The purpose of this glossary is to provide clarification of the most commonly used terms related to cancer research and treatment using gene therapy and immunotherapy. The terms in this glossary are listed alphabetically, with illustrations where appropriate.

**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 molecular target recognized by T cells or antibodies. “Self-antigens” are all of the normal molecular structures found in healthy tissue; under normal conditions the immune system does not attack self-antigens. “Non-self-antigens” are molecular 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 molecular 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 molecular 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.

**Appendix M**: a section of the NIH Guidelines that specifically addresses points to consider in the design and submission of human gene transfer trials, including the standards and procedures to which investigators (and sponsors) must adhere.

**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).

**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 infectious materials recommended by the Centers for Disease Control (CDC) according to the potential hazard posed to laboratory workers and the community. Biosafety levels range from least hazardous (BSL-1) to most hazardous (BSL-4). Current gene therapy technology is almost always conducted at BSL-2.

**CAR (Chimeric Antigen Receptor)-T Cell Therapy**: a cutting-edge form of immunotherapy using 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 **CD8** killer T cells are armed with a chimeric antigen receptor, they become **CAR-T Cells**: artificial tumor-killing machines. CARs may be designed to recognize any of several target molecules (“antigens”) found on tumors. Some of the first CAR therapies to be tested in humans are directed against the **CD19** lymphoma antigen.

**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 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. This is the main cell type used for CAR-T Cell Therapy.

**CD19:** a protein expressed on the surface of B cells, including the abnormal cells of many B cell lymphomas. Several first-generation CAR-T cell treatments use CARs that recognize CD19 and thus direct immune attack against the lymphoma 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™.
**Chromosomes:** thread-like structures located inside the nucleus of human cells and containing almost the entire DNA of the cell.

**CRISPRs (Clustered Regularly-Interspaced Short Palindromic Repeats):** natural features of bacterial DNA that bacteria use to protect themselves from infection. CRISPR sequences in bacterial DNA provide signals that guide editing proteins such as CAS9 (CRISPR-Associated Protein 9) to edit genetic material that may be harmful to the bacterium.

**CRISPR-CAS9:** one of a number of naturally occurring systems that bacteria use to edit 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.

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

**CRS (Cytokine Release Syndrome):** an effect that occurs in some subjects receiving 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.

**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. Most human gene transfer studies involve delivery of recombinant DNA. (See RNA).

**Electroporation:** a means of causing cells to take up DNA by very brief exposure to high voltage. 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 field. 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*).

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**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 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.

**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.

**Germline:** with respect to human gene transfer, “germline” is a term used to describe genetic material contained in sperm and ova. 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.

**HGT (Human Gene Transfer):** as defined by NIH, “the deliberate transfer into human research participants of either recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or synthetic nucleic acid molecules.” DNA and RNA form the alphabet of the genetic code that tells living things how to make proteins and organize themselves.

**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 convened at the site of research involving recombinant or synthetic nucleic acids. The NIH Guidelines mandate IBC review of NIH-funded research involving recombinant and synthetic DNA or RNA, including human gene transfer projects.

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.

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).
**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 (i.e., retroviral and lentiviral vectors).

**Lentivirus vector:** a vector derived from a lentivirus (usually HIV) particularly useful for delivering DNA to non-dividing cells. Because it is a form of retrovirus, lentiviruses insert DNA sequences into chromosomes of infected cells.

**Leukemia:** any of several types of cancer 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 leukemia caused by the uncontrolled growth of lymphocytes. Almost all lymphomas are either T cell lymphomas or B cells 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 molecular target, which makes them useful in drug development as antitumor agents or as checkpoint inhibitors.
**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.

**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.

**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.

**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.
**Plasmid:** circular rings of DNA that naturally occur in bacteria. Historically plasmids have also been essential tools for manipulating recombinant DNA in the lab. In the clinic, direct injection of plasmid DNA is the simplest form 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. All clinical research projects involving human gene transfer must be registered with the RAC; each project requires submission of Appendix M.

**Recombinant DNA or RNA:** DNA or RNA that was produced by manipulating or recombining DNA or RNA fragments, usually with reproduction steps occurring in plasmids that grow in bacteria. Historically the vast majority of genetic engineering has involved recombinant DNA. (Compare with synthetic).

**Retroviral vector:** a vector derived from a retrovirus. Retroviruses are a large family of viruses that reproduce by inserting their own genetic sequence into the host DNA. From the standpoint of human infectious disease, the most important retrovirus is HIV, a lentivirus. Lentiviruses are a natural subgroup of the retrovirus family, but in gene therapy the term “retroviral vector” is often (sometimes confusingly) used to refer to other vectors (e.g., gamma-retroviral vectors) in contradistinction to “lentiviral vectors” derived from HIV.

**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.
**Synthetic DNA or RNA:** artificial DNA or RNA that is manufactured by a chemical process in the absence of bacteria or other microbes. Some of the newest gene transfer technologies involve synthetic DNA. Synthetic DNA may be chemically and biologically equivalent to recombinant DNA, or it may incorporate artificial chemical components that change the biological activity of the treatment. (Compare with **Recombinant DNA or RNA**).

**TALENs (Transcription activator-like effector nuclease):** proteins that can be engineered to make specific changes to cellular DNA. Along with CRISPR-CAS9 and Zinc-Finger Nucleases, TALENS are used to develop gene-editing technology.

**“T-VEC” (Imlygic; Talimogene laherparepvec):** the first oncolytic virus approved by the FDA for the treatment of cancer. T-VEC is a viral construct modified to express a cytokine called GM-CSF, which stimulates production and activation of dendritic cells at the tumor site.

**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 gene therapy, a special kind of virus that has been engineered 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.

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

Vectors Used in Gene Therapy Clinical Trials

- Adenovirus 22.2% (n=506)
- Retrovirus 18.4% (n=420)
- Naked/Plasmid DNA 17.4% (n=397)
- Vaccinia virus 7.2% (n=165)
- Adeno-associated virus 6% (n=137)
- Lipofection 5% (n=115)
- Lentivirus 5% (n=114)
- Poxvirus 4.4% (n=101)
- Herpes simplex virus 3.2% (n=73)
- Other vectors 7.6% (n=174)
- Unknown 3.3% (n=76)

**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 Nuclease are used to develop gene-editing technology.
About WCG Biosafety™

The most experienced and trusted provider of Biosafety solutions, the WIRB-Copernicus Group® (WCG™) has evaluated over 250 human gene transfer protocols to date – more than any organization outside of the Food and Drug Administration (FDA) and the National Institute of Health (NIH). We also maintain a global presence, operating Institutional Biosafety Committees (IBCs) in the United States and Canada, as well as in eight countries in Africa, South America, Central America, the Caribbean, and Europe. WCG Biosafety’s consulting division has led laboratory and biosafety projects in more than 50 countries over three decades, assisting government, academic and private institutions in maintaining the highest level of safety, security and compliance.

Gene Therapy research services are provided to Sponsors, CROs and Institutions involved in clinical trials involving recombinant and synthetic nucleic acids.

- Our Institutional Biosafety Outsourcing service provides management and administration of local Institutional Biosafety Committees (IBCs). Unlike IRBs, IBCs must be local to each institution evaluating a study. The management and administration of each IBC, however, can be outsourced to WCG Biosafety.

- If an institution wants to retain the management of their local IBC but lacks human gene therapy expertise, WCG Biosafety can provide an expert to review protocols and assess risks, and present to the convened IBC.

- Clinical protocols for gene therapy products have additional and different regulatory requirements that must be addressed in the protocol and consent. WCG Biosafety experts provide review of draft documents and comments/suggestions to ensure that requirements are met.
Laboratory Biosafety and Biosecurity services are provided to smaller hospitals and institutions working with biological agents and other hazards.

- WCG Biosafety provides on-site biosafety officer staffing for inspections, training, and biosafety program development, as well as an off-site mentoring program to train existing institutional staff.

- Our Biosafety Assessment service provides a snapshot analysis of the institution’s biosafety program to identify the strengths and weaknesses of the organization’s biosafety program. If gaps are detected in the program, WCG Biosafety sends experts onsite to develop a new, or review an existing, biorisk management program including development/refinement of biorisk management policies, SOPs, biosafety manuals, emergency response plans, and oversight committee structure.

- WCG Biosafety assists institutions looking to pursue or renew Select Agent Program (SAP) registrations. Select Agents are those that are used in important research, but could also cause significant public health risk if uncontrolled or misused.

- When an institution has a breach in biosafety processes, or a regulatory inspection that identifies problems, WCG Biosafety can provide immediate support to help correct problems and re-train staff.

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