounts they can also cause serious, or even life-threatening, effects due to immune system over-activation.

Cytokine: a broad class of small proteins produced by cells of the immune system that act as signals to accelerate or suppress immune responses.

Dendritic Cells: leukocytes that act as sentries of the immune system. They recognize danger signals from cancer and infection, and prime the lymphocyte cells to attack.

Dendritic Cell Vaccine: a form of immunotherapy based on ex vivo production of dendritic cells. For experimental treatment of cancer, tumor antigens are delivered to dendritic cells, often by gene transfer, and the dendritic cells are reinfused into the subject, where they can prime anti-cancer T cell responses.

DNA (Deoxyribonucleic Acid): the long-term storage medium for genetic information that defines the form and function of living cells and some viruses. Many human gene transfer studies involve delivery of recombinant DNA molecules and/or DNA viral vectors. DNA molecules are made of a chain of nucleotides (Adenine, Guanine, Cytosine, or Thymine; represented by A, G, C, and T in genetic notation). The most common form of DNA is double-stranded—composed of two antiparallel strands that form a double helix and where nucleotides from the each strand form "base pairs" with the corresponding nucleotides on the other strand. (See RNA).

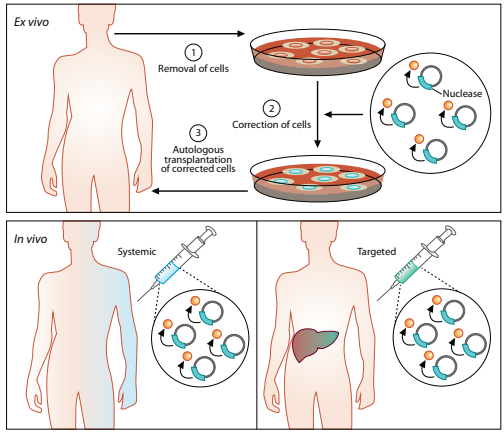
Dominant Mutation: with respect to human disease, a dominant mutation is a polymorphism or change in a person's DNA that results in disease even if present on only one of two chromosomal copies. (Compare with recessive).

Electroporation: a means of causing cells to take up DNA by very brief exposure to an electric current. Electroporation usually is applied ex vivo, but there are some special devices that are used to perform electroporation on live cells in the intact skin or muscle of subjects. One commonly-used small hand-held device contains a syringe needle and four electrodes recessed in the main shaft. The clinician holds the applicator against the patient's upper arm and presses a button. The device then deploys the needle and electrodes at a fixed depth and location and delivers the DNA and the electrical current. Compared to gene transfer by viral vectors, electroporation allows only limited quantities of gene transfer to a limited selection of anatomical sites.

Ex Vivo: of or pertaining to procedures performed on cells that have been removed from the body. Current approaches to CAR-T cell treatment are ex vivo: T cells are first removed from the body, subjected to gene transfer, and then put back into the patient's body. (Compare with in vivo).

Ex vivo and in vivo gene transfer

In ex vivo therapy, cells are removed from a person and genetically modified in a test tube or petri dish in the lab. In the figure shown, the cells are removed from a person and subjected to gene editing, and then put back into the same donor. This is an example of autologous ex vivo gene therapy. Other approaches to ex vivo therapy can include allogeneic therapy (where the modified cells come from a different person other than the recipient) and/or other forms of human gene transfer rather than gene editing. In vivo gene transfer occurs when genetic material is delivered directly to cells in a person's body- for example by electroporation, with a gene gun, or with a viral vector.

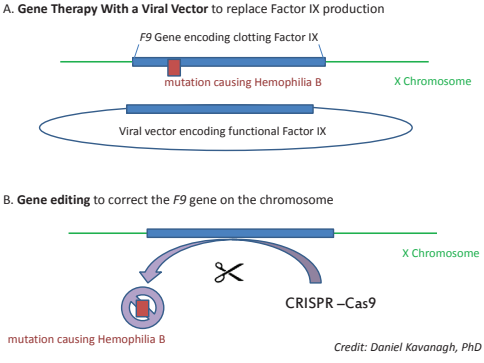


Gene: a gene is a portion of the DNA on a chromosome that encodes a particular protein. Human chromosomes contain around 20,000 genes.

Gene Editing: the process of "re-writing" portions of a subject's DNA, frequently to correct a disease-associated defect or to create new functional codes in the DNA. This term is widely used in reference to genetic engineering mediated by CRISPR-CAS9, TALENs, and Zinc-Finger Nucleases. "Gene Editing" sometime refers narrowly to editing of coding DNA within a gene. Depending on whether DNA sequence is added, deleted, or altered, the final edited gene may be longer, shorter, or the same length as the original. (Compare with Genome Editing).

Gene Gun: a device that is used for needle-free delivery of DNA to skin. DNA is coated onto microscopic gold "bullets" prior to being "fired" into the skin. This method is sometimes known as Particle-Mediated Epidermal Delivery (PMED). In contrast to electroporation, no electrical field is applied to the injection site. Compared to gene transfer by viral vectors, gene guns allow only limited quantities of gene transfer to a limited selection of anatomical sites.

Gene Therapy: any of several techniques to treat or prevent disease by altering the DNA content of some of a person's cells. Traditional approaches to gene therapy generally involve using a vector to introduce DNA into target cells to replace the function of a nonfunctional gene in a subject with a genetic disease. These older approaches do not alter the sequence of the dysfunctional gene in the subject's chromosome. Some authorities limit the term "gene therapy" to interventions intended to treat genetic disease. Others use "gene therapy" to refer to a variety of techniques involving gene transfer, including CAR-T technology. The consensus definition of "gene therapy" is likely to evolve over time. Some people draw a distinction between gene therapy, which introduces DNA into a cell without specifically targeting a particular site on a chromosome, and gene editing or genome editing, which involve specific modifications to targeted sites on a chromosome.



Gene Therapy and Gene Editing

Hemophilia B is caused by a mutation in the F9 gene, encoding clotting Factor IX, on the X chromosome. If a boy inherits an X chromosome with a particular defect (mutation) in the DNA sequence of the F9 gene, he will suffer from Hemophilia B due to an inability to produce functional Factor IX. Two genetic approaches to treat Hemophilia B are shown. **A. Gene Therapy With a Viral Vector:** recombinant DNA encoding a functional F9 gene is introduced into some of the subject's cells (for example, liver cells). The gene transfer leads to production of functional Factor IX without altering the DNA sequence of the chromosome. **B. Gene editing:** CRISPR-Cas9 system is used to edit the DNA on the chromosome– the mutation is corrected in situ and the resulting "edited" chromosome encodes fully functional clotting factor.

Genome: the total DNA information within a cell, including the coding DNA within the genes, and all noncoding DNA outside of the genes. The vast majority of the human genome is noncoding.

Genome Editing: the process of "re-writing" small portions of a subject's DNA, frequently to correct a disease-associated defect or to create new functional codes in the DNA. This term is widely used in reference to genetic engineering mediated by CRISPR-CAS9, TALENs, and Zinc-Finger Nucleases. "Genome Editing" is a broad term encompassing Gene Editing (editing of coding DNA within genes) as well as the editing of noncoding DNA outside of genes. Genome Editing may result in addition of DNA, deletion of DNA, or alteration of a DNA sequence without changing length.

Genetic Disease or Disorder: a condition caused by factors encoded in a person's DNA. A genetic disorder can be attributed to one or more differences in the DNA sequence of the affected person compared to the DNA sequence found in unaffected persons. Genetic disorders may be monogenic or polygenic, dominant or recessive, and autosomal or sex-linked. Most genetic disorders qualify as rare diseases, with an affected population in the USA ranging from tens of thousands of persons (cystic fibrosis, sickle cell anemia) to ultra-rare conditions only observed in single individuals. As scientists discover the relationship between a particular genetic sequence and the corresponding medical condition, the disease may become a candidate for experimental gene therapy.

Genetic Polymorphism: a difference in the DNA sequence at a particular location in a person's genome compared to a reference sequence. Each form of a polymorphism at a particular genetic location represents a unique allele. A genetic polymorphism can represent a switch in the genetic code from one nucleotide letter to another, or an addition, or a deletion compared to the reference sequence. Some polymorphisms are extremely rare, and some are extremely common, being present in up to half of the human population. Because humans are genetically, racially, and ethnically diverse, there is no unique "normal" reference sequence for the human genome.

Germline: with respect to human gene transfer, "germline" is a term used to describe genetic material contained in sperm and ova, or the cells that produce them. Genetic changes in an embryo may affect the germline if the embryo becomes an adult. Germline genetic changes can be passed on to offspring and succeeding generations. Deliberate germline gene transfer in humans is forbidden in the United States using federal funds and in most countries around the world. Because the risk of accidental germline modification exists in human gene transfer, subjects undergoing gene transfer are advised not to become pregnant or to make anyone else pregnant.

Hemophilia: an inherited blood disorder characterized by inadequate blood clotting and excessive bleeding. Of the two major forms, Hemophilia A and Hemophilia B, both are X-linked recessive and mostly affect males.

Heterozygous: having two distinct DNA sequences (alleles) at a particular gene or genetic locus on each of two paired chromosomes. (Compare to Homozygous).

Homozygous: having identical DNA sequences (alleles) at a particular gene or genetic locus on each of two paired chromosomes. (Compare to Heterozygous).

HGT (Human Gene Transfer): a category of clinical research requiring special oversight and review under the *NIH Guidelines*. The *NIH Guidelines* define HGT as the deliberate transfer into human research participants of recombinant or synthetic nucleic acid molecules or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, with the exception of certain agents based on size or function. HGT research subject to the *NIH Guidelines* must be approved by an IBC prior to enrollment of participants, and, with the exception of certain microbial vaccine studies, must also be registered with the NIH Office of Science Policy. Importantly, HGT includes traditional gene therapy as well as many applications in the areas of immunotherapy, oncolytics, vaccines, and regenerative medicine.

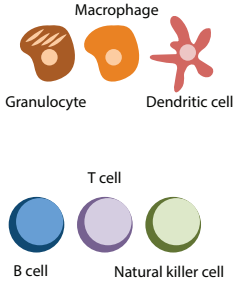
HIV (Human Immunodeficiency Virus): a member of the *Lentivirus* genus of the Retrovirus family, and the causative agent of Acquired Immune Deficiency Syndrome (AIDS). HIV causes AIDS by infecting and destroying a victim's CD4 T cells. Because this virus is so efficient at infecting certain kinds of cells, researchers have used this virus as the basis to construct and engineer lentiviral vectors for gene transfer.

IBC (Institutional Biosafety Committee): a local safety review committee that is registered on behalf of a research site using recombinant or synthetic nucleic acid molecules. The *NIH Guidelines* mandate IBC review of NIH-funded research involving recombinant or synthetic DNA or RNA. IBC review is required at each dosing site for any human gene transfer study that is supported by NIH funding, or that uses a product developed with NIH funding. IBC review of human gene transfer studies is also required at any dosing site associated with an institution that receives NIH funding for research involving recombinant and synthetic DNA or RNA.

Immune System: the body's primary defense against infection and cancer, made up of different kinds of white blood cells or leukocytes. Some of the most important white blood cells are B cells, CD4 T cells, CD8 T cells, Natural Killer (NK) Cells, and Dendritic Cells.

Cells of the Immune System

The cells of the immune system are known as "white blood cells" or "leukocytes". White blood cells include lymphocytes: B cells, T cells (including CD4 and CD8 T cells), and natural killer cells. Another group of white blood cells other than lymphocytes is comprised of "myeloid cells". For current immunotherapy applications, the most important myeloid cells are dendritic cells.



Myeloid cells

Patrol where infections might arise
Act quickly to kill infections
Alert lymphocytes of danger

Lymphoid cells

Move around the body in the lymph
Act more slowly to kill more specifically

Immunotherapy: any type of medical intervention that harnesses the immune system to treat disease. Some but not all immunotherapy involves human gene transfer. The term immunotherapy is broadly used to refer to treatments designed to alter or exploit immune functions, such as those involving monoclonal antibodies, checkpoint inhibitors, chimeric antigen receptors, or oncolytic viruses.

In Vivo: with reference to gene transfer, in vivo refers to gene transfer that is delivered directly to cells in the subject's body, such as via a vector or electroporation. (Compare with ex vivo).

Inborn Errors of Metabolism: any of several distinct genetic diseases that interfere with the proper processing of food and nutrients, resulting in a deficit of essential biochemical components, and an excess of toxic bioproducts. These diseases are often traditionally managed with specialized and restrictive diets. Several experimental approaches aim to correct inborn errors of metabolism by targeting gene therapy to the liver or gut.

Inherited Blood Disorder: any of several distinct genetic diseases, including hemophilia, sickle-cell anemia, and beta-thalassemia, affecting important functions of the blood. Because blood is a liquid tissue that flows throughout the body and is rapidly replaced, it is relatively easier to develop genetic therapies for inherited blood disorders than for disorders that affect solid organs. Inherited blood disorders are some of the primary indications for experimental gene therapy clinical trials.

Insertional Mutagenesis: a change in the chromosomal DNA of a cell due to insertion of foreign DNA. Mutagenesis is any process that introduces changes (mutations) in DNA. Accidental insertional mutagenesis is a theoretical risk of

any type of gene therapy, but the practical risk is greatest for vectors that are designed to incorporate recombinant DNA into a subject's DNA (e.g., gamma retroviral and lentiviral vectors).

Leukemia: any of several types of cancer that affect the blood and bone marrow, in particular those caused by abnormal growth of white blood cells (also known as leukocytes). Even though the healthy immune system is an important defense against cancer, immune cells can also turn into cancer cells if they suffer damage to their DNA. Leukemias are among the most promising targets of experimental immunotherapy for cancer.

Leukocytes: white blood cells. Leukocytes comprise all the major cell types of the human immune system.

Lymphocyte: a type of white blood cell including B cells, T cells, and natural killer cells.

Lymphoma: any of several types of cancer caused by the uncontrolled growth of lymphocytes. Almost all lymphomas are either T cell lymphomas or B cell lymphomas.

Monoclonal Antibody: an antibody produced artificially by B cells specially grown in the lab. In contrast to mixed "polyclonal" antibodies in blood that recognize a very diverse repertoire of targets, each monoclonal antibody recognizes only one protein target, which makes them useful in drug development as antitumor agents or as checkpoint inhibitors.

Monogenic Disease: a genetic disease primarily caused by a polymorphism or mutation occurring at a single location in the affected person's DNA on one or both chromosomes. Compared to polygenic diseases, it is much easier to

understand the causal relationship between the DNA sequence and the disease state, and thus it is generally easier to design experimental gene therapy to treat a monogenic disease. Although monogenic diseases can be primarily attributed to individual genetic differences, all medical conditions can be affected by multiple genetic factors, and persons affected with monogenic diseases can present with a diversity of severities and symptoms.

Mutation: a "change" at a specific location in a person's DNA sequence relative to a reference sequence. The term mutation is often used as a synonym for "polymorphism" but it is important to remember that there is no unique "normal" human reference sequence. Disease-associated polymorphisms are often referred to as "mutations" by default. Some mutations are inherited from a subject's parents, and some are spontaneous or somatic mutations that occur at any point in life after conception.

Nanoparticle: an artificial microscopic package engineered to deliver cargo, such as drugs or DNA, to cells in the body of a subject. In many ways a nanoparticle resembles a small, simplified viral vector.

NIH Guidelines: also known as *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*, an extensive and detailed guidance document published by the National Institutes of Health (NIH) describing recommended safety practices and containment procedures for basic and clinical research involving recombinant or synthetic DNA or RNA. All institutions receiving NIH funding for research involving recombinant or synthetic nucleic acid molecules must comply with the *NIH Guidelines* as a condition of funding. Furthermore human gene transfer studies, even at institutions without NIH funding, are also subject to the *NIH Guidelines* if the study is NIH-supported, or if the investigational product was developed with NIH funds, or if the sponsor receives NIH funding for any research involving recombinant or synthetic DNA or RNA.

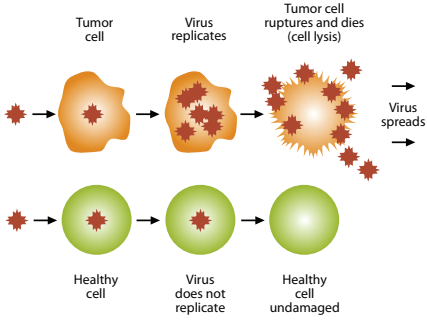
Changes to the *Guidelines* went into effect in April 2016—notably these changes altered the previous process for registration of human gene transfer studies with the NIH, and for review of such studies by the Recombinant DNA Advisory Committee (RAC).

NK Cells (Natural Killer Cells): a type of white blood cell with a similar function to the CD8 Killer T cell, except that NK cells do not recognize targets via a specific antigen receptor. Several experimental immunotherapies rely on autologous or allogeneic NK cells to kill tumors.

Oncolytic Virus: a cancer-killing virus. Recombinant oncolytic viruses are engineered to preferentially infect or reproduce in cancer cells rather than healthy normal cells. When an oncolytic virus infects a cancer cell, it may be programmed to initiate a variety of anti-cancer activities, such as directly killing the target cell, making the cell sensitive to chemotherapy, or producing cytokines that prime and enhance the immune response. Although only some oncolytic viruses are engineered to engage the immune system, oncolytic viruses are frequently categorized as "immunotherapy" in commercial literature.

An oncolytic virus selectively destroys cancer cells

Cancer cells are always abnormal compared to healthy cells. Genetic engineers can exploit these abnormalities to create viruses that reproduce much better inside cancer cells than in normal cells. When an oncolytic virus is injected at the site of a tumor, it rapidly reproduces inside cancer cells, making them vulnerable to direct destruction and/or immune attack. Because the engineered virus replicates poorly in normal cells, healthy tissue is left undamaged.



OSP (Office of Science Policy): a staff office within the Office of the Director of the NIH that advises the Director on issues involving biosafety, biosecurity, human subjects protections, and the organization and management of the NIH. With the exception of certain vaccine studies, all human gene transfer studies subject to the *NIH Guidelines* must be registered with the OSP. At the time of registration of a new study by the initial dosing site(s), the NIH Director makes a determination whether or not the study will be referred to the RAC. This determination is informed by advisory letters provided by oversight bodies (the IBC and the IRB) representing the initial dosing site(s), indicating whether or not the study would benefit from RAC review.

Polygenic Disease: a genetic disease caused by the combined effects of polymorphisms or mutations occurring at more than one location in the affected person's chromosomes. Compared to monogenic diseases, it is much more difficult to determine the genetic cause of polygenic diseases, and also much more challenging to design experimental gene therapies.

Plasmid: a type of circular ring of DNA that naturally occurs in bacteria and also has been extensively used for artificial manipulation of recombinant DNA in the lab. In the clinic, direct injection of plasmid DNA is one of the simplest forms of gene transfer.

RAC (Recombinant DNA Advisory Committee): a federal advisory committee that provides recommendations to the NIH Director on matters related to basic and clinical research involving recombinant or synthetic nucleic acid molecules. Under the revised *NIH Guidelines* as amended in April 2016, the NIH Director selects certain human gene transfer studies for review by the RAC at the time that the initial site registers the study with the Office of Science Policy. The decision of the NIH director is partly informed by required letters from the oversight bodies (IRB and IBC) involved in review of the protocol at the

initial dosing site(s). If an oversight body determines that the protocol would significantly benefit from public RAC review and also meets review criteria relating to novelty, safety, or potential toxicity, then the principal investigator must submit additional documentation by a specified deadline in order to be reviewed at the next RAC meeting.

Rare Disease: in the United States, rare diseases are defined under the Orphan Drug Act of 1983 as those conditions affecting fewer than 200,000 people. There are up to 7,000 different identified conditions that qualify as rare diseases. While some rare diseases are caused by infection or other factors, the majority of rare diseases are believed to be genetic diseases. Many rare diseases are potential indications for gene therapy.

Recessive Mutation: with respect to human disease, a recessive mutation is a polymorphism or change in a person's DNA that results in disease only in the absence of a normal copy of the sequence on a paired chromosome. (Compare with dominant). A person who has a disease-associated mutation on one chromosome, but has no symptoms because of a normal genetic sequence on a paired chromosome, is known as a "carrier" for that disease.

Recombinant and Synthetic Nucleic Acid Molecules: in the context of the *NIH Guidelines*, recombinant and synthetic nucleic acid molecules are defined as: i) recombinant nucleic acid molecules: DNA or RNA molecules that are constructed by joining nucleic acid molecules and that can replicate in a living cell; or ii) synthetic nucleic acid molecules: DNA or RNA molecules that are produced chemically or by other means and can form certain molecular structures with naturally occurring DNA or RNA; or iii) molecules that result from the replication of those described in (i) or (ii) above.

Risk Group (RG): under the *NIH Guidelines*, one of four categories into which infectious agents or gene transfer vectors are categorized according to their potential relative pathogenicity for healthy adult humans. Risk Group 1 (RG1) agents are not associated with disease in healthy adult humans. Risk Group 2 (RG2) agents are associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available. Risk Group 3 (RG3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available. Risk Group 4 (RG4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

RNA (Ribonucleic Acid): a type of nucleic acid molecule similar to DNA and serving various purposes in living cells and some viruses. Notably messenger RNA (mRNA) serves as a short-lived medium for information encoded in the chromosomal DNA of a cell. In contrast to DNA, the most common form of RNA is single-stranded, and does not persist in stable base-paired double-stranded formats. Many human gene transfer studies involve delivery of recombinant mRNA molecules and/or RNA viral vectors.

RNA Interference (RNAi): any of several natural or synthetic mechanisms for controlling gene expression that rely on interactions between RNA molecules. Technology for RNAi therapy includes using synthetic short interfering RNA (siRNA) molecules to block expression of harmful genes. The effects of siRNA therapy are designed to be much more transient than those of most gene transfer technologies, and siRNA therapies are generally intended to require systematic re-dosing to treat chronic disease. Notably, due to the short length of the molecules, siRNA therapies are generally not classified as human gene transfer under the *NIH Guidelines* and thus do not require IBC or RAC review. Nevertheless some siRNA-based approaches to treat disease are referred to as "gene therapy" in popular media.

Sex-Linked Disease: a genetic disease associated with the sex (X or Y) chromosomes. Almost all human sex-linked diseases are X-linked diseases. (Compare with autosomal).

Shedding: the spreading or dispersal of a gene therapy vector from the person who is receiving treatment to the broader environment. Design of clinical trials using gene therapy vectors must take into account the possibility of shedding, and must include plans to mitigate risks to household contacts and the general public. In general the risk of shedding is greatest when a fully replicating viral vector is used, and/or when very high doses of a non-replicating vector are used.

Sickle Cell Anemia: an inherited blood disorder characterized by abnormal function of red blood cells. Sickle cell anemia is autosomal recessive, causing severe disease in homozygous individuals but having little or no deleterious effect in heterozygous carriers, who are said to have "sickle cell trait". The polymorphisms that cause sickle cell disease in homozygous individuals have been shown to protect against malaria in heterozygous individuals, and sickle cell disease is most common in ethnic groups originating from regions where malaria is endemic. Sickle cell anemia is a potential target for gene therapy or gene editing approaches targeted to blood stem cells.

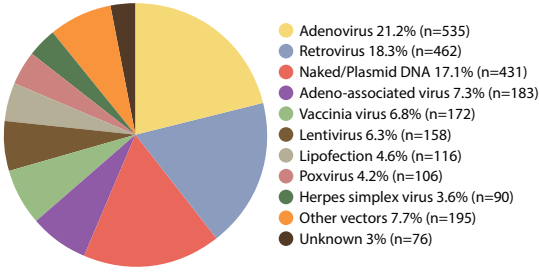
TALENs (Transcription Activator-Like Effector Nucleases): proteins that can be engineered to make specific changes to cellular DNA. As with CRISPR-CAS9 and Zinc-Finger Nucleases, TALENS are used to develop gene editing technology.

Vaccine: a treatment designed to prime an immune response against one or more antigens. Cancer vaccines are specifically designed to drive immune responses against cancer cells. For example, some cancer vaccines utilize viral vectors expressing tumor antigens. Some are based on tumor cells that have been given DNA encoding immune-activating cytokines. Others utilize plasmid DNA encoding tumor antigens and/or cytokines, delivered with a gene gun or by electroporation.

Vector: in genetic engineering, a microbe—usually a virus—that has been designed specifically to deliver DNA or RNA for the purpose of human gene transfer (HGT). To make HGT work, scientists must get recombinant DNA into the subject's cells (and to the right place inside the subject's cells). Sometimes this can be accomplished with "naked" DNA, but usually the DNA needs to be packaged in a vector. To reduce the risk of side effects, or of accidentally infecting other people, viral vectors are usually non-replicating vectors. This means that the vectors cannot reproduce themselves inside the human body—each vector particle provides gene transfer to only one target cell in the patient. In some cases, there are potential benefits to having the vector able to reproduce itself, in which case live/replicating vectors may be used. Potential advantages of replicating vectors include longer-lasting gene delivery, a stronger or more durable immune response, and enhanced destruction of tumors. Potential risks of using replicating vectors include the possibility of the vector shedding, and the risk of causing "collateral damage" to healthy tissue. The following table shows some of the most common vectors used in gene therapy.

Vector Type	Genetic Material	Capacity	Chromosomal Integration	Notes
Adenoviral	DNA	++++	No	efficient short term expression
Vaccinia	DNA	++++	No	derived from smallpox vaccine, primes strong immune responses
HSV	DNA	++++	No	deliver genes to neural tissues; basis of some oncolytic virus
AAV	DNA	+	Rare	short term expression with minimal immune response
Retroviral	RNA	++	Yes	persistent gene transfer in dividing cells
Lentiviral	RNA	++	Yes	persistent gene transfer in dividing and non-dividing cells

Vectors Used in Gene Therapy Clinical Trials



 www.wiley.co.uk/genmed/clinical

X-linked disease: a genetic disease associated with a mutation or polymorphism on the X chromosome. Most X-linked diseases are recessive, which means that they result in symptomatic disease almost exclusively in males. Examples of relatively common X-linked diseases include red-green colorblindness and hemophilia. X-linked diseases occur in females only in the relatively rare cases where a woman is homozygous for the mutation or where the X-linked condition is dominant.

Zinc Finger Nuclease: a type of protein that can be engineered to make specific changes to cellular DNA. Along with CRISPR-CAS9 and TALENs, Zinc-Finger Nucleases are used to develop gene editing technology.

About WCG Biosafety™

WCG Biosafety (a division of WIRB-Copernicus Group) provides IBC management and review services to partners around the world. Since 2001, our IBC Services division has managed externally-administered, NIH-registered IBCs on behalf of over 600 institutions within the USA and abroad. Our expertise derives from decades of combined experience in gene therapy and molecular biology in academic and commercial research.

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