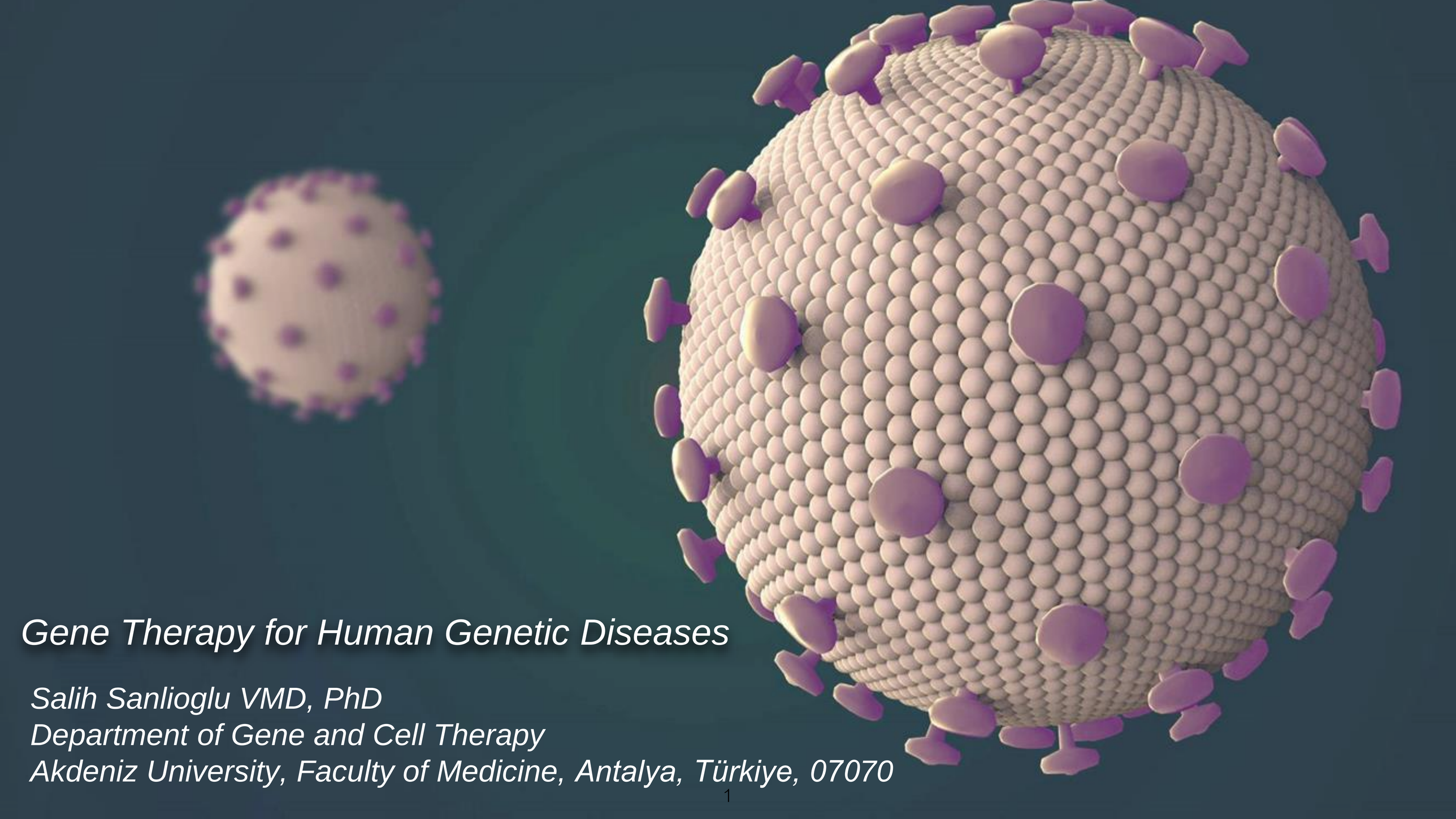

Gene Therapy for Human Genetic Diseases

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The Aim and The Learning Objectives

The Aim:

How to design and develop ***state of the art molecular genetic therapeutic strategies*** to treat currently incurable human diseases.

Learning Objectives:

- Characteristic features of gene therapy drugs,
- ***How to treat diseases*** like malignant melanoma, bubble boy disease, acute lymphoblastic leukemia, genetic blindness, SMA and Beta Thalassemia etc. ***using gene delivery***,
- How to design, construct, purify and administer ***gene therapy viral vectors*** for the treatment of emerging pandemic diseases.

Presentation Schema

- FDA and EMA ***definition of gene therapy***
- FDA and/or EMA approved ***gene therapy drugs marketed for clinical use***
- ***Pros and cons*** of clinically used gene therapy viral vectors
- Most formidable ***diseases of the century targeted by gene therapy***
- Testing of ***experimental gene therapy approaches*** developed at Akdeniz University against cancer, and diabetes.
- Summary and the take home message

GENE THERAPIES:

The Next Generation of Medicine

The genes in your body's cells play a key role in your health. Indeed, a defective gene or genes can make you sick. Recognizing this, scientists have worked for decades on ways to modify genes or replace faulty genes with healthy ones to treat, cure, or prevent a disease or medical condition. This research is paying off, as advancements in science and technology today are changing the way we define disease, develop drugs, and prescribe treatments. ***Human gene therapy*** seeks to **modify** or **manipulate the expression** of a gene or to alter **the biological properties** of living cells for **therapeutic** use.

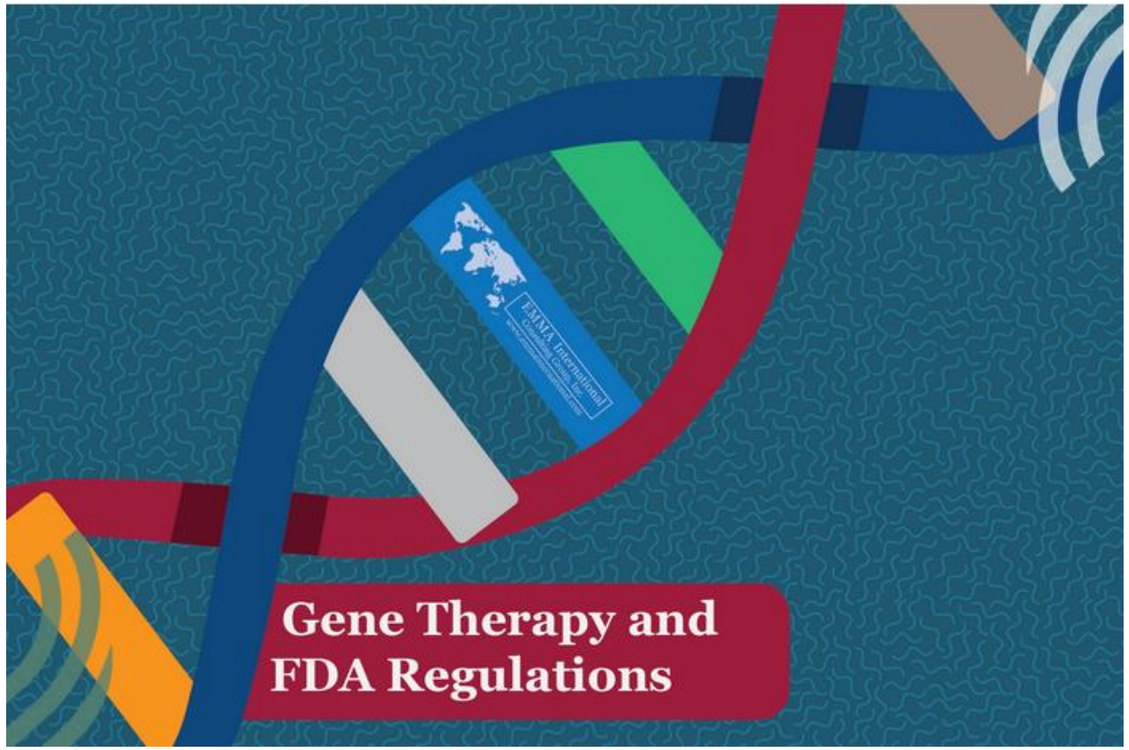
Gene therapy is a medical field which focuses on the **genetic modification** of cells to produce a therapeutic effect or the treatment of disease by repairing or **reconstructing defective genetic material**. The concept of gene therapy is to fix a genetic problem at its source. If, for instance, a mutation in a certain gene causes the production of a dysfunctional protein resulting (usually recessively) in an inherited disease, gene therapy could be used to deliver a copy of this gene that does not contain the deleterious mutation and thereby produces a functional protein. This strategy is referred to as ***gene replacement therapy***.

Currently, programmable nuclease (CRISPR/Cas9) based ***genome editing***, a revolutionary approach to developing medicines, has been becoming one of the most promising tools for treating human genetic diseases. These novel treatments named ***next generation gene therapy strategies*** gained significant attention gradually replacing classical vector mediated gene therapy approaches.

Gene therapy may be classified into two types:

In somatic cell gene therapy (SCGT), the therapeutic genes are transferred into any cell other than a gamete, germ cell, gametocyte, or undifferentiated stem cell. Any such modifications affect the individual patient only, and are not inherited by offspring. Somatic gene therapy represents mainstream basic and clinical research, in which therapeutic DNA (either integrated in the genome or as an external episome or plasmid) is used to treat disease.

In germline gene therapy (GGT), germ cells (sperm or egg cells) are modified by the introduction of functional genes into their genomes. Modifying a germ cell causes all the organism's cells to contain the modified gene. The change is therefore heritable and passed on to later generations. Many counties prohibit GGT for application in human beings, for technical and ethical reasons, including insufficient knowledge about possible risks to future generations and higher risks versus SCGT.



Cell, tissue and gene therapy products (CTGTPs) are **therapeutic products** intended for use in humans for prophylactic, palliative, diagnostic, or curative purposes. Generally, they are **breakthrough therapies** with immense potential in treating diseases with **no cure** or **rare diseases** with high treatment burdens. Gene therapy products are ***biological products*** regulated by the FDA's ***Center for Biologics Evaluation and Research*** (CBER). Before a gene therapy can be marketed for use in humans, the product must be tested in clinical studies for safety and effectiveness so FDA scientists can consider whether the risks of the therapy are acceptable considering the potential benefits.

Clinical studies in humans require the submission of ***an investigational new drug application*** (IND) prior to initiating clinical studies in the United States. Marketing a gene therapy product also requires submission and approval of ***a biologics license application*** (BLA). The Center for Biologics Evaluation and Research (CBER) regulates **cellular therapy products**, **human gene therapy products**, and **certain devices** related to cell and gene therapy.

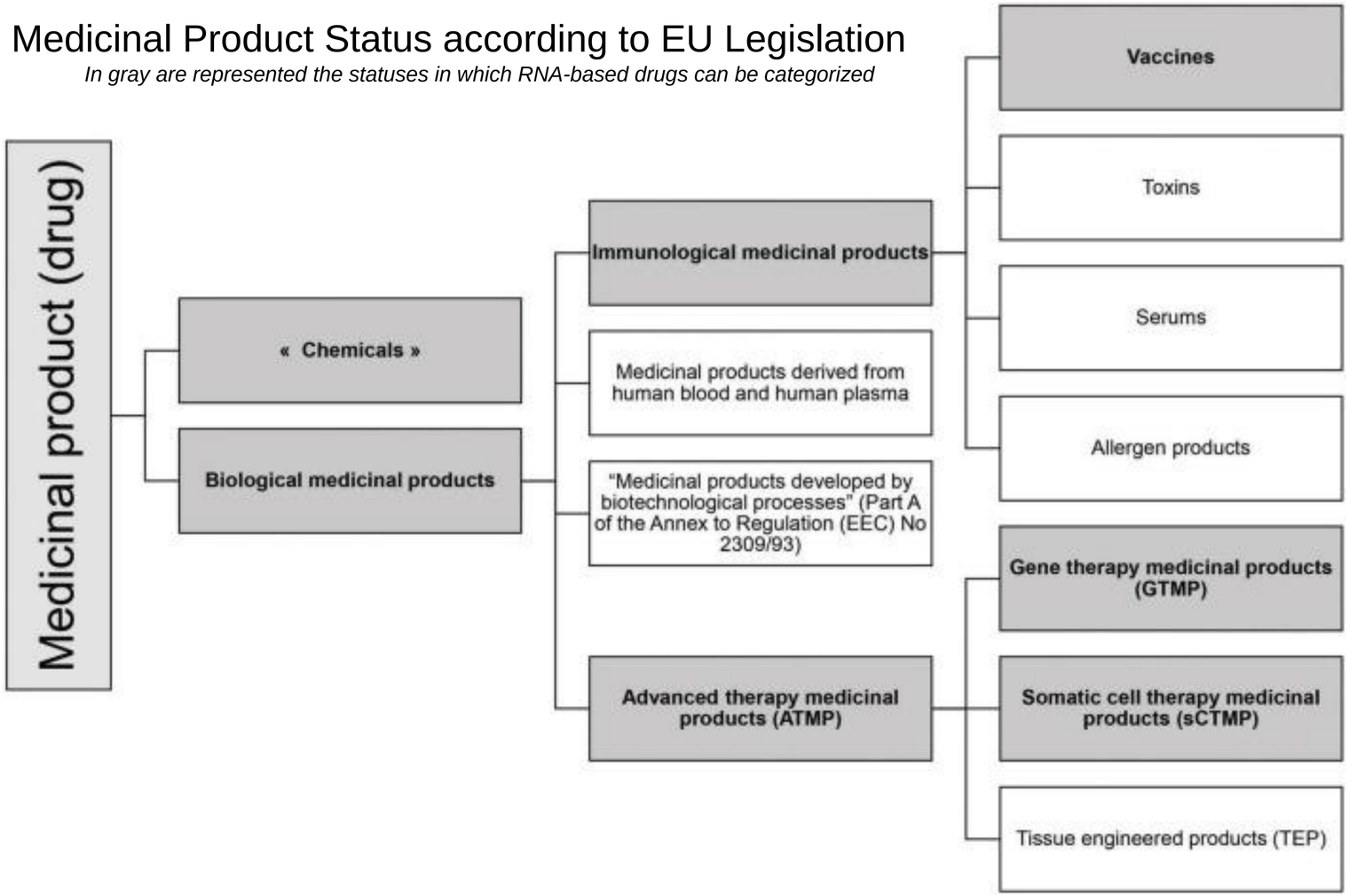
According to FDA, ***gene therapy*** is **a medical intervention based on modification of the genetic material of living cells.** FDA defines ***gene therapy drugs*** as: products "**that mediate their effects by transcription and/or translation of transferred genetic material and/or by integrating into the host genome and that are administered as nucleic acids, viruses, or genetically engineered microorganisms**. Cells may be modified ex vivo for subsequent administration to humans or may be altered in vivo by gene therapy given directly to the subject. When the genetic manipulation is performed ex vivo on cells which are then administered to the patient, this is also a form of **somatic cell therapy.**

The genetic manipulation may be intended to have a **therapeutic** or **prophylactic effect** or may provide a way of marking cells for later identification. Recombinant DNA materials used to transfer genetic material for such therapy are considered components of gene therapy and as such are subject to regulatory oversight. **Cellular therapy products** include **cellular immunotherapies**, **cancer vaccines**, and other types of both autologous and allogeneic cells for certain therapeutic indications, including **hematopoietic stem cells** and **adult** and **embryonic stem cells**. In addition to regulatory oversight of clinical studies, CBER provides proactive scientific and regulatory advice to medical researchers and manufacturers in the area of novel product development. As scientists continue to make great strides in this therapy, the FDA is committed to helping speed up development by interacting with those developing products and through prompt review of groundbreaking treatments that have the potential to save lives.

The National Institutes of Health (NIH) reports that nearly **7,000 rare diseases** affect more than 25 million Americans. Approximately **80% of rare diseases** are caused by **a single-gene defect**, and **about half** of all rare diseases **affect children**. Since **most rare diseases have no approved therapies**, there is a significant **unmet need for effective treatments**, and **many rare diseases are serious or life-threatening conditions**. As a general matter, developing safe and effective products to treat rare diseases can be challenging. For example, it might be **more difficult to find and recruit patients with rare diseases into clinical trials**. Additionally, patients may have highly **diverse clinical manifestations** and **rates of disease progression** that are difficult to predict. **These challenges are also present for the development of gene therapy (GT) products**. However, despite these challenges, GT-related research and development in the area of rare diseases continues to grow at a rapid rate.

Medicinal Product Status according to EU Legislation

In gray are represented the statuses in which RNA-based drugs can be categorized



EMA role: All **advanced therapy medicines** are authorized centrally via **the European Medicines Agency (EMA)**. They benefit from a single evaluation and authorization procedure. As with all medicines, the Agency continues to monitor **the safety and efficacy of advanced therapy medicines** after they are **approved** and **marketed**. The Agency also gives scientific support to developers to help them design pharmacovigilance and risk management systems used to monitor the safety of these medicines.

Committee for Advanced Therapies: The Agency's **Committee for Advanced Therapies (CAT)** plays a central role in the scientific assessment of advanced therapy medicines. It provides the expertise that is needed to evaluate advanced therapy medicines. During the assessment procedure, the CAT prepares a draft opinion on **the quality, safety and efficacy** of the advanced therapy medicine. It sends this to **the Committee for Medicinal Products for Human Use (CHMP)**. Based on the CAT opinion, the CHMP adopts an opinion recommending or not the authorization of the medicine by the European Commission. **The European Commission** makes its final decision on the basis of the CHMP opinion.

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Gene Therapy Definition: First of all, the drug must contain **active ingredients** consisting of **recombinant nucleic acids (DNA-RNA)** that will be involved in the **modification** (editing, repair, replacement, insertion or deletion) **of any genetic sequence** when administered to humans. In addition, **the therapeutic, prophylactic or diagnostic effect** of the relevant drug should be directly **derived from the recombinant nucleic acid sequence** it contains or from **the product** (RNA, protein, etc.) formed by the genetic expression of this sequence.

Advanced therapy medicinal products (ATMPs) are medicines for human use that are based on genes, tissues or cells. They offer groundbreaking new opportunities for the treatment of disease and injury.

ATMPs can be classified into three main types:

- ***gene therapy medicines:*** these contain genes that lead to a therapeutic, prophylactic or diagnostic effect. They work by inserting 'recombinant' genes into the body, usually to treat a variety of diseases, including genetic disorders, cancer or long-term diseases. A recombinant gene is a stretch of DNA that is created in the laboratory, bringing together DNA from different sources;
- ***somatic-cell therapy medicines:*** these contain cells or tissues that have been manipulated to change their biological characteristics or cells or tissues not intended to be used for the same essential functions in the body. They can be used to cure, diagnose or prevent diseases;
- ***tissue-engineered medicines:*** these contain cells or tissues that have been modified so they can be used to repair, regenerate or replace human tissue;

Gene therapy medicinal products

Gene therapy medicinal products (GTMPs) have been defined in the Annex I, Part IV, §2.1 of the Directive 2001/83/EC consolidated. This definition is complex and involves several criteria:

1) the drug must belong to the status of **a biological medicinal product**. The active substance must therefore be a biological substance,

2) the drug must contain or **consist of a nucleic acid**, in other words, DNA or RNA, and this nucleic acid must be recombinant,

3) the sequence must be **administered to regulate, repair, replace, add, or delete a genetic sequence**,

4) the action must be **therapeutic, prophylactic, or diagnostic** and must be directly **dependent on the nucleic acid used**.

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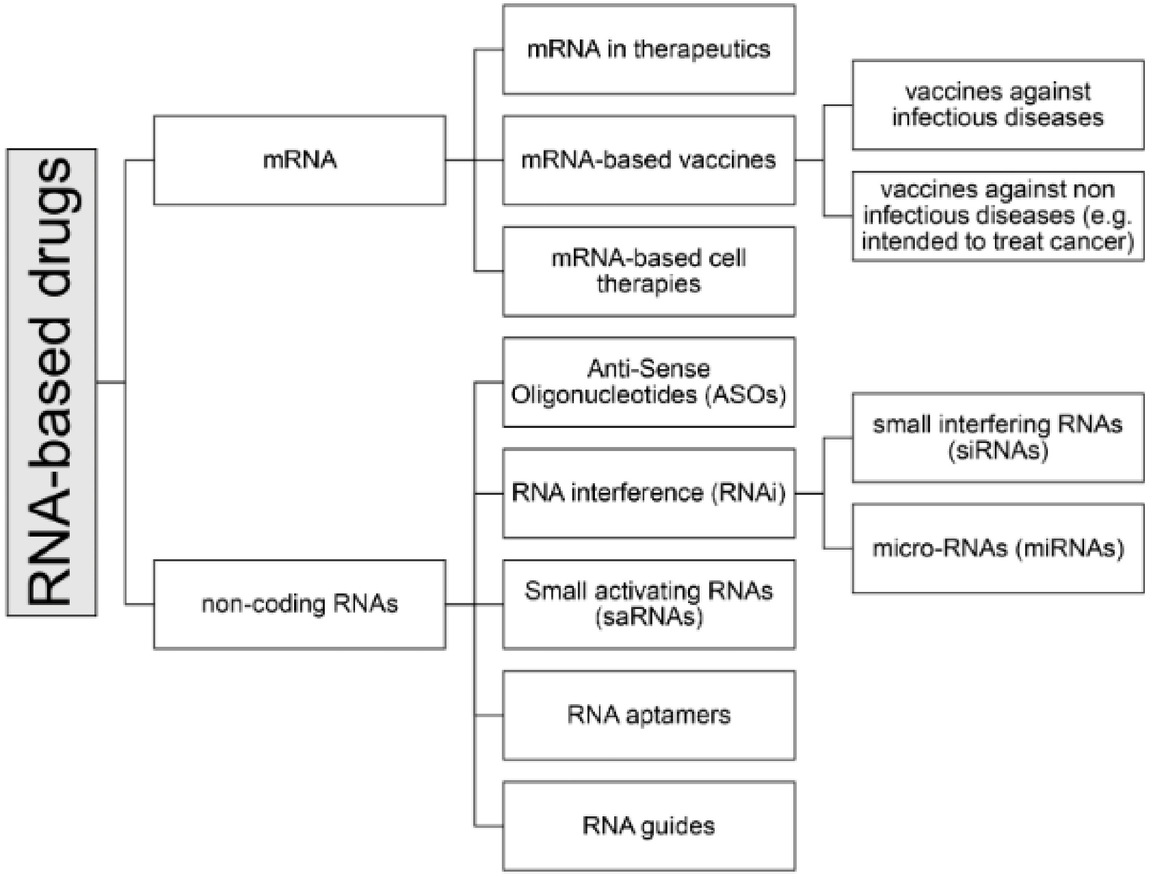
It should be noted that vaccines against infectious diseases **are not included** in GTMPs**:**

"Gene therapy medicinal products shall not include vaccines against infectious diseases."

Guerriaud M and Kohli E (2022) Front. Med. 9:1012497.

RNA-based Drugs and Regulation



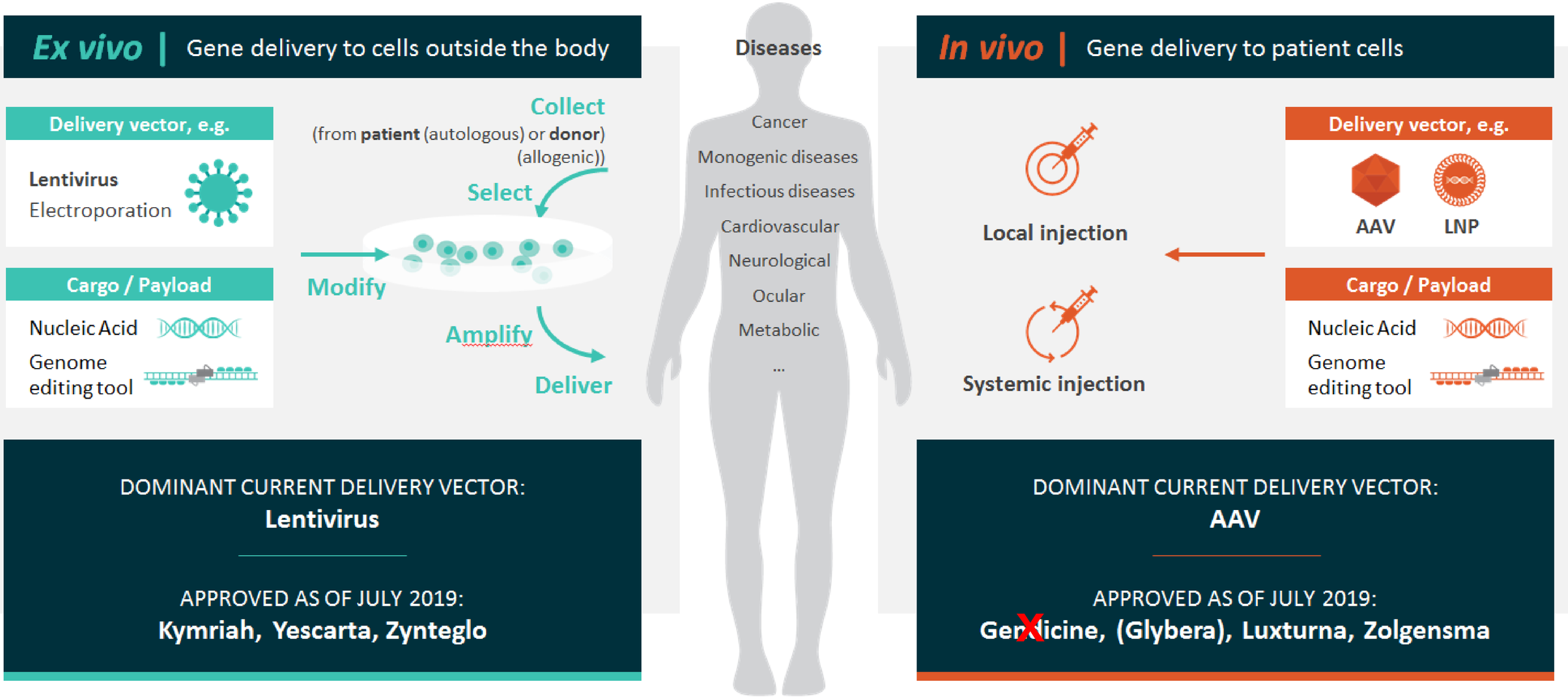
Many RNA-based drugs, both vaccines and non-vaccines, are under development or even approved. They include coding mRNAs and non-coding (nc) RNAs among them antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), micro-RNAs (miRNAs), small activating RNAs (saRNAs), RNA aptamers and RNA guides. According to the EU legislation, RNA-based drugs fall under several different statuses depending, for vaccines, on their targets – infectious vs. non-infectious diseases – and for non- vaccine drugs, on the type of RNA substance and its production. For vaccines, although subject to discussion, the target criterion is easy to use for classification. However, **the current rules raise several problems, in particular the risk, because technology is evolving, to have similar RNA drugs being covered by very different legal statuses and the lack of international harmonization.**

For both the EU and the USA, **the biological origin of the drug** matters, so ASOs (Spinraza-nusinersen etc.), siRNA, miRNA, saRNA and aptamers, which are **chemically produced**, are considered as "**chemical drugs**." RNA-based medicinal products question the relevance of maintaining the current European union definition of a biological medicinal product. **In theory, a drug containing a chemically synthesized RNA cannot claim the status of a biological drug**, and therefore in extension of a vaccine or a GTMP. In the case where some RNA could be both chemically and biologically synthesized, this could lead to having two medicinal products with the same indication and the same composition (with the only difference being the source of the RNA), but which would not have the same status at all. This would be problematic and cruelly lacking in consistency. According to CAT; "long chain mRNAs cannot yet be produced via chemical synthesis. However, when this becomes possible, the regulatory status of such synthetic RNAs needs to be considered, as it should be avoided to have similar products being covered by different legal frameworks".

Advanced therapy medicinal products (ATMP) are defined in Article 2 of Regulation (EC) No 1394/2007 as any of the following medicinal products for human use: a ***gene therapy medicinal product (GTMP),*** a somatic cell therapy medicinal product (sCTMP), tissue engineered product (TEP). Among these, **GTMP is the most frequently used status for RNA-based drugs**, **but sCTMP also represents a possible status.** Examples: ***NTLA-2001*** (**mRNA for Cas9 combined with a single short guide RNA**) for **hereditary transthyretin amyloidosis** and **mRNA-based vaccines for the treatment of cancer**: direct injection of mRNA ***IVAC MUTANOMER*** for **advanced melanoma**. Current **CAR-T cells**, which are genetically modified by adding DNA ex vivo, are classified as **GTMP**.

Comirnaty (Pfizer-Biontech) is a vaccine for preventing coronavirus disease 2019 (COVID-19) in people from the age of 6 months. Comirnaty contains tozinameran, a messenger RNA (mRNA) molecule with instructions for producing Spike protein from the original strain of SARS-CoV-2, the virus that causes COVID-19. **Spikevax** (previously COVID-19 Vaccine Moderna) is a vaccine for preventing coronavirus disease 2019 (COVID-19) in people from the age of 6 months. Spikevax contains elasomeran, a molecule called messenger RNA (mRNA) with instructions for producing a Spike protein from the original strain of SARS-CoV-2, the virus that causes COVID-19. ***Both Comirnaty and Spikevax are categorized under "Vaccines" category of medicinal products in EU.*** mRNA vaccines against infectious diseases are also categorized as vaccines in the USA. Nusinersen, marketed as **Spinraza**, is an **anti-sense oligonucleotide** based medication used in treating spinal muscular atrophy (SMA), a rare neuromuscular disorder. It is categorized under "**Chemicals**" section of medicinal products. It is not considered to be GTMP.

Guerriaud M and Kohli E (2022) Front. Med. 9:1012497.



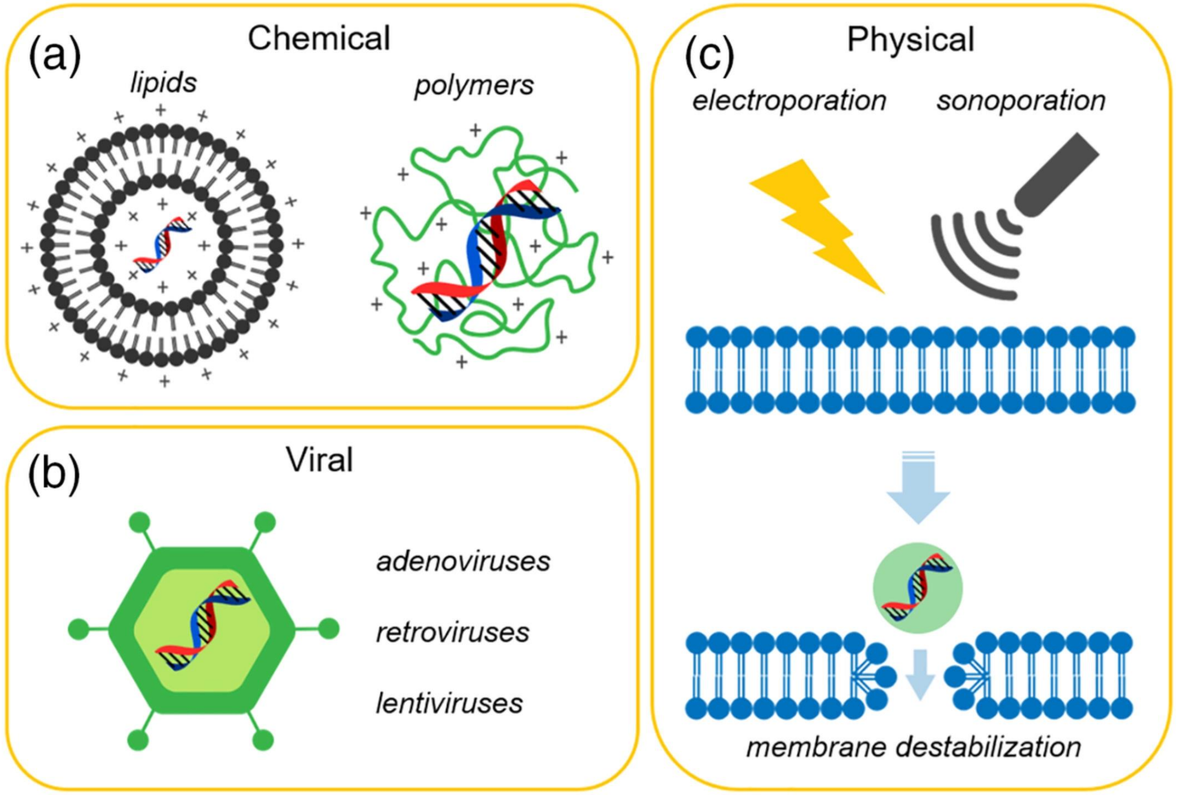
Gene therapy can be used to modify cells inside or outside the body. The vector can be delivered in one of two ways: **ex-vivo treatment** removes the person's own cells and delivers the genetic material to these cells outside the body. The modified cells are then returned to the body. When gene therapy is used to modify cells outside the body, doctors take blood, bone marrow, or another tissue, and separate out specific cell types in the lab. The vector containing the desired gene is introduced into these cells. The cells are later injected into the patient, where the new gene is used to produce the desired effect. **In-vivo treatment** means the genetic material is delivered directly into the person, such as through an injection. When a gene therapy is used to modify cells inside the body, a doctor will inject the vector carrying the gene directly into the patient.

How does Gene Therapy Work?

Genes are the blueprints providing instructions on how to make proteins for the body. Unfortunately, these blueprints are not always correct. Sometimes the whole or part of a gene is defective or missing from birth. This is typically referred to as a genetically **inherited mutation**. In addition, healthy genes can change (mutate) over the course of our lives. These **acquired mutations** can be caused by environmental exposures.

The good news is that most of these genetic changes do not cause disease. But some inherited and acquired mutations can cause developmental disorders, neurological diseases, and cancer. Depending on what is wrong, scientists can do one of several things in gene therapy: They can replace a gene that is missing or is causing a problem. They can add genes to the body to help treat disease. Or they can turn off genes that are causing problems.

To insert new genes directly into cells, scientists use a vehicle called a "**vector**." Vectors are genetically engineered to deliver the necessary genes for treating the disease. A vector is like a package used to deliver a specific message. The genetic material that is delivered, DNA or RNA, has instructions to change how a protein—or group of proteins—is produced by the cell. For some diseases, this means making changes to account for too much, not enough, or incorrect essential proteins being produced within cells. Gene and cell therapies need to express the gene in the right tissue, at the right level, for the right amount of time.



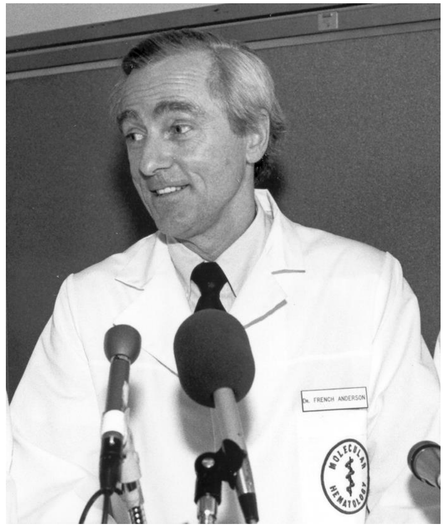
Summary of gene delivery approaches (viral, physical, and chemical): (a) chemical systems involve cationic lipids or polymers which complex negatively charged nucleic acids; (b) biological systems utilize replication defective viral vectors; and (c) physical methods, such as electroporation and sonoporation, create temporary pores in the cell membrane using electronic pulses or ultrasound.

There are a variety of types of gene therapy products, including:

- **Plasmid DNA:** Circular DNA molecules can be genetically engineered to carry therapeutic genes into human cells.
- **Viral vectors:** Viruses have a natural ability to deliver genetic material into cells. Modified viruses can be used as vectors (vehicles) to carry therapeutic genes into human cells.
- **Bacterial vectors:** Bacteria can be modified to prevent them from causing infectious disease and then used as vectors (vehicles) to carry therapeutic genes into human tissues.
- **Human gene editing tools** (CRISPR/Cas**)**: The goals of gene editing are to disrupt harmful genes or to repair mutated genes.
- **Patient-derived cellular gene therapy products** (CAR-T Cells)**:** Cells are removed from the patient, genetically modified (often using a viral vector) and then returned to the patient.

Salameh et al. WIREs Nanomed Nanobiotechnol. 2019;e1598

Results From First Human Gene Therapy Clinical Trial



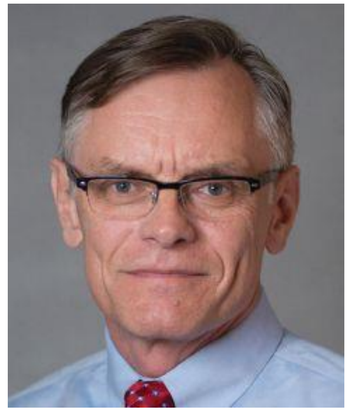
ADA-SCID, a rare, inherited disorder in which the immune system is damaged, causing a person to have a complete lack of B lymphocytes and T lymphocytes (types of white blood cells that help the body fight infection). Born with a rare genetic disease, **severe combined immune deficiency (SCID)**, **Ashanti DeSilva** lacked a healthy immune system and was extremely vulnerable to infection. Children with SCID usually develop overwhelming infections and **rarely survive to adulthood**; even a common childhood illness like chicken pox is life-threatening. Ashanti led a cloistered existence, avoiding contact with people outside her family, remaining in the sterile environment of her home, and battling frequent illnesses with **massive amounts of antibiotics**. Ashanti DeSilva started receiving **PEG-ADA** injections (enzyme replacement therapy-ERT) at the age of two, and initially she responded well. Her T-cell count rose sharply and she developed some resistance to disease. The standard treatment for ADA deficiency is frequent injections of PEG-ADA, a synthetic form of the ADA enzyme. Unfortunately, although it usually produces a rapid improvement when first used, children tend to respond less and less to the drug each time they receive a dose. But by the age of four, she was slipping away, no longer responding strongly to her injections. If she was to live, she'd need something more than PEG-ADA. The only other option at the time, **a bone-marrow transplant**, was ruled out by **the lack of matching donors**.

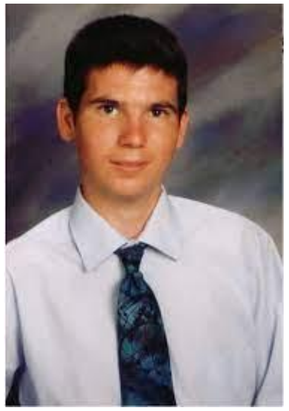
Dr. **W. French Anderson** from the National Heart, Lung, and Blood Institute was **the first to treat a four year old girl with gene therapy**. This girl had **adenosine deaminase (ADA) deficiency** which affected her immunity to sickness since she lacked sufficient white blood cells. Anderson and his colleagues **Michael Blaese** and **Kenneth Culver** slowly extracted some of Ashanti's **blood cells**. Safely outside the body, the cells had new, working copies of **the ADA gene** inserted into them by a **retrovirus**. Finally, starting on the afternoon of September 14, 1990, Culver injected the cells back into Ashanti's body. This was **the first FDA approved gene therapy procedure conducted in the world**. In Ashanti's gene therapy procedure, her own **white blood cells** were genetically modified and then infused back into her bloodstream. Laboratory tests show that the therapy has **strengthened** Ashanti's **immune system**. She **no longer has recurrent colds**, has been allowed to **attend school**, and has been immunized against whooping cough. This gene therapy procedure is not a cure, however. **The genetically-modified white blood cells only survive for a few months** and must then be replaced, but Ashanti's future was much brighter because of the new therapy.

Ashanti received her first infusion of cells on September 14, 1990, with no complications. She received **10 more infusions over the next 2 years**. Her immune evaluation studies became normal and she became **healthy** with no major infections. A thorough immune status follow-up was done **after 12 years**: she remained healthy with **20% of her lymphocytes** still carrying **an active retroviral ADA gene** – a sufficient percentage to **ensure immunologic protection**. She is now 33 years old, married, and works as a genetic counselor and a journalist.

The Biggest Disappointment in Gene Therapy Clinical Trials

Gene Therapy: An Interview with an Unfortunate Pioneer





Jesse Gelsinger was **the first person** publicly identified as **having died in a clinical trial for gene therapy**. Gelsinger suffered from **ornithine transcarbamylase deficiency**, an **X-linked genetic disease of the liver**, the symptoms of which include **an inability to metabolize ammonia** – a byproduct of protein breakdown. The disease is usually **fatal at birth**, but Gelsinger had **a milder form of the disease**, in which the ornithine transcarbamylase gene is mutated in only part of the patient's cells, a condition known as **somatic mosaicism**. As his deficiency was partial, Gelsinger managed to survive on **a restricted diet** and **special medications**.

The trial Dr. Wilson conducted tested the safety of a therapy for **ornithine transcarbamylase (OTC) deficiency**, a rare disorder in which the liver lacks a functional copy of the OTC gene. The defect prevents the body from **eliminating ammonia**, **a toxic breakdown product** of protein metabolism. The Penn scientists had engineered a weakened **adenovirus, or cold virus**, to deliver a normal copy of **the OTC gene into the liver**. **Seventeen patients had undergone treatment** before Gelsinger, who was in the final cohort—the one receiving **the highest dose of the therapy**.

Gelsinger joined a clinical trial run by the University of Pennsylvania that aimed at developing a treatment for infants born with the severe form of the disease. **On September 13, 1999**, Gelsinger was injected with **an adenoviral vector** carrying a corrected gene to **test the safety of the procedure**. **He died four days later at the age of 18**, **on September 17**, apparently having suffered **a massive immune response triggered by the use of the adenoviral vector** to transport the gene into his cells, leading to **multiple organ failure and brain death**. But Wilson now believes that the teen died from **a rare phenomenon** called **antibody-dependent enhancement**. He may have been exposed to a similar adenovirus in the past, which caused his body to create antibodies against it. Normally antibodies control a virus when the body encounters it again. **But occasionally they elicit a dangerous immune response**. However, that there is no way to prove it, because none of Gelsinger's pretreatment blood samples remain.

A Food and Drug Administration (FDA) investigation concluded that the scientists involved in the trial, including the co-investigator James Wilson (Director of the Institute for Human Gene Therapy), **broke several rules of conduct**:

- Inclusion of Gelsinger as a substitute for another volunteer who dropped out, despite Gelsinger's having **high ammonia** levels that **should have led to his exclusion from the trial**.
- Failure by the university to report that **two patients had experienced serious side effects from the gene therapy**.
- Failure to disclose, in the informed-consent documentation, **the deaths of monkeys given a similar treatment**.

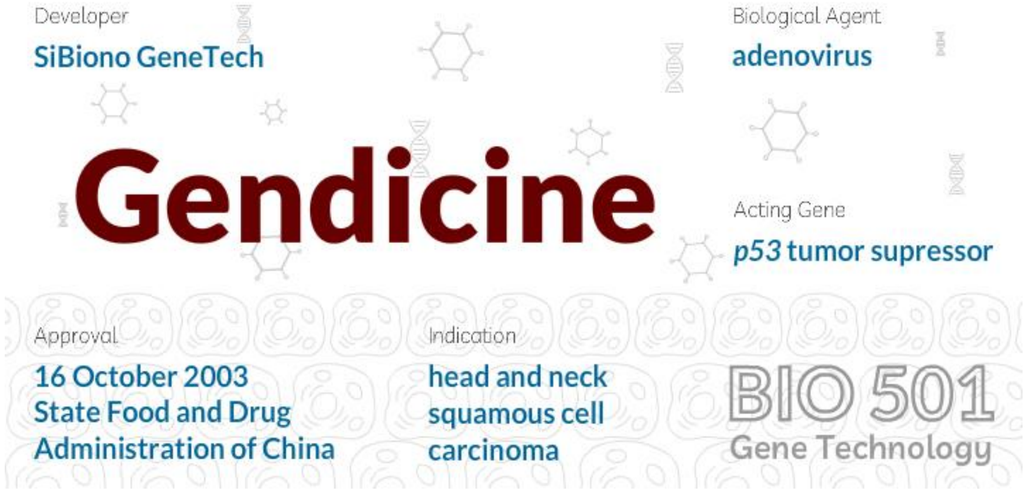
The University of Pennsylvania later issued a rebuttal, but the university and Children's National Medical Center each agreed to pay more than **$500,000 to the government**. Both Wilson and the University are reported to have had financial stakes in the research. **After his death, all gene therapy trials in the United States halted for 5 years**. The Gelsinger case was a severe setback for scientists working in the field and a reminder of the risks involved.

Wilson hasn't given up on the field, though—**he is trying to make it safer**. Since 1999, **with a grant from GlaxoSmithKline**, his lab has identified **120 new adenovirus-associated viruses including AAV9 (used for a spinal muscular atrophy (SMA) gene therapy)** that can more easily sneak past the immune system and deliver gene therapies with lower risk, and he has distributed them to 700 investigators around the world for further study. Today, **his lab's $70 million annual budget** and bevy of biotech partnerships are **fueling the gene therapy explosion**.

Scientific American 301, 3, 14-15 (September 2009)

The First Commercial Gene Therapy Product

Gendicine is a **recombinant E1-deleted adenovirus** engineered to express **wildtype-p53** (rAd-p53). This virus is designed to treat patients with tumors which have mutated p53 genes. **Gendicine is the first commercial gene therapy product approved for clinical use in humans**. Gendicine is manufactured by Shenzhen SiBiono GeneTech. Gendicine was approved **in 2003** by **the Chinese State Food and Drug Administration** to treat **head and neck squamous cell carcinoma**. **Advexin**, a similar gene therapy developed by Introgene that also uses adenovirus to deliver the p53 gene, was **turned down by the FDA in 2008**.



Mechanism of action

The p53 gene is one of the most important **tumor-suppressor** genes existing in normal cells. In normal cells, **the expression level of p53 protein is very low.** p53 expression is activated upon oncogene activation, growth-factor deprivation, hypoxia, and DNA damage. The upregulation of p53 gene expression occurs at the posttranslational level and is achieved through stabilization of the expressed protein. **The activation of p53 gene expression results in either cell cycle arrest or apoptotic cell death**. The p53 gene is **mutated or deleted** (null) in approximately **50% to 70% of human tumors**. **Mutant forms** of the p53 gene are **not necessarily inactive** and can **gain oncogenic functions** that contribute to **tumorigenicity**. Most importantly, mutant p53 proteins have been associated with the upregulation of the **multidrug resistance (MDR)** gene, which results in **tumor resistance** to a variety of **chemotherapeutics**.

Gendicine enters the tumor cells by way of receptor-mediated endocytosis and begins to over-express genes coding for the p53 protein needed to fight the tumor. **Ad-p53** seems to act by stimulating the **apoptotic pathway** in tumor cells, which increases the expression of **tumor suppressor genes** and **immune response factors** (such as the ability of **natural killer (NK) cells** to exert "**bystander**" effects). it can function as a **tumor-antigen** by stimulating human immune cells (**cytotoxic T cells**) to selectively kill cancer cells that **over-express the p53 gene**. It also **decreases the expression of multi-drug resistance**, **vascular endothelial growth factor** and **matrix metalloproteinase-2 genes** which are involved in tumor progress, metastasis, and chemo-drug resistance, and blocking transcriptional survival signals. **Ad-p53 appears to act synergistically** with conventional treatments such as **chemo- and radiotherapy**.

The First Approved Gene Therapy Product for Cancer Ad-p53 (Gendicine): 12 Years in the Clinic

Based on **12 years of commercial use** in more than **30,000 patients**, and more than **30 published clinical studies**, Gendicine has exhibited an exemplary safety record, and when combined with chemotherapy and radiotherapy has demonstrated significantly **higher response rates** than for standard therapies alone. Thirteen published studies that include long-term survival data showed that Gendicine combination regimens yield **progression-free survival** times that are significantly longer than standard therapies alone. Although the **only currently approved used of Gendicine is in combination with radiotherapy for treatment of HNSCC**, clinical studies have been carried out for more than 20 other applications of Gendicine in treating cancer, including treatment of advanced lung cancer, advanced liver cancer, malignant gynecological tumors, and soft tissue sarcomas.

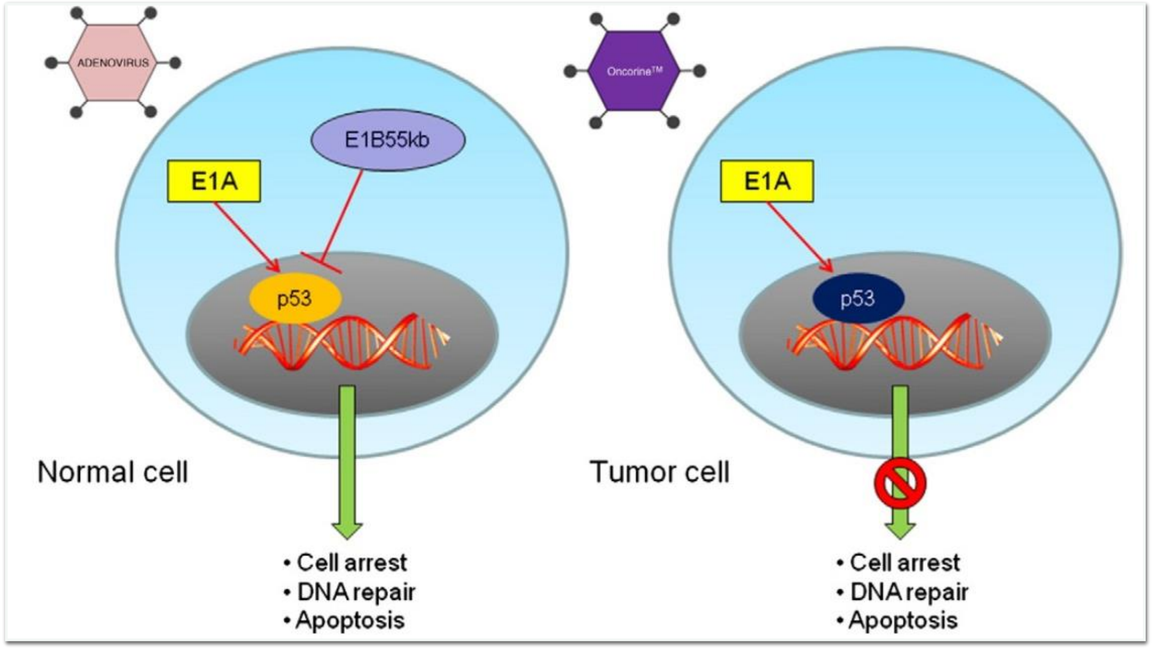
Human Gene Therapy. Feb 2018.160-179.

Oncorine®

Oncolytic viruses, a new class of therapeutic agents, are **engineered viruses** with abilities of both **directly killing the tumor cells** and stimulating **host anti-tumor immune responses**. These oncolytic viruses can be **adenovirus**, autonomous **parvoviruses**, **vaccinia virus**, **vesicular stomatitis virus**, etc. Oncolytic viruses can replicate in cancer cells but not in normal cells, causing lysis of the tumor mass. On the other hand, **the lysed tumor cells release more infectious virus particles** to infect and destroy the remaining tumor. They also release **tumor-associated antigens** into the microenvironment to **stimulate the immune response**.

Among these oncolytic viruses, **adenovirus** is **the first** as well as **the most extensively tested vector in the clinical trials**. **Oncorine**, also referred to as **Recombinant Human Adenovirus Type 5 Injection**, was **the first approved oncolytic virus** by the Chinese State Food and Drug Administration (CFDA) for the cancer treatment in **November 2005**. Oncorine is **a genetically modified adenovirus** named H101 (**E1B-deletion**) developed by Shanghai Sunway Biotech in China, which is used **in conjunction with chemotherapy** for the treatment of **nasopharyngeal carcinoma (NPC)** and **head and neck cancer**. The approval and successful application of Oncorine greatly promote the development of cancer immunotherapy based on the oncolytic virus.

T. Wirth et al. / Gene 525 (2013) 162–169



Mechanism of Action of Oncorine®: In normal cells, early proteins (E1A and E1B) are expressed when they are infected by adenovirus. And early proteins are related with virus replication. The cell response for expression of E1A protein is to stimulate P53 expression. P53 is an important transcription regulation factor and a product of tumor suppressor gene. P53 overexpression results three changes in the cell: 1) **cell arresting** 2) **DNA damage-repair** 3) **Inducing apoptosis**. **Those reactions prevent replication of viruses in normal cells. E1B-55KD can degrade P53 protein, which favors for virus replication**. Comparing with wild virus, replication ability of E1B-55KD deleted virus was reduced; meanwhile, P53 couldn't be degraded effectively when E1B-55KD is deleted. **So that Oncorine® can't be replicated in normal cells**. **In P53 deficient cancer cells , apoptosis can not be induced because of P53 deficiency**, **it is conducive to replication of reconstructed virus**. The current view is that it is not only P53 mutation itself, but also P53 pathway deficiency are conducive for selective replication of Oncorine®.

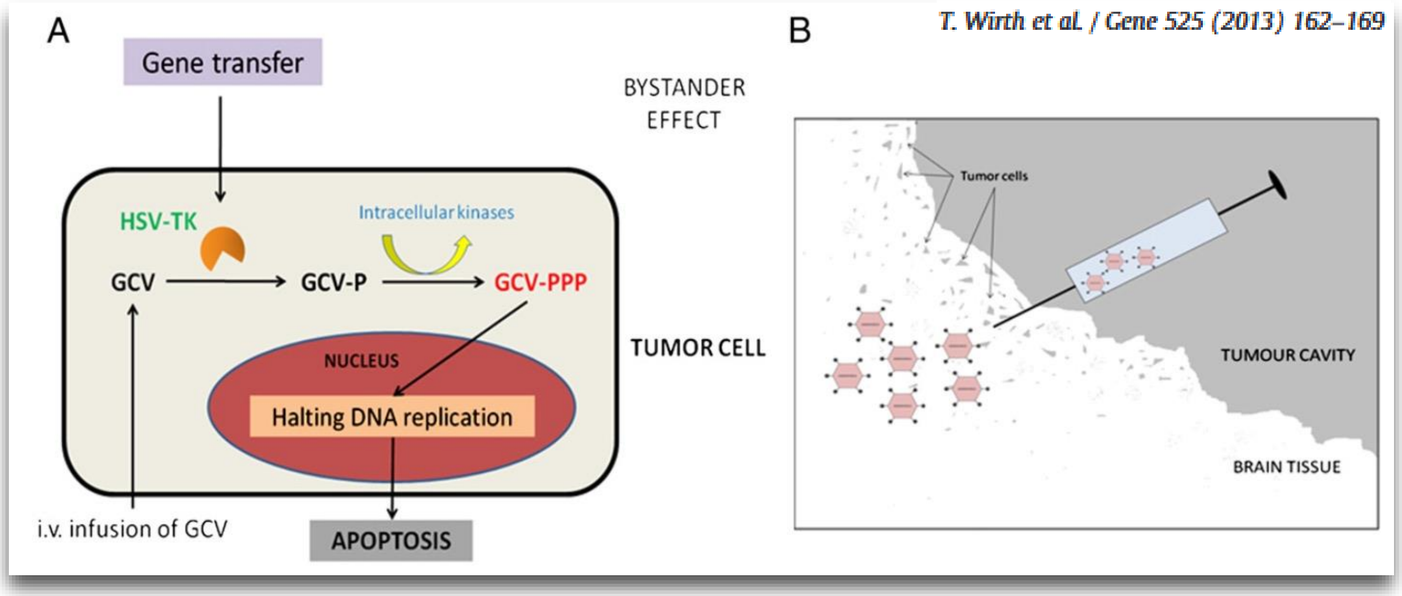
Oncolytic viruses are promising new categories of anticancer agents, and the present clinical studies have shown that **they are safe and well tolerated**. Importantly, **oncolytic virotherapy** can **boost the tumor killing effects of chemotherapy**, without enhancing the toxicity of the chemotherapy drug, and also **stimulate immune response against cancer**. This property makes oncolytic viruses an ideal treatment option that can be safely combined with conventional treatment regimens used to treat cancer patients. Oncorine was the only oncolytic virus product approved in China till now, and ten years earlier than the approval of IMLYGIC (T-VEC) by FDA in October 2015. Despite such breakthrough, **the sales of Oncorine are limited in the Chinese market**. Although the sales of Oncorine are constantly growing year by year, Oncorine still has not had a great impact on the market of cancer therapeutics, due to the very **limited sales** in the beginning. This may be caused by the fact that **the majority of NPC patients in China are treated with radiotherapy**, and **can not benefit from the approval of Oncorine with chemotherapy**. Therefore, to better target this part of the Chinese market, **future clinical trials are needed to evaluate the efficacy of Oncorine combined with radiotherapy in NPC patients**. In the long run, it may be useful to collect data on the long-term efficacy of Oncorine, such as patient's survival benefit, not only to improve the virus designs or treatment regimens in the future, but also to enhance consumer's confidence in this class of novel cancer therapeutics. The development in the USA of **a very similar virus** called **Onyx-15** was halted at the outset of a phase III trial due to **a funding crisis**. This funding crisis has resulted in a ten year lag behind China in the approval of an oncolytic virus.

Current Cancer Drug Targets, 2018, 18, 171-176

Suicide Gene Therapy

The thymidine kinase (TK)/ganciclovir (GCV) system is a **gene-directed enzyme prodrug therapy**. **The herpes simplex virus 1 thymidine kinase (HSV-TK) gene**, called **the suicide gene**, introduced into cells via **local gene transfer** phosphorylates **a prodrug, ganciclovir (GCV),** to the monophosphate form in the introduced cells. After that, it is phosphorylated by intracellular TK to the triphosphorylated form, which **inhibits DNA synthesis** and causes **cell apoptosis**.

In addition, phosphorylated GCV is **passively transferred** to surrounding cells not expressing HSV-TK through **gap junction** intercellular communication and induces **surrounding cell death**. This is the so-called **bystander effect** and is important for **enhancing the antitumor effect** of the TK/GCV system. Since the phosphorylated form of GCV inhibits DNA synthesis, suicide gene therapy is likely to be effective against **cells with active DNA synthesis**, such as **tumor cells**.



Glioma is a type of brain tumor that begins in **'glial'** cells (the cells that surround and support nerve cells). **Glioblastoma (GBM)** is **the most common** and **aggressive primary brain tumor** in the adult population. The mainstay of treatment for GBMs is **surgery**, followed by radiation and chemotherapy. The primary objective of surgery is to remove as much of the tumor as possible without injuring the surrounding normal brain tissue needed for normal neurological function. However, GBMs are surrounded by a zone of migrating, infiltrating tumor cells that invade surrounding tissues, **making it impossible to ever remove the tumor entirely**. **Inefficient drug delivery** across the blood brain barrier (BBB), an **immunosuppressive tumor microenvironment** (TME) and **development of drug resistance** are key barriers to successful glioma treatment. Since gliomas occur through sequential acquisition of genetic alterations, **gene therapy**, which enables to modification of the genetic make-up of target cells, appears to be a promising approach to overcome the obstacles encountered by current therapeutic strategies.

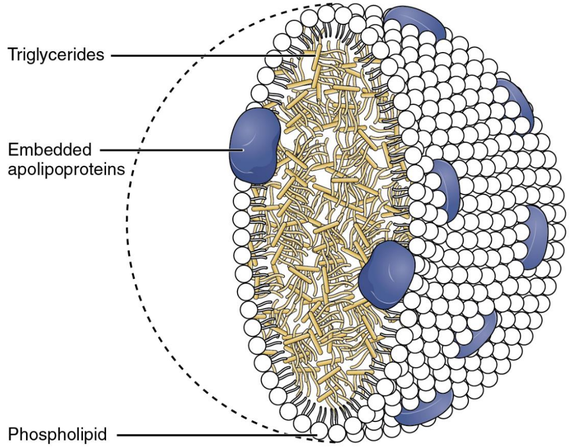
Cerepro® is an adenoviral mediated gene-based medicine given by multiple injections into the healthy brain tissue of patients following surgical removal of the solid tumor mass to treat **high-grade glioma**. **Cerepro®** contains the gene for the enzyme **'thymidine kinase'** from the herpes virus. In the following days, ganciclovir, is given intravenously. Once treated, healthy brain cells surrounding the site where the tumor was removed express the enzyme thymidine kinase. This converts the ganciclovir to a substance which **specifically kills dividing cells**. **The healthy neurons** surrounding the tumor in the brain are **non-dividing** and are therefore **not susceptible** to this substance. In this way Cerepro® harnesses healthy brain cells to help prevent a new tumor from growing.

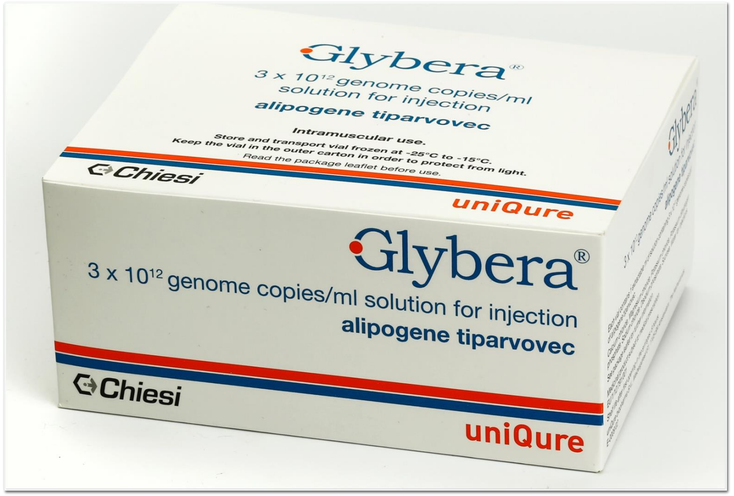
In 2008, Cerepro® became **the first** and so far the only **adenoviral vector** that has completed **a phase III clinical trial**. Cerepro® has been studied in **36 patients** with **high-grade glioma**. The study compared the effects of adding Cerepro® and ganciclovir sodium to standard treatment with the effects of standard treatment alone. The main **measure of effectiveness** was **how long the patients survived after the first operation**. Unfortunately, based on the review of the data, the **CHMP** had given **a negative opinion** and **did not recommend a marketing authorization** for Cerepro® for the treatment of patients with **operable high-grade glioma**. The CHMP had concerns that **a benefit of Cerepro® had not yet been shown**. It was concerned over **the low number of patients** included in the main study of Cerepro®. In addition, the CHMP considered there to be **insufficient information on the safety of Cerepro®**, and, **since the benefits of the medicine had not been demonstrated**, that **its risks, when used in combination with ganciclovir, could be of concern**. On 13 July 2007, **Ark Therapeutics** officially notified the Committee for Medicinal Products for Human Use (CHMP) that it wishes **to withdraw its application for a marketing authorization for Cerepro**, for the treatment of patients with operable high-grade glioma.

Mol Ther Methods Clin Dev 2022 Jul 6;26:253-265

Familial Lipoprotein Lipase Deficiency

Familial Lipoprotein Lipase Deficiency is an **inherited disorder** caused by a defective gene responsible for an enzyme called **lipoprotein lipase**. As a result, the affected individuals lack the ability to produce lipoprotein lipase enzyme that is needed to **breakdown the fat molecules**. The disorder is characterized by **a massive accumulation** of fatty particles (**chylomicrons**) in blood and a corresponding increase of fatty substances called **triglycerides**. Chylomicrons also known as ultra low-density lipoproteins (ULDL), are lipoprotein particles that consist of triglycerides (85–92%), phospholipids (6–12%), cholesterol (1–3%), and proteins (1–2%). They transport dietary lipids from the intestines to other locations in the body. The signs and symptoms associated with Familial Lipoprotein Lipase Deficiency include **recurrent attacks of pancreatitis** resulting in abdominal pain, nausea, vomiting, and loss of appetite.

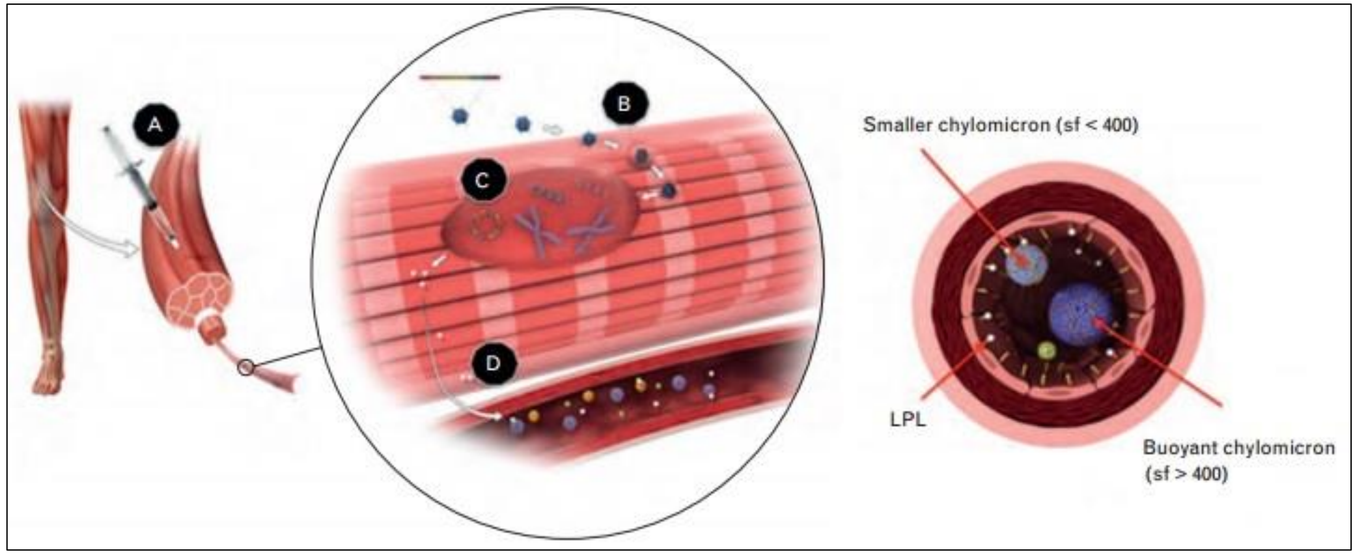


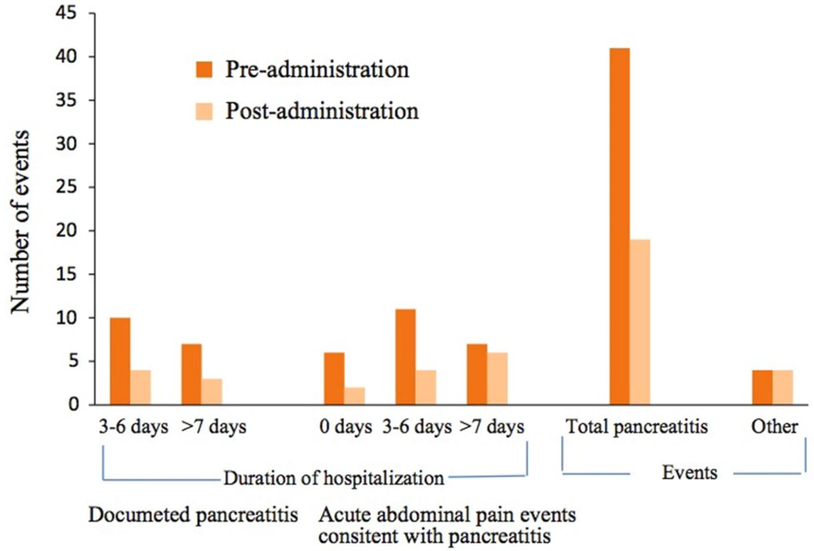


Lipoprotein lipase deficiency is a genetic disorder in which a person has **a defective gene for lipoprotein lipase**, which leads to very **high triglycerides**, which in turn causes stomach pain and deposits of fat under the skin, and which can lead to **problems with the pancreas** and liver, which in turn can lead to diabetes. Lab tests show massive accumulation of chylomicrons in the plasma and corresponding **severe hypertriglyceridemia**. Typically, the plasma in a **fasting blood sample appears creamy**. **Familial LPL deficiency** should be considered in anyone with **severe hypertriglyceridemia** and the **chylomicronemia syndrome**. Patients with this disease need to be **on a strict low-fat diet** and are prone to **recurring attacks of pancreatitis**, which is a **severe** and **life-threatening complication**.

Glybera (Glibera; AMT-011; **AAV1-**LPLS447X) is a type of advanced therapy medicine called a '**gene therapy product**'. This is a type of medicine that works by delivering genes into the body. The active substance in Glybera, alipogene tiparvovec, is derived from an **AAV1 virus** that has been modified so it can carry **the lipoprotein lipase gene** into the body's cells. Glybera is used to treat **adults** with **lipoprotein lipase deficiency** who have severe or multiple attacks of **pancreatitis** (inflammation of the pancreas) **despite maintaining a low-fat diet**. When injected into the muscles, it corrects the lipoprotein lipase deficiency by enabling the muscle cells to produce the enzyme. The drug is administered via a series of injections into the leg muscles – as many as 60, all in one session. The amount of the medicine to inject and the number of injections depend on the patient's weight. The enzyme produced by these cells can then help to **break down fats in the blood**, **reducing the number of pancreatitis attacks** and the severity of the disease. For **three days before** Glybera treatment and for **twelve weeks after,** patients are given **immunosuppressive treatment** to reduce the reaction of the body's immune system against the medicine. Due to the multiple injections required, it is advisable to give it with **a spinal** or **regional anesthetic**. The modified viral material used in Glybera does not cause infections and cannot make copies of itself.

Glybera is only for patients whose disease has been **confirmed by** appropriate **genetic testing** and who **have detectable levels of the lipoprotein lipase enzyme in their blood**. Because the number of patients with lipoprotein lipase deficiency is low, the disease is considered '**rare**', and Glybera was designated an '**orphan medicine**' (a medicine used in rare diseases) on 8 March 2004. The medicine can only be obtained with a **prescription**. Glybera should only be prescribed and given under the supervision of a doctor with **expertise in treating lipoprotein lipase deficiency** and **in gene therapy**. It is a one-time treatment intended **to last at least ten years**. Alipogene tiparvovec, sold under the brand name **Glybera** was recommended for approval by the European Medicines Agency **in July 2012**, and approved by **the European Commission in November** of the same year. **It was the first marketing authorization for a gene therapy treatment in Europe**.



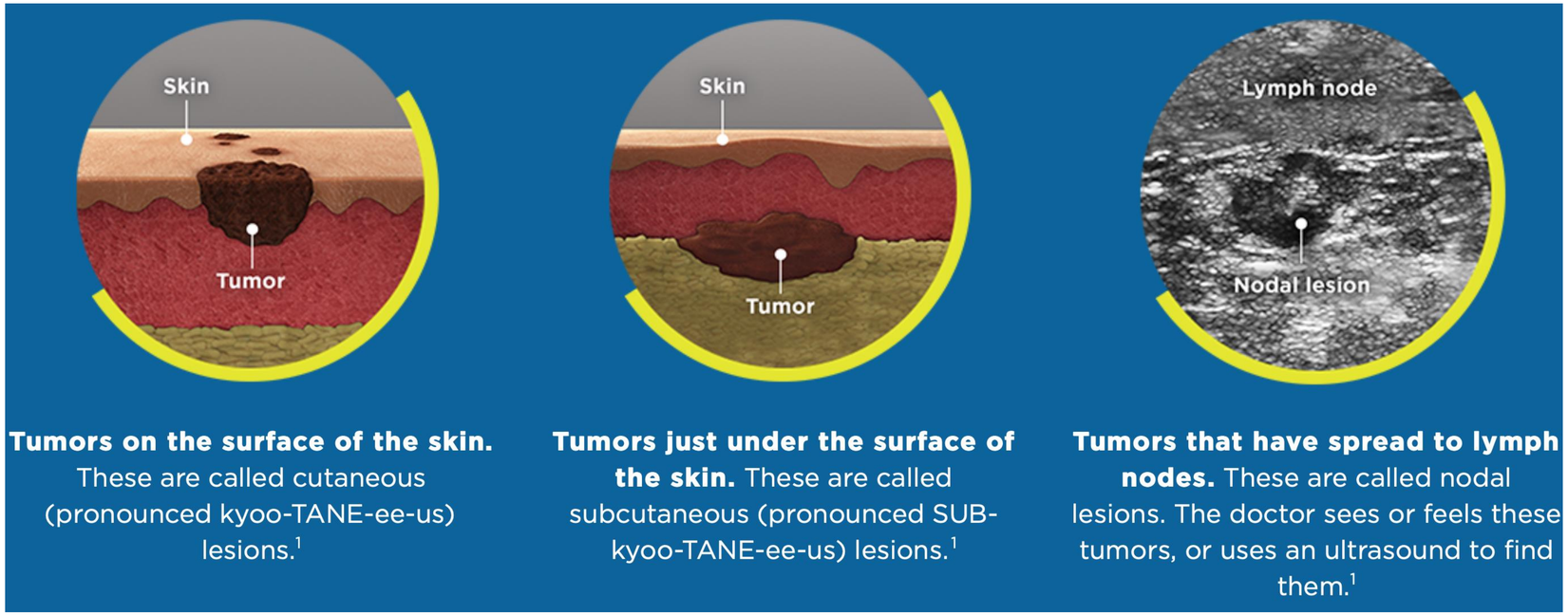


Glybera has been studied in **27 patients** with **lipoprotein lipase deficiency on a low-fat diet**. The majority of patients who received Glybera also received **immunosuppressive** treatment. The main measures of effectiveness were the **reduction in blood fat levels** after meals and **the reduction in the number of pancreatitis attacks**. The data showed **a reduction in blood fat levels** after meals in some patients. There was also **a reduction in the number of pancreatitis attacks** in some patients, as well as **fewer hospital admissions and stays** in intensive care units. Although there were data for only a small number of patients, the results indicate that Glybera would be of **benefit** to those patients with **severe or multiple pancreatitis attacks**.

After careful consideration of all the evidence and the circumstances of the disease, including its extreme rarity, the CHMP concluded that the results from studies showed that **the benefits of Glybera outweigh its risks in patients with severe or multiple pancreatitis attacks despite following a low-fat diet**. This is a subgroup of severely affected patients with a high unmet medical need. The CHMP therefore recommended that the medicine be granted marketing authorization. Glybera has been **authorized under 'exceptional circumstances**' as it has not been possible to obtain complete information about the medicine, because of the rarity of the disease. Every year, the European Medicines Agency will review any new information that becomes available and this summary will be updated as necessary. Under the terms of the authorization, the company is required to provide further data on **fat levels in the blood** after meals and on **the immune response to Glybera** in new patients. The company will also provide data from a registry to monitor the outcome of patients treated with Glybera, and will add a step to the manufacturing of the product to improve the safety profile.

With treatment **cost of €1m+ per patient**, Glybera was **the most expensive therapy ever approved in Europe**. In April 2017, Uniqure has decided **to terminate post-marketing studies** required for prolongation of its existing EU conditional market approval. Glybera gained infamy as the "**million-dollar drug**" and **proved commercially unsuccessful** for a number of reasons. Its cost to patients and payers, together with **the rarity of LPLD**, **high maintenance costs** to its manufacturer uniQure, and **failure to achieve approval in the US**, led to uniQure **withdrawing the drug after two years on the EU market**. The company has in fact been losing money on the drug, as **the cost of manufacturing** each dose is so high, and claims it will now **save $2 million annually** by withdrawing the drug. As of 2018, **only 31 people** worldwide have ever been **administered Glybera**, and uniQure has no plans to sell the drug in the US or Canada.

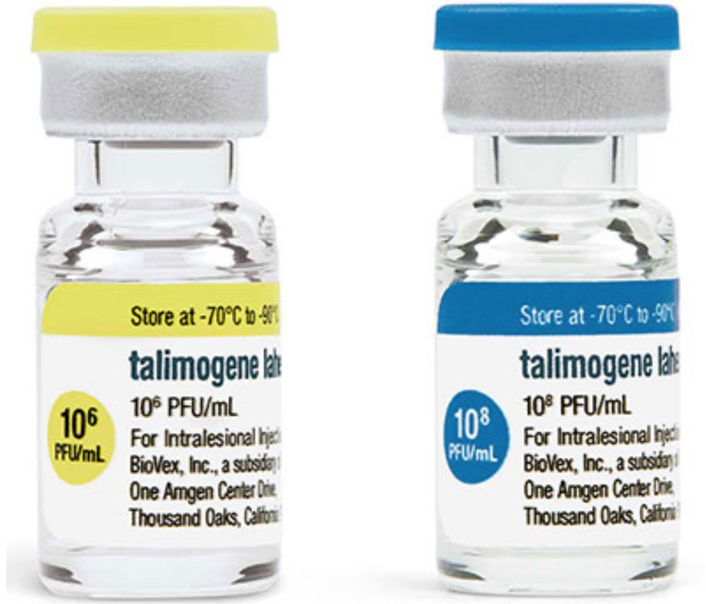
Drugs volume 75, pages 175–182 (2015)



Tumors on the surface of the skin. These are called cutaneous (pronounced kyoo-TANE-ee-us) lesions.[1]

Tumors just under the surface of the skin. These are called subcutaneous (pronounced SUB-kyoo-TANE-ee-us) lesions.[1]

Tumors that have spread to lymph nodes. These are called nodal lesions. The doctor sees or feels these tumors, or uses an ultrasound to find them.[1]

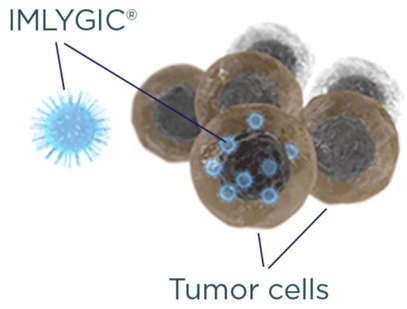


Melanoma, also redundantly known as **malignant melanoma**, is a type of **skin cancer** that develops from **the pigment-producing cells** known as **melanocytes**. Melanomas typically occur in the skin, but may rarely occur in the mouth, intestines, or eye. Melanoma is the most dangerous type of skin cancer. **Metastatic melanoma** continues to be one of **the most difficult-to-treat cancers** because it is often **insensitive to chemotherapy**, can be **highly aggressive** and can require several different types of treatment depending on the stage and location of the disease and health of the patient. Despite new therapeutic options, additional treatments are needed – particularly for patients with metastatic disease.

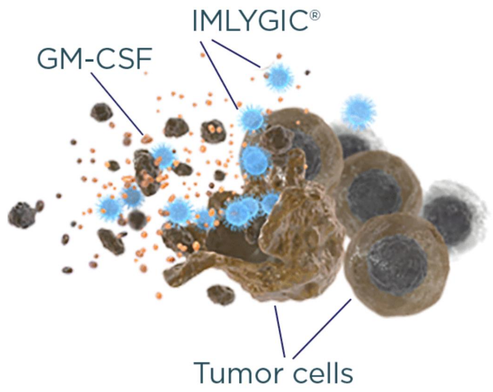
IMLYGIC (talimogene laherparepvec **T-VEC**) is a genetically modified **oncolytic viral therapy** indicated for the local treatment of **unresectable cutaneous, subcutaneous,** and **nodal lesions** in patients with **melanoma** recurrent after initial surgery. T-VEC is administered through injection into the cutaneous, subcutaneous and nodal lesions that are visible or detected by ultrasound. It is injected into the tumors directly, where it replicates and produces an immunostimulatory protein **granulocyte macrophage colony stimulating factor (GMC-SF)** that causes rupturing and killing of the tumor cells in a process known as **cell lysis**. **Tumor-derived antigens** are released through the ruptured tumors that along with virally derived GM-CSF protein may produce an **anti-tumor immune response**. While T-VEC is using the cell's translation machinery to replicate, it also uses it to make the cell create GM-CSF. GM-CSF is secreted or released when the cancer cell bursts, **attracting dendritic cells to the site**, which pick up the tumor antigens, process them, and then present them on their surface to **cytotoxic (killer) T cells** which in turn sets off an immune response.

T-VEC is a biopharmaceutical drug, **an oncolytic herpes virus** that was created by genetically engineering a strain of **herpes simplex virus 1** (**HSV-1**) **taken from a person infected with the virus**, rather than a laboratory strain. **Both copies** of the viral gene coding for **the infected cell protein** (**ICP34.5**) were deleted and replaced with the gene coding for **human GM-CSF**, and the gene coding for **ICP47** was **removed**. Deletion of ICP 34.5 gene promotes **specific replication** of IMLYGIC® in tumor tissue. In wild herpes virus, ICP47 suppresses the immune response to the virus via down-regulation of antigen presentation; it was removed because the drug was designed with the intention of activating the immune system. Inserted GM-CSF may **recruit and activate antigen-presenting cells**, which can process and present TDA to promote **an effector T-cell response**.

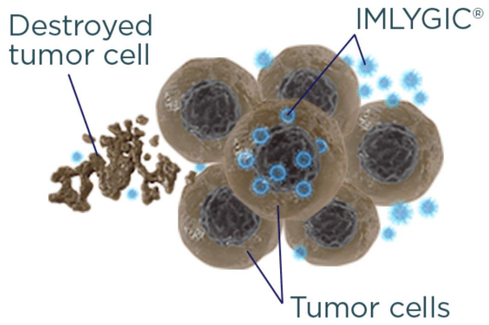
IMLYGIC®
(talimogene laherparepvec)
INTRALESIONAL INJECTION



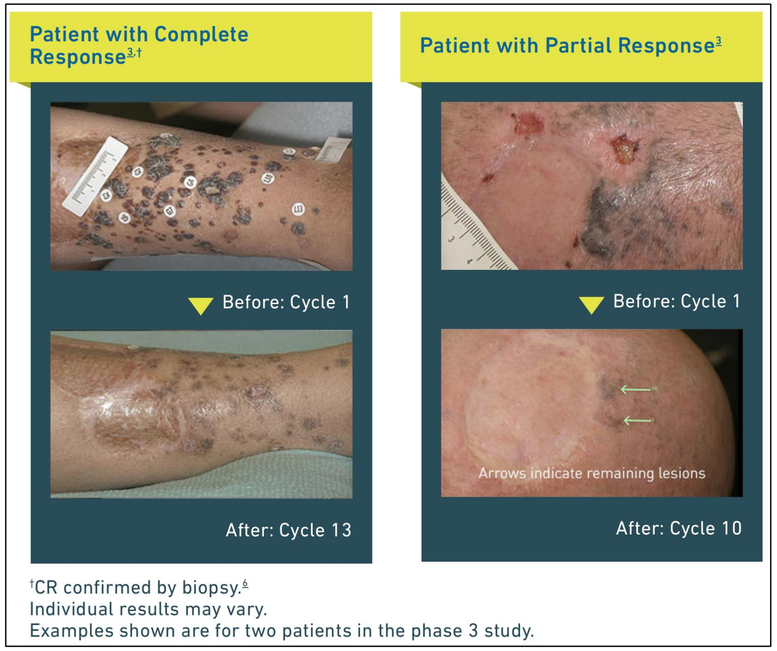
IMLYGIC® is injected directly into melanoma tumors on your skin or lymph glands.[1]



IMLYGIC® multiplies inside the tumor cells and may trigger your immune system to fight the cancer.[1]



IMLYGIC® continues to invade neighboring tumor cells.[1,6]

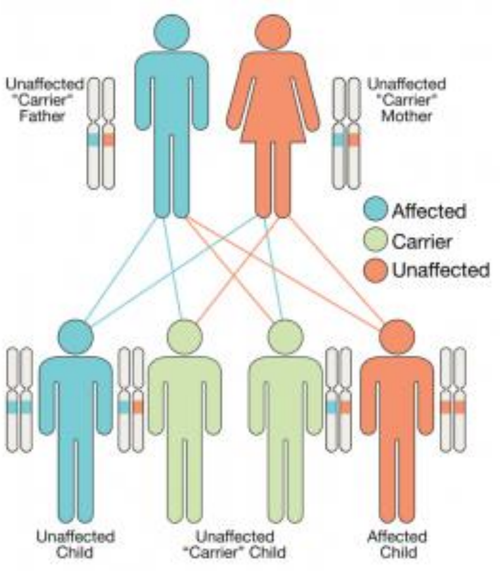


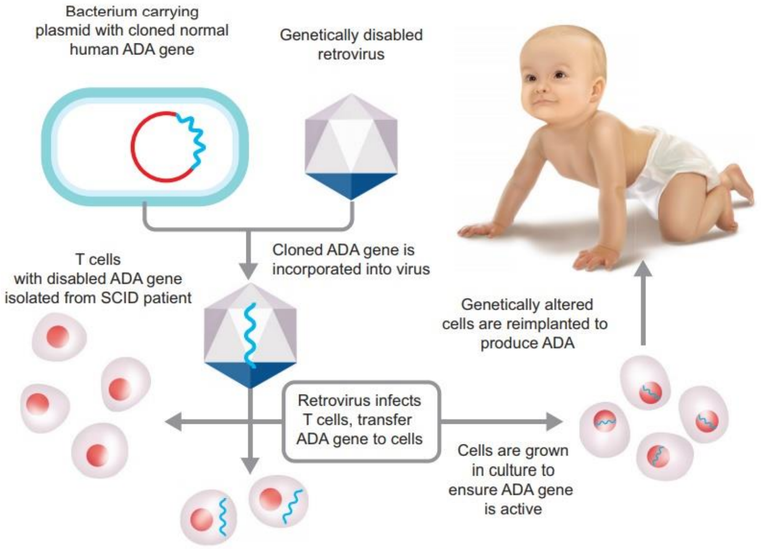
[†]CR confirmed by biopsy.[4]
Individual results may vary.
Examples shown are for two patients in the phase 3 study.

The virus invades both cancerous and healthy cells, but it **cannot** productively replicate in healthy tissue because it **lacks Infected cell protein 34.5 (ICP34.5)**. When cells are infected with a virus they shut down and die, but ICP34.5 blocks this stress response, allowing the virus to hijack the cell's translation machinery to replicate itself. A herpes virus lacking the gene coding for ICP34.5 cannot replicate in normal tissue. However, in many cancer cells the stress response is already disrupted, so **a virus lacking ICP34.5 can still replicate in tumors**. After the virus has replicated many times, the cell swells and finally bursts, killing the cell and releasing the copies of the virus, which can then infect nearby cells.

The approval of IMLYGIC is based on data involving **436 patients** from Study 005/05, referred to as **OPTiM**. **OPTiM** was a **Phase 3**, multicenter, open-label, randomized clinical trial **comparing IMLYGIC to GM-CSF** in patients with **advanced melanoma** (Stage IIIB, IIIC, or IV) **that was not surgically resectable**. The primary endpoint of the study was durable response rate (DRR), defined as the percent of patients with complete response (CR) or partial response (PR) **maintained continuously for a minimum of six months**. **The final four year analysis** from the pivotal phase 3 study upon which T-VEC was approved by the FDA showed a **31.5% response rate with a 16.9% complete response (CR) rate**. There was also a substantial and statistically significant **survival benefit in patients with earlier metastatic disease** (stages IIIb-IVM1a) and in patients who hadn't received prior systemic treatment for melanoma. The earlier stage group had a reduction in the risk of death of approximately 50% with one in four patients appearing to have met, or be close to be reaching, the medical definition of cure. **Real world use of T-VEC has shown response rates of up to 88.5% with CR rates of up to 61.5%.** Around half of people treated with T-VEC in clinical trials experienced fatigue and chills; around 40% had fever, around 35% had nausea, and around 30% had flu-like symptoms as well as pain at the injection site.

T-VEC was approved by the **U.S. Food and Drug Administration** to treat melanoma **in October 2015**. **It was the first approval of an oncolytic virus in the West.** As of 2016, T-VEC has been studied in early stage clinical trials in **pancreatic cancer**, **soft-tissue sarcoma**, and **head and neck squamous-cell carcinoma**; it had also been tested in combination with **checkpoint inhibitors** ipilimumab and pembrolizumab.





Adenosine Deaminase Severe Combined Immunodeficiency Disorder (**ADA-SCID**), also known as **bubble-boy disease**, severely impacts **the immune system function**, which **potentiates everyday infections to life-threatening illnesses**. ADA-SCID is **a rare inherited condition** in which there is a change (**mutation**) in the gene needed to make an enzyme called **adenosine deaminase**. Because ADA is **essential** for maintaining **healthy lymphocytes** (white blood cells that fight off infections), the immune system of people with ADA-SCID does not work properly and without effective treatment they **rarely survive more than two years**. It is an **ultra-rare immune disorder**, caused by a faulty gene inherited from both parents that stops the production of adenosine deaminase. Without this enzyme, the body is unable to break down a toxic substance called **deoxyadenosine**. The toxin builds up and **destroys** infection-fighting **lymphocytes**. Children born with ADA-SCID are severely **impaired** in their ability to **fight infections**. The disorder can also lead to various non-immunological health problems, including a failure to grow and develop normally, hearing loss and liver and kidney problems. Symptoms normally appear in the first six months of life. The disease is usually fatal in the first two years of life, unless the function of the immune system can be restored.

Immune reconstitution in ADA deficiency can be achieved by **bone marrow transplantation**, **enzyme replacement**, or **gene therapy**, nonetheless recovery of immune functions may vary depending on the applied treatment and patient's characteristics. Typically, ADA-SCID sufferers who receive stem cell transplants from genetically-matched siblings have a good chance of survival and recovery of the immune system. However, survival of patients who have no related matched donor is poor, mainly because of the risk of **graft versus host disease**, whereby the T-cells in the donated tissue attack the body cells of the recipient. This requires **immunosuppressant** treatment and increases **the risk of infection**, the main **cause of death after transplantation**. Some patients received enzyme replacement therapy with **pegylated adenosine deaminase (PEG-ADA)** on a compassionate use basis, although this treatment is not authorized anywhere in the EU. Enzyme replacement requires **lifelong weekly injections**. Based on the experience so far, there appears to be **a loss in immune function over time** in patients receiving PEG-ADA, making them again more susceptible to infections. ADA-SCID has been **the pioneer disease for the development of human gene therapy**. It is based on the reinfusion of **autologous HSC transduced** with **a retroviral vector** containing the **ADA cDNA**. Variable degrees of immune reconstitution can be achieved by these treatments, but onset of autoimmunity is of concern in post-treatment ADA-SCID patients.

Strimvelis is manufactured from a patient's own **immature bone marrow cells** (called **CD34+ cells**) into which a normal adenosine deaminase enzyme gene has been inserted. After these cells are injected back into the patient, the cells are able to develop into **the different types of blood and immune cells**. This is expected to give the patient **life-long ability to produce lymphocytes** that can fight off infections. Using a patient's own cells **avoids the risk of graft versus host disease**, and **lowers the risk of infections due to immunosuppression**. It also **reduces the dose of chemotherapy** needed to prepare a patient for treatment compared to bone marrow transplant. Furthermore, gene therapy is **not dependent upon a donor search**, so it can be made available to any patient.

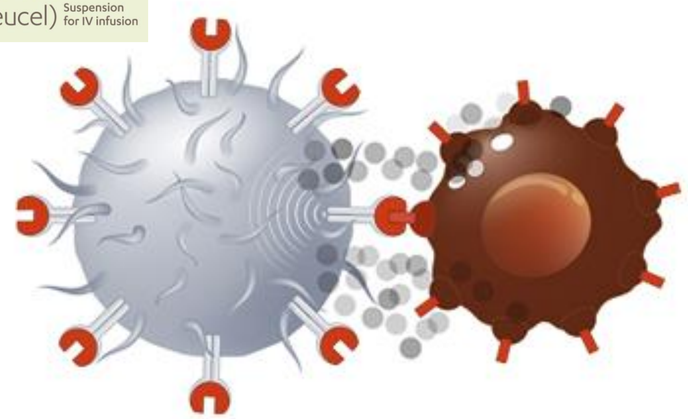
The treatment is personalized for each person; hematopoietic stem cell (HSCs) are extracted from the person and purified so that only **CD34-expressing cells** remain. Those cells are cultured with **cytokines and growth factors** and then transduced with **a gamma-retrovirus containing ADA gene** and then reinfused into the person. These cells take root in the person's bone marrow, replicating and creating cells that mature and create normally functioning adenosine deaminase protein, resolving the problem. Prior to extraction, the person is treated with **granulocyte colony-stimulating factor** in order to **increase the number of stem cells and improve the harvest**; after that but prior to reinfusion, the person is treated with **busulfan or melphalan** to kill as many of the person's existing HSCs to increase the chances of the new cells' survival.

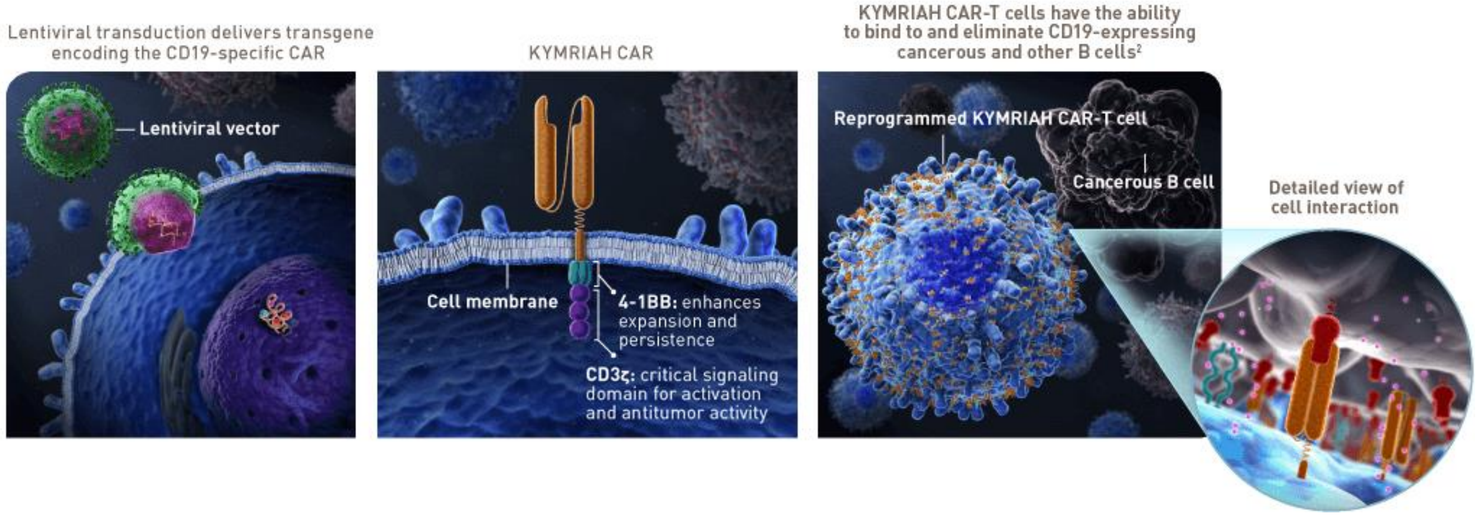
The treatment was developed at **San Raffaele Telethon Institute for Gene Therapy** and developed by **GlaxoSmithKline (GSK**). **In April 2016**, a committee at the **European Medicines Agency** (**EMA**) recommended marketing approval for **its use in children with adenosine deaminase deficiency, for whom no matched HSC donor is available**, on the basis of a clinical trial that produced a **100% survival rate**; the median follow-up time was **7 years** after the treatment was administered. 75% of people who received the treatment needed no further enzyme replacement therapy. Around 80% of patients have no matched donor. **Strimvelis was approved by the European Commission on 27 May 2016**. The price for the treatment was set at **€594,000**, twice the annual cost of enzyme replacement therapy injections. **Enzyme replacement therapy** for ADA requires weekly injections and costs about **US$4.25 million for one patient over ten years**. The treatment is an **ex vivo gene therapy** for severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID), and is the **second gene therapy** to ever be approved **in Europe** after the 2012 green light for the Glybera. There are currently only around **15 cases** of the disorder across Europe **each year**, with an estimated **350 patients worldwide**.

As of today, **some challenges exist** for scientists employing **Strimvelis** such as challenges associated with **using retroviruses** as "**vectors**" that deliver the genes. For example, there is a small chance that a deactivated virus may insert the corrected gene into an incorrect region of the genome, which could lead to **the activation of cancer genes**. This is what happened in the 2000 trial of **SCID-X1**, which cured patients of SCID, but simultaneously dysregulated cell growth resulting in **leukemia**. The reason why **gamma retroviral vectors are prone to increased genotoxicity** might be something to do with their genome containing long-terminal repeats, harboring intact promoter and enhancer sequences which, **upon integration in the vicinity of a growth promoting gene** of the host cell, can enhance its transcription, leading to **abnormal cell growth** (Verma I, Science 2013).

The potential of gene therapies as cures for some hard-to-treat genetic diseases can be very attractive. But one such product is now suspected of causing a serious safety problem. **Orchard Therapeutics** stated its Strimvelis treatment, approved by European authorities in 2016 to treat the rare inherited condition ADA-SCID, has been linked to **a patient's leukemia** (2020). **Preliminary findings** suggest this diagnosis may be attributable to **an insertional event related to treatment with Strimvelis**. It's now investigating whether there's indeed a causal relationship. Since its 2016 EU approval—when it was owned by original developer GlaxoSmithKline—only **16 patients** have been treated with Strimvelis. The patient who developed leukemia had apparently been treated under a GSK compassionate use program in 2016. No more patients will get the therapy before the investigation is complete. **The drug was never approved in the U.S**. Besides Strimvelis, **Orchard is also developing** OTL-101, which uses **a lentivirus** to insert a functional copy of the ADA gene into a patient's cells. The drug is currently undergoing a registrational trial and has won breakthrough and **orphan drug designations from the FDA**. All the gene therapy candidates in Orchard's pipeline use **lentiviral vectors that have been specifically designed to avoid insertional oncogenesis after administration**. **No dangerous gene insertion** has been reported around **lentiviral vector-based stem cell gene therapy** in **any indication**.

KYMRIAH™ (tisagenlecleucel) Suspension for IV infusion





Acute lymphoblastic leukemia (ALL) is a **cancer** of the **lymphoid line of blood cells** characterized by the development of large numbers of **immature lymphocytes**. The cancerous cell in ALL is **the lymphoblast**. Normal lymphoblasts develop into mature, **infection-fighting B-cells or T-cells**, also called **lymphocytes**. Signals in the body control the number of lymphocytes so neither too few nor too many are made. In ALL, both the normal development of some lymphocytes and the control over the number of lymphoid cells become defective. Symptoms may include **feeling tired**, pale skin color, **fever**, easy bleeding or bruising, **enlarged lymph nodes**, or **bone pain**. As an acute leukemia, ALL progresses rapidly and is **typically fatal** within weeks or months if left untreated. The disease progresses quickly and is **the most common childhood cancer in the U.S**. The National Cancer Institute estimates that approximately **3,100 patients** aged 20 and younger are diagnosed with ALL **each year**. ALL can be of either T- or B-cell origin, with **B-cell the most common**. ALL is typically treated initially with **chemotherapy** aimed at bringing about remission. This is then followed by **further chemotherapy** typically **over a number of years**. Treatment usually also includes **intrathecal chemotherapy** since systemic chemotherapy can have limited penetration into the central nervous system and **the central nervous system is a common site for relapse** of acute lymphoblastic leukemia. Treatment can also include **radiation therapy** if spread to the brain has occurred. **Stem cell transplantation** may be used if the disease recurs following standard treatment.

Chimeric antigen receptors (CARs) have been developed as a promising **immunotherapy for ALL**. This technology uses a specific **chimeric cell surface receptor** with components from both a **T-cell receptor** and an **antibody** specific to CD19 protein on the cancer cell. **CD19** is a molecule found on **all B-cells** and can be used as a means of distinguishing the **potentially malignant B-cell population**. The T cells are engineered to target CD19 that is common on B cells. In this scenario, a chimeric T cell receptor (CAR-T) is expressed on the surface of the T cell. Inserting the transgenic DNA encoding CAR into the immune effector T cell can be accomplished by several methods. Most commonly, this is done using **a lentivirus** that encodes the transgene. **Pseudotyped**, **self-inactivating (SIN) lentiviruses** are an effective method for the stable insertion of a desired transgene into the target cell. Other methods include **electroporation** and **transfection**, but these are limited in their efficacy as transgene expression diminishes over time.

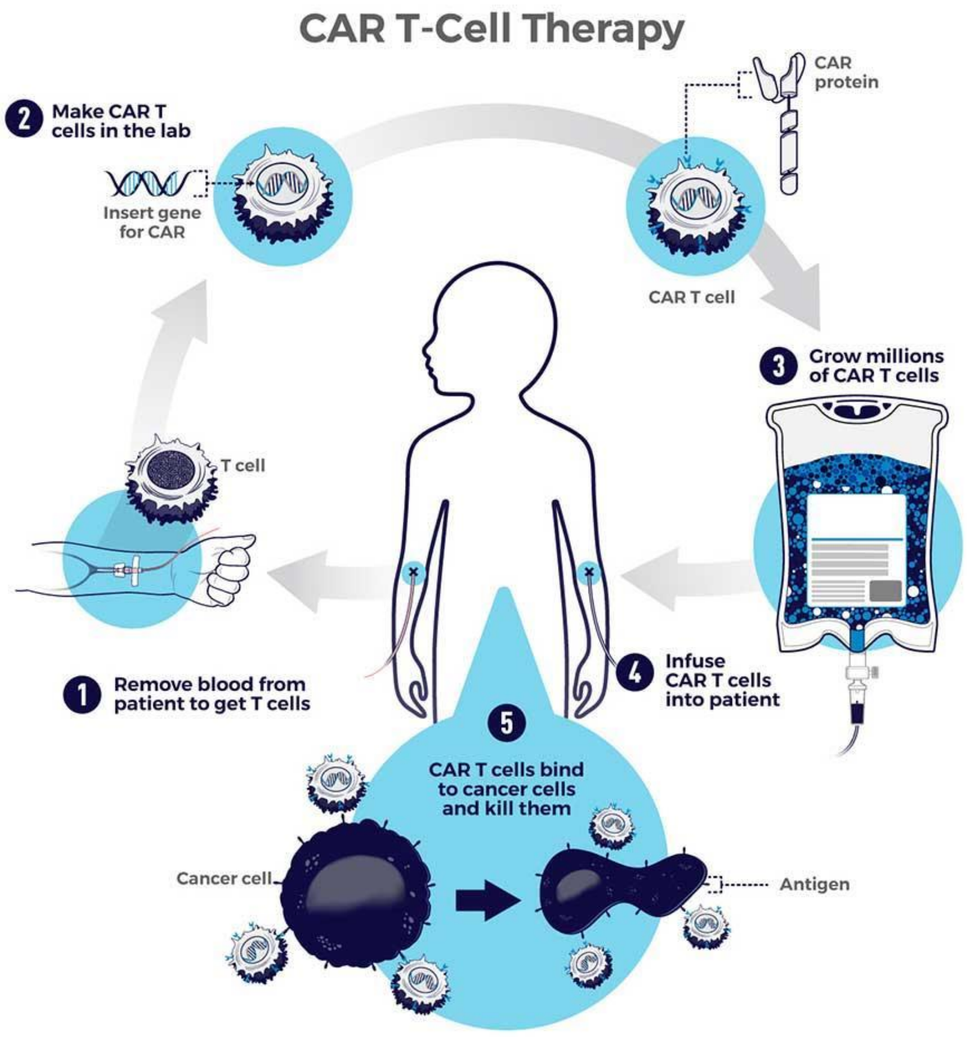
Kymriah is a genetically-modified autologous T-cell immunotherapy. It is approved for use in pediatric and young adult patients **up to 25 years of age** with **B-cell ALL** and is intended for patients whose cancer has not responded to (refractory) or has returned after initial treatment (relapsing), which occurs in an estimated 15-20 percent of patients. It is also used in patients with **large B-cell lymphoma or follicular lymphoma** (FL), two types of non-Hodgkin lymphoma, that have relapsed or are refractory after having at least two other kinds of treatment. It was invented and initially developed at the **University of Pennsylvania**; **Novartis completed development**, **obtained FDA approval, and markets the treatment**. **In August 2017,** it became **the first FDA-approved treatment** of CAR-T cell therapy that included a gene therapy step in the United States.

Each dose of Kymriah is a customized treatment created using an **individual patient's own T-cells**, a type of white blood cell known as a lymphocyte. The patient's T-cells are collected and sent to **a manufacturing center** where they are **genetically modified** to include a new gene that contains a specific protein (a chimeric antigen receptor or **CAR**) that directs **the T-cells to target** and **kill leukemia cells** that have a specific antigen (CD19) on the surface. It uses **the 4-1BB co-stimulatory domain** in its CAR **to improve response**. Once the cells are modified, they are infused back into the patient to kill the cancer cells.

The safety and efficacy of Kymriah were demonstrated in one multicenter clinical trial of **63 pediatric and young adult patients** with relapsed or refractory B-cell precursor ALL. **The overall remission rate** within three months of treatment was **83 percent**. **After one year 79 percent of patients were still alive**. Patients with **ALL who fail chemotherapy** typically have **only a 16 percent to 30 percent chance of survival**.

Treatment with Kymriah has the potential to cause **severe side effects**. Serious side effects occur in most patients. It carries a boxed warning for **cytokine release syndrome (CRS)**, which **is a systemic response** to the activation and proliferation of CAR T-cells causing **high fever** and **flu-like symptoms,** and **for neurological events**. Both **CRS** and **neurological events** can be **life-threatening**. Other severe side effects of Kymriah include **serious infections**, low blood pressure (**hypotension**), **acute kidney injury**, **fever**, and decreased oxygen (**hypoxia**). Most symptoms appear **within one to 22 days** following infusion of Kymriah. Since the CD19 antigen is also present on normal B-cells, and Kymriah will **also destroy those normal B cells** that **produce antibodies**, there may be **an increased risk of infections** for a prolonged period of time. Serious infections occur in around three in ten diffuse large B-cell lymphoma (DLBCL) patients. According to Novartis, the treatment will be administered at **specific medical centers** where staff have been trained to manage possible reactions to this new type of treatment.

The FDA is **requiring** that **hospitals and their associated clinics** that dispense Kymriah **be specially certified**. As part of that certification, staff involved in the prescribing, dispensing, or administering of Kymriah are required to be **trained to recognize and manage CRS and neurological events**. Additionally, **the certified health care settings** are required to have protocols in place to ensure that Kymriah is only given to patients after verifying that **tocilizumab** is available for immediate administration. **The Risk Evaluation and Mitigation Strategy program (REMS)** specifies that patients be informed of the signs and symptoms of CRS and neurological toxicities following infusion – and of the importance of promptly returning to the treatment site if they develop fever or other adverse reactions after receiving treatment with Kymriah. To further evaluate the long-term safety, Novartis is also required to conduct **a post-marketing observational study** involving patients treated with Kymriah.



CAR T-cell therapy is a type of treatment in which a patient's T cells are genetically engineered in the laboratory so they will bind to specific proteins (antigens) on cancer cells and kill them. **(1)** A patient's T cells are removed from their blood. Then, **(2)** the gene for a special receptor called a chimeric antigen receptor (CAR) is inserted into the T cells in the laboratory. The gene encodes the engineered CAR protein that is expressed on the surface of the patient's T cells, creating a CAR T cell. **(3)** Millions of CAR T cells are grown in the laboratory. **(4)** They are then given to the patient by intravenous infusion. **(5)** The CAR T cells bind to antigens on the cancer cells and kill them.

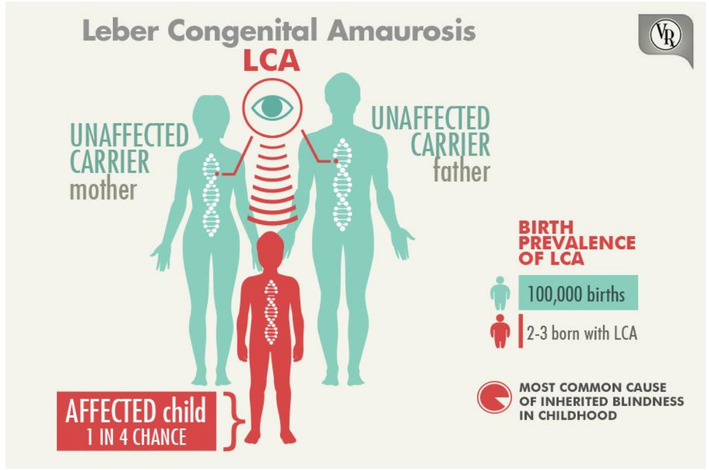
cancer.gov

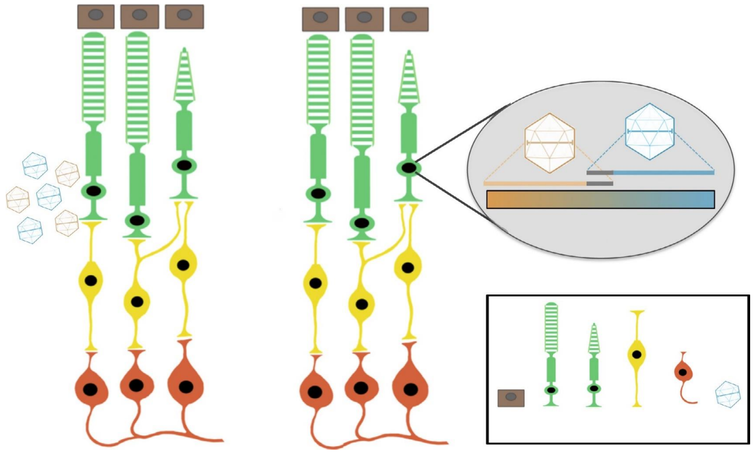
FDA-Approved CAR T-Cell Therapies

Generic Name	Brand Name	Target Antigen	Targeted Disease	Patient Population
Tisagenlecleucel	Kymriah	CD19	B-cell acute lymphoblastic leukemia (ALL)	Children and young adults with refractory or relapsed B-cell ALL
			B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
Axicabtagene ciloleucel	Yescarta	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
			Follicular lymphoma	Adults with relapsed or refractory follicular lymphoma
Brexucabtagene autoleucel	Tecartus	CD19	Mantle cell lymphoma (MCL)	Adults with relapsed or refractory MCL
			B-cell acute lymphoblastic leukemia (ALL)	Adults with refractory or relapsed B-cell ALL
Lisocabtagene maraleucel	Breyanzi	CD19	B-cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B-cell NHL
Idecabtagene vicleucel	Abecma	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma
Ciltacabtagene autoleucel	Carvykti	BCMA	Multiple myeloma	Adults with relapsed or refractory multiple myeloma

CAR T cells are the equivalent of giving patients **a living drug**. As their name implies, **T cells**—which help orchestrate the immune response and directly kill cells infected by pathogens—are **the backbone** of CAR T-cell therapy. Currently available CAR T-cell therapies are customized for each individual patient. They are made by **collecting T cells from the patient** and **re-engineering them in the laboratory** to produce proteins on their surface called chimeric antigen receptors, or **CARs**. The CARs recognize and bind to specific proteins, or **antigens**, **on the surface of cancer cells**. **These receptors are synthetic molecules, they don't exist naturally**. After the revamped T cells are “expanded” into the millions in the laboratory, they’re then infused back into the patient. If all goes as planned, the CAR T cells will continue to multiply in the patient's body and, with guidance from their engineered receptor, recognize and kill any cancer cells that harbor the target antigen on their surfaces. The CAR T-cell therapies approved **by FDA** to date target one of two antigens on B cells, **CD19** or **B-cell maturation antigen (BCMA)**.

Since 2017, **six CAR T-cell therapies** have been approved **by the Food and Drug Administration (FDA).** All are approved for the treatment of blood cancers, including **lymphomas**, some forms of **leukemia**, and, most recently, **multiple myeloma**. Nevertheless, after years of painstaking research, CAR T-cell therapies have entered **the mainstream of cancer treatment**. CAR T cells are now widely available in the United States and other countries and have become a standard treatment for patients with **aggressive lymphomas**.





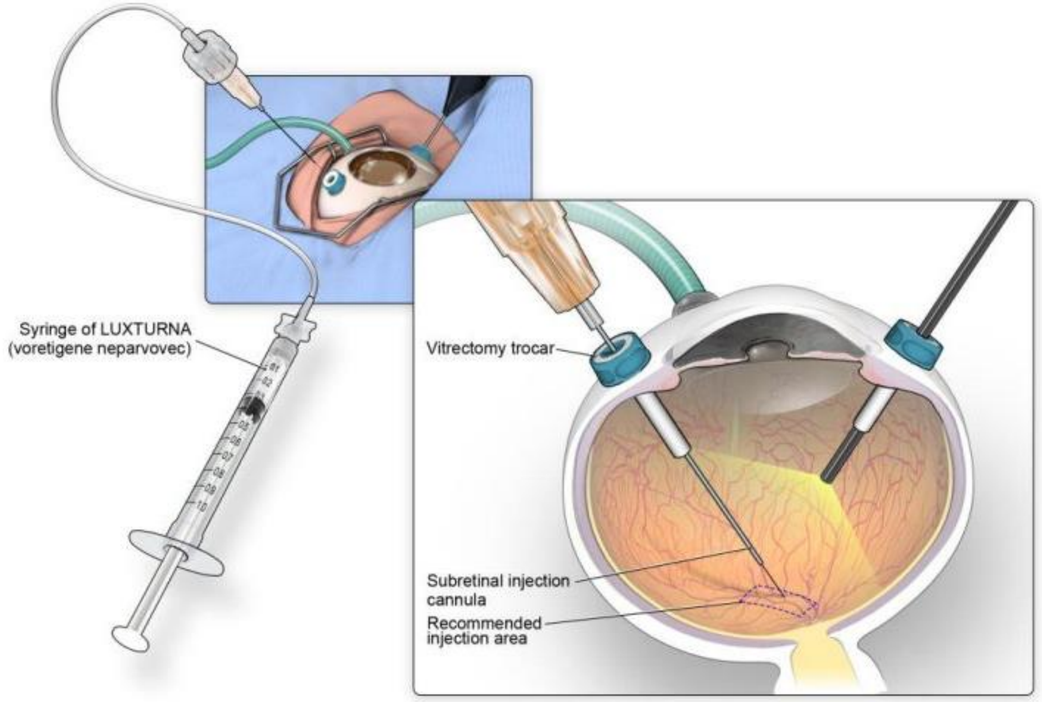
Trends in Molecular Medicine, August 2018, Vol. 24, No. 8

LUXTURNA™
voretigene neparvovec-rzyl
for subretinal injection

Retinal dystrophies are a group of **eye disorders** characterized by the **degeneration of** different parts of **the retina**. They are **genetically heterogeneous**, meaning **mutations in many different genes** may be responsible. Determining the specific gene mutation(s) is critical to understand the range of symptoms and treatment possibilities. **Leber congenital amaurosis (LCA)** is **the earliest and most severe form** of all **inherited retinal dystrophies** responsible for **congenital blindness**. The term **congenital** refers to a **condition present from birth** (not acquired) and **amaurosis** (Greek meaning darkening, dark, or obscure) refers to **a loss of vision** not associated with a lesion. However, beyond these general descriptions, the presentation of LCA can vary, because it is associated with **multiple genes**. LCA is typically characterized by **nystagmus**, sluggish or absent pupillary responses, and **severe vision loss or blindness**. It affects about 1 in 40,000 newborns.

One of the causes of these conditions is mutations in the gene encoding **retinal pigment epithelium–specific protein 65 kDa (RPE65)**. RPE65 is located in the retinal pigment epithelial cells and converts all-trans-retinol to 11-cis-retinol, which subsequently forms the chromophore, 11-cis-retinal, during the visual (retinoid) cycle. These steps are critical in the biological **conversion of a photon of light into an electrical signal within the retina**. Mutations in the RPE65 gene lead to reduced or absent RPE65 all-trans-retinyl isomerase activity, blocking the visual cycle and resulting in vision loss. Over time, accumulation of **toxic precursors** leads to **the death of retinal pigment epithelial cells**, and subsequently to progressive photoreceptor cell death. Individuals with **biallelic RPE65 mutation-associated retinal dystrophy** exhibit **vision loss**, including impaired visual function parameters such as **visual acuity** and visual fields often during childhood or adolescence; **this loss of vision ultimately progresses to complete blindness**. **RPE65 deficiency causes photoreceptor cell dysfunction** and **impaired vision from birth**. Severe dysfunction of **rod photoreceptor cells**, which are wholly reliant on retinal pigment epithelium–derived RPE65, causes **severely impaired night vision**. The function of **cone photoreceptor cells**, which mediate **vision in daylight**, **is relatively preserved in childhood** because cones have access to an alternative source of 11-cis retinal. However, progressive degeneration of both rod and cone photoreceptor cells, in association with local accumulation of **toxic retinyl esters**, results in severe **sight impairment** by early adulthood. There are an estimated **1,000–2,000 individuals in the US** with RPE65-IRDs.

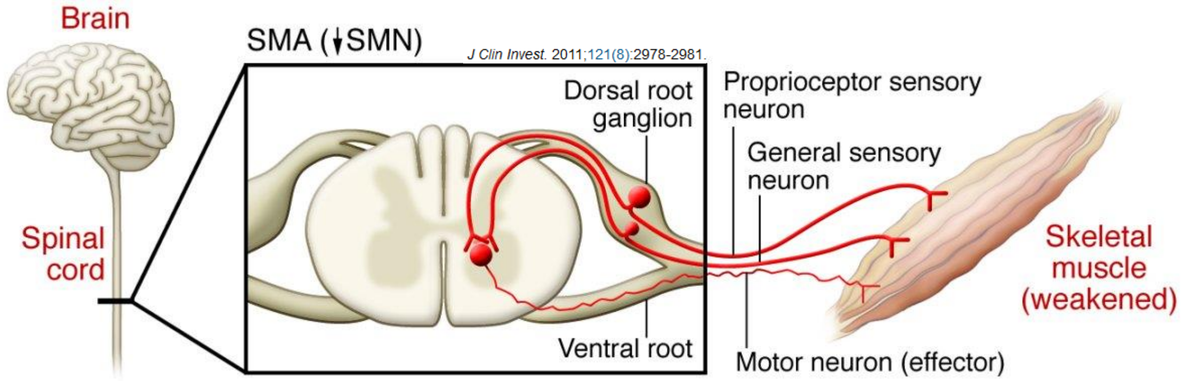
Injection of an **AAV2 vector containing human RPE65 cDNA** into the **subretinal space** results in transduction of retinal pigment epithelial cells with a cDNA encoding normal **human RPE65 protein** (**gene augmentation therapy**), providing the potential to restore the visual cycle. Augmentation of RPE65 in animal models of RPE65 deficiency can **improve retinal and visual function**, as assessed by means of **electroretinography (ERG)** and **observation of vision-guided behavior**, respectively. Since the target retinal cells are post mitotic cells, it is expected that a one-time administration of the gene product will provide benefit as long as the retina cells are viable. Gene therapy treatment does not produce new tissue so it is vital the patient **have sufficient viable retinal cells prior to administration**. This can be measured by **optical coherence testing (OCT)** documenting **a retinal layer > 100µm thick**.

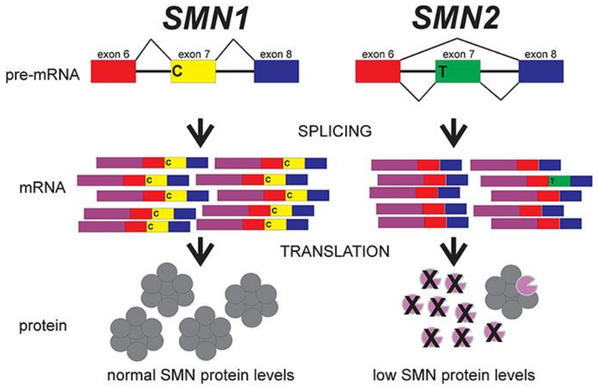


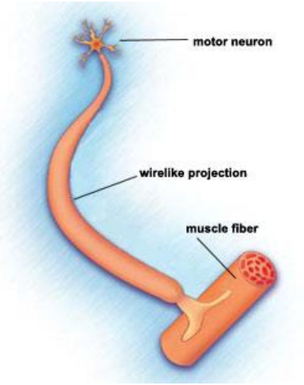
One form of LCA, in patients with **LCA2** bearing a mutation in the **RPE65 gene**, has been successfully treated in clinical trials using gene therapy. The results of **three early clinical trials** were published in 2008 demonstrating the safety and efficacy of using **adeno-associated virus** to deliver RPE65 gene therapy to restore vision in **LCA patients**. **In all three clinical trials**, patients **recovered functional vision** without apparent side effects. These studies, which used adeno-associated virus, have spawned a number of new studies investigating gene therapy for human retinal disease. The results of **a phase 1 trial** conducted **by the University of Pennsylvania and Children's Hospital of Philadelphia** and **published in 2009** showed **sustained improvement in 12 subjects** (ages 8 to 44) with RPE65-associated LCA after treatment with **AAV2-hRPE65v2**, a gene replacement therapy. **Early intervention was associated with better results**. In that study, patients were excluded based on the presence of particular **antibodies to the vector AAV2** and treatment was only administered to one eye as a precaution. **A 2010 study** testing the effect of administration of AAV2-hRPE65v2 in both eyes **in animals with antibodies present** suggested that **immune responses may not complicate use of the treatment in both eyes**.

Luxturna (Voretigene neparvovec) is an **adeno-associated virus vector-based gene therapy** indicated for the treatment of people with vision loss due to **inherited retinal dystrophy** caused by **confirmed biallelic RPE65 mutations. FDA approved Luxturna** developed by **Spark Therapeutics and Children's Hospital of Philadelphia** o**n 19 December 2017. Patients must have viable retinal cells** as a prerequisite for the intraocular administration of Luxturna. It is given as a **subretinal injection**. Not only has treatment with Luxturna **changed** the lives of people previously destined to live a life of blindness, but it has fueled interest in developing additional gene therapy reagents targeting numerous other genetic forms of inherited retinal disease. Clinical trial evidence shows that, in the short term, **Luxturna can improve vision and prevent the condition from getting worse**. **There is no long-term clinical evidence**, but it is biologically plausible that the treatment effect is likely to continue for a considerable time. The gene therapy is not a cure for the condition, but **substantially improves vision** in those treated. Treatment with LUXTURNA is **not recommended for patients younger than 12 months of age** because the retina is still growing, which may affect how LUXTURNA works.

The cost of the drug is around **850.000 USD per therapy** (425.000 USD per eye) which hinders its access to most patients. It is important to note that the treatment does not restore normal vision, and some patients may experience permanent vision loss or reduction of central visual acuity.







Spinal muscular atrophy (SMA) is a rare neuromuscular disorder that results in **the loss of motor neurons** and progressive muscle wasting. It is usually diagnosed in infancy or early childhood and if left untreated it is the **most common genetic cause of infant death**. It may also appear later in life and then have a milder course of the disease. The common feature is progressive weakness of voluntary muscles, with **arm, leg and respiratory muscles** being affected first. Associated problems may include poor head control, difficulties **swallowing**, scoliosis, and joint contractures. SMA is due to an abnormality (mutation) in the **survival of motor neuron protein (SMN1)** gene which encodes SMN, a protein necessary for survival of motor neurons. Loss of these neurons in the spinal cord **prevents signaling between the brain and skeletal muscles**. Another gene, **SMN2**, is considered a **disease modifying gene**, since usually the more the SMN2 copies, the milder is the disease course.

Human **chromosome 5** contains two nearly identical genes at location 5q13: a telomeric copy **SMN1** and a centromeric copy **SMN2**. In healthy individuals, the SMN1 gene codes the survival of motor neuron protein (SMN) which, as its name says, plays a crucial role in **survival of motor neurons**. The human genome harbors a paralogous SMN1 gene, SMN2, that differs only by a few nucleotides, and in particular by a **C to T transition in exon 7**. This base change causes the **skipping of exon 7** in most SMN2 transcripts and generates **a truncated unstable protein (SMNΔ7)** with low levels (approximately 10%) of full-length, functional SMN protein produced. In individuals affected by SMA, the SMN1 gene is mutated in such a way that it is unable to correctly code the SMN protein. Almost all people, however, have at least one functional copy of the SMN2 gene (with most having 2–4 of them) which still codes 10–20% of the usual level of the SMN protein, **allowing some neurons to survive**. In the long run, however, the reduced availability of the SMN protein results in gradual death of motor neuron cells **in the anterior horn of spinal cord and the brain**. Skeletal muscles, which all depend on these motor neurons for neural input, now have decreased innervation (also called **denervation**), and therefore have **decreased input from the central nervous system (CNS).** Decreased impulse transmission through the motor neurons leads to **decreased contractile activity of the denervated muscle**. Consequently, denervated muscles undergo **progressive atrophy**. Muscles of lower extremities are usually affected first, followed by muscles of upper extremities, spine and neck and, in more severe cases, pulmonary and mastication muscles.

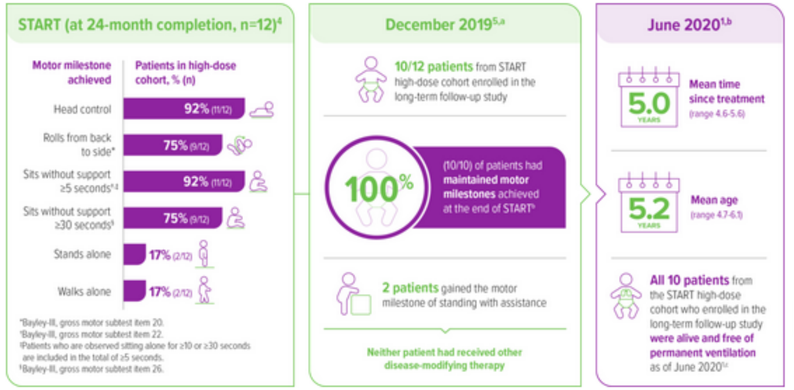
The severity of SMA symptoms is broadly related to how well **the remaining SMN2 genes** can **make up for the loss of function of SMN1**. This partly depends on the number of copies of the SMN2 gene present on the chromosome. While healthy individuals usually carry **two SMN2 gene copies**, people with SMA can have **anything between 1 and 5 (or more)** of them; the greater the number of SMN2 copies, the milder the disease severity. Thus, most **SMA type I babies have one or two SMN2 copies; people with SMA II and III usually have at least three SMN2 copies; and people with SMA IV normally have at least four of them**. However, the correlation between symptom severity and SMN2 copy number is not absolute and there seem to exist other factors affecting the disease phenotype. The management of SMA varies based upon the severity and type. In the most severe forms (types 0/1), individuals have the greatest muscle weakness requiring prompt intervention. Whereas the least severe form (type 4/adult onset), individuals may not seek the certain aspects of care until later (decades) in life. The diagnosis of SMA is based on symptoms and confirmed **by genetic testing**. Usually, the mutation in the SMN1 gene is inherited from both parents in **an autosomal recessive manner**, although in around 2% of cases it occurs during early development (de novo). About 1 in 6,000 to 10,000 babies worldwide are born with SMA. More than **25,000 people** in the United States have SMA.

ZOLGENSMA continues to provide durable efficacy over 5 years after treatment[1]

The long-term follow-up study is an ongoing follow-up of the Phase 1 START trial.

All 10 patients from the START high-dose cohort who enrolled in the long-term follow-up were alive and free of permanent ventilation as of June 2020[1,b]

[a]As of the last visit prior to December 2019 data cut.
[b]Based on adverse event reporting death, withdrawal, or permanent ventilation. There were no in-person assessments between December 2019 and June 2020.



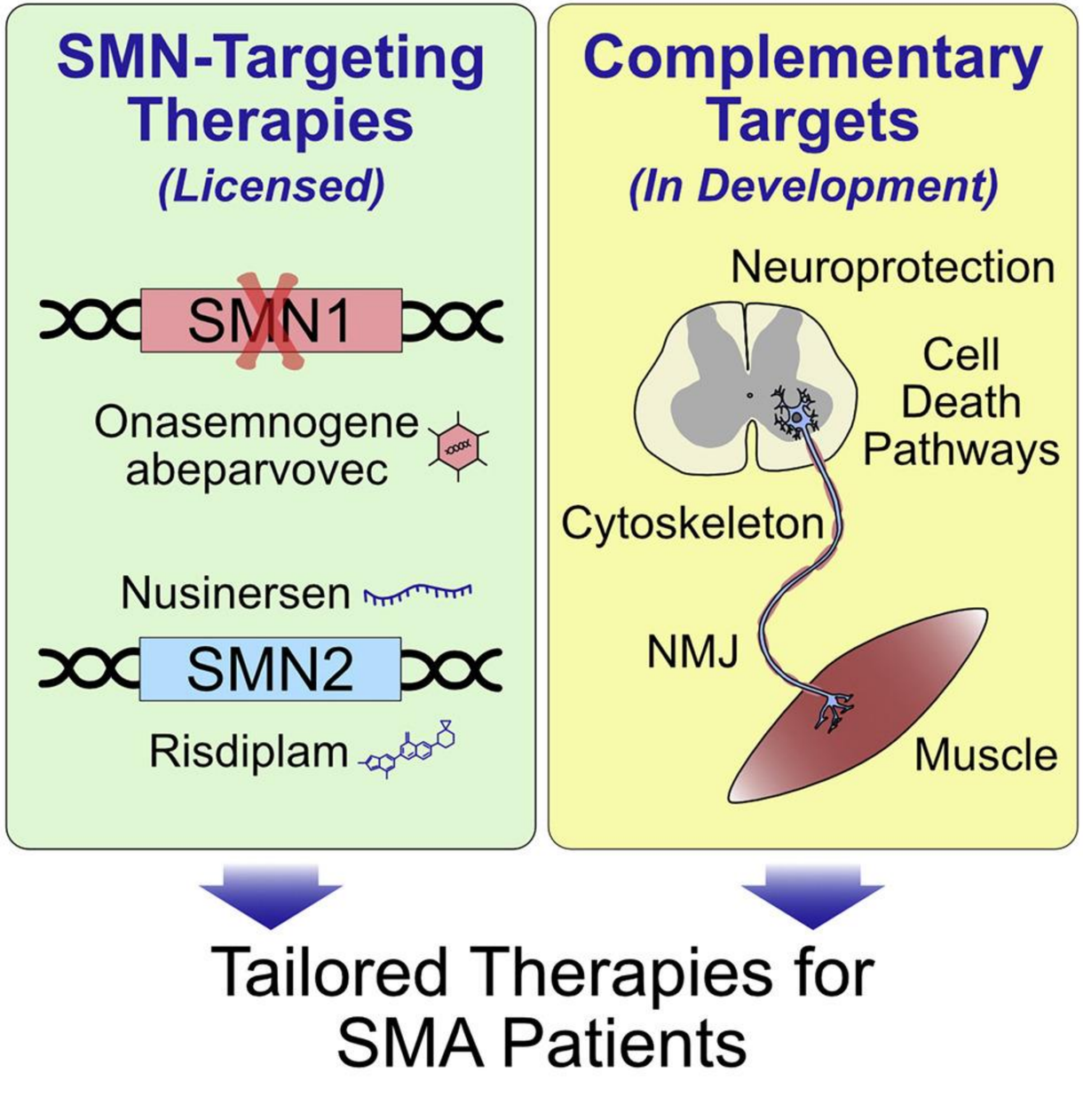
SMA is a progressive **childhood neuromuscular disease** that is caused by a mutation in a single gene that attacks nerve cells. It causes major physical limitations including the inability **to breathe, swallow, talk or sit up**. Children born with SMA typically die or need permanent breathing assistance by the time they turn 2 years old. **Zolgensma** (Onasemnogene abeparvovec) is a gene therapy treatment which uses **self-complementary adeno-associated virus type 9 (scAAV-9)** as a vector to deliver **the SMN1 transgene**. **the AAV9 vector can cross the blood-brain barrier** when injected into the vascular system and can **deliver genes directly to motor neurons**. The therapy was first approved in the US **in May 2019** as **an intravenous formulation** for children **below 24 months of age**. Zolgensma works by providing a new copy of the gene that makes the human SMN protein. The treatment must be accompanied by a course of **corticosteroids of at least two months**. In June 2022, Novartis published the final results from **the phase 3 SPR1NT trial** (NCT03505099), of the gene therapy, with data demonstrating that those with **presymptomatic SMA** with **either 2 or 3 copies of the SMN2 gene** achieved age-appropriate motor milestones, including **sitting independently**, **standing**, and **walking** up to 18 months after infusion.

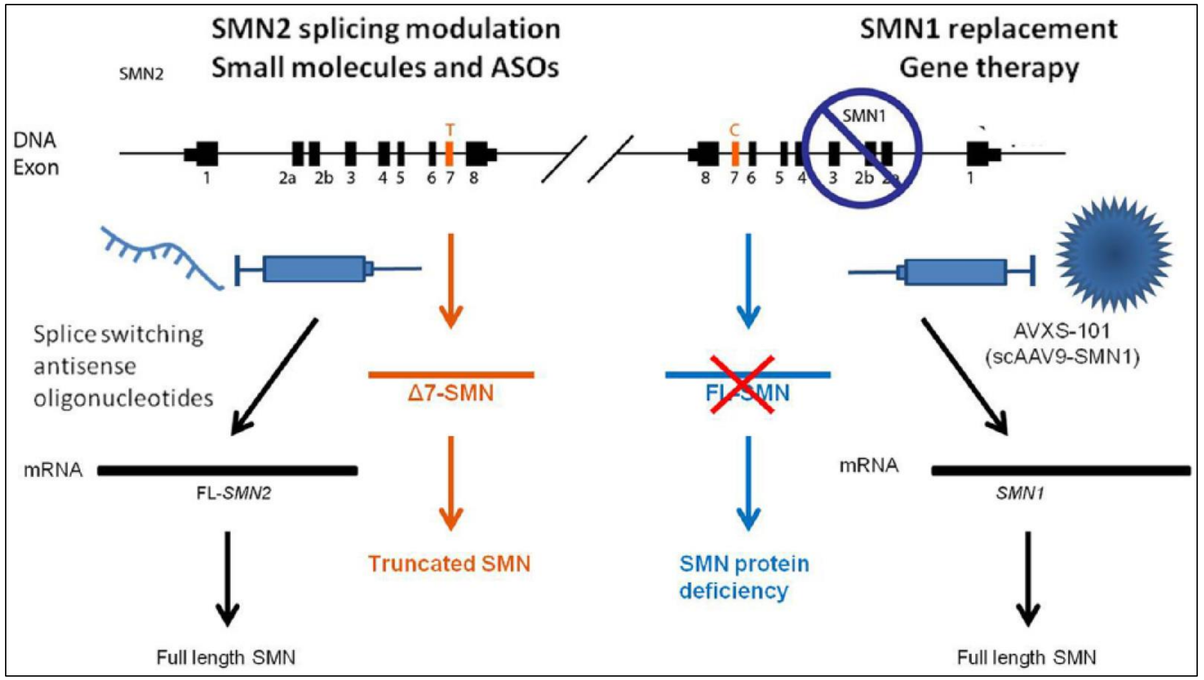
Novartis Gene Therapies is currently testing **a second way of delivering gene therapy**. This is via an **intrathecal (IT) injection**, which is an injection into directly into the cerebrospinal fluid through the lower back. This method of delivery which is currently being studied in **a Phase 3 clinical trial** could eventually make this treatment available to **older and larger patients**. There are some limitations concerning the use of ZOLGENSMA. **Repeat dosing** of ZOLGENSMA has not been evaluated. The use of ZOLGENSMA in patients with **advanced SMA** (e.g., complete paralysis of limbs, permanent ventilator dependence) has not been evaluated. ZOLGENSMA should not be used in **premature infants** due to neurologic developmental concerns related to using corticosteroids in premature infants.

Common side effects include vomiting and **increased liver enzymes**. Serious adverse reactions may include liver problems and **low platelets**. **Liver function** should be monitored for three months after administration. **Two children** in Russia and Kazakhstan **died** about five to six weeks after receiving Zolgensma. Both patients died of **acute liver failure**, a known side effect of Zolgensma that's included in a boxed warning on the one-time therapy's label. Both had received **corticosteroid taper to restore liver function**. **Liver toxicity** is one of the most common **side effects** of gene therapies like Zolgensma, which rely **on adeno-associated viruses (AAVs)** as vectors to deliver therapeutic genes. AAVs are mostly directed at the liver and are currently **the most commonly used platform for gene therapies**. While this is important safety information, **the overall risk/benefit profile** of Zolgensma is favorable since to date it has been used to treat more than **2,300 patients** worldwide across clinical trials. **Immunogenicity of AAV vectors** is thought to cause or exacerbate some of the more serious adverse events associated with AAV gene therapy, such as **hepatotoxicity** and **thrombotic microangiopathy** (TMA). Moreover, these adverse events tend to be correlated with **vector dose**, increasing in both prevalence and severity with higher doses.

Zolgensma was developed by Dr. Mendell and team in collaboration with colleagues at The Ohio State University **the Center for Gene Therapy** at the Wexner Research Institute **at Nationwide Children's Hospital**. The drug carries a list price of **US$2.125 million per treatment**, making it the most expensive medication in the world as of 2019. Since an initial FDA nod in 2019, Zolgensma has been cleared in over 40 countries and has become a blockbuster therapy.

Cell Reports Medicine 2, 100346, July 20, 2021





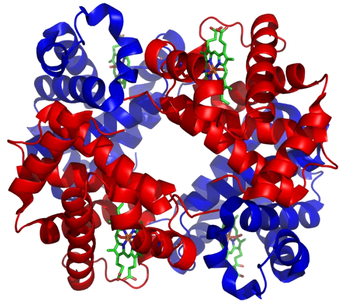
Ther Adv Neurol Disord 2018, Vol. 11: 1–13 DOI: 10.1177/1756285618754450

Spinraza (nusinersen), marketed by Biogen, was the first FDA-approved therapy to treat SMA. It is an SMN-enhancing therapy (**a synthetic anti-sense oligonucleotide**) that works by targeting the SMN2 gene, causing it to make more complete protein.

Evrysdi (Risdiplam) is an orally bioavailable **mRNA splicing modifier** used for the treatment of spinal muscular atrophy (SMA). **Since they are synthesized synthetically, Spinraza and Evrysdi are not considered to be Gene Therapy Products**.

Normal blood

Thalassemia



FATIGUE/ WEAKNESS

PALE SKIN

LIVER DAMAGE

ENLARGEMENT OF SPLEEN

WEAKENING AND FRAGILE BONES

HIGHER RISK OF INFECTIONS

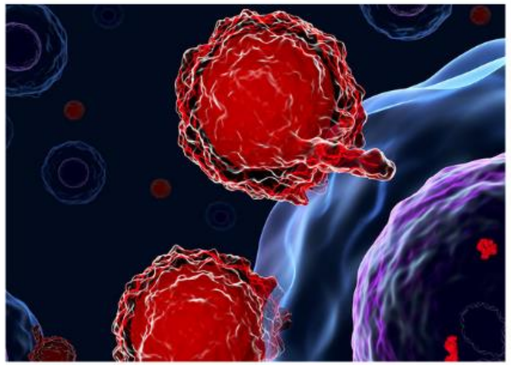
Beta thalassemias (β thalassemias) are **a group of inherited blood disorders**. The severity of the disease depends **on the nature of the mutation**. They are forms of thalassemia caused by reduced or absent synthesis of **the beta chains of hemoglobin** that result in variable outcomes ranging from **severe anemia** to **clinically asymptomatic individuals**. The body's inability to construct new beta-chains leads to **the underproduction of HbA** (adult hemoglobin). Reductions in HbA available overall to fill the red blood cells in turn leads to **microcytic anemia**. Severe symptoms include **liver cirrhosis**, **liver fibrosis**, and, **liver cancer**. Beta thalassemia is most prevalent in the "**thalassemia belt**" which includes areas in **the Mediterranean** extending into **the Middle East** and Southeast Asia.

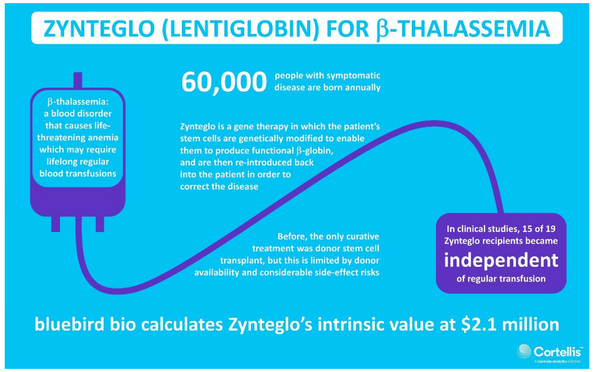
Three main forms have been described: thalassemia minor, thalassemia intermedia, and thalassemia major which vary from asymptomatic or mild symptoms to severe anemia requiring lifelong transfusions. Individuals with **beta thalassemia major** (those who are **homozygous for thalassemia mutations**, or inheriting 2 mutations) usually present within the first two years of life with symptomatic **severe anemia**, **poor growth**, and **skeletal abnormalities**. Untreated thalassemia major eventually leads to death, usually by heart failure; therefore**, prenatal screening** is very important. Those with **beta thalassemia intermedia** (those who are **compound heterozygotes** for the beta thalassemia mutation) usually present later in life with **mild to moderate symptoms of anemia**. **Beta thalassemia minor** (thalassemia **silent carriers**) involves **heterozygous inheritance** of a beta-thalassemia mutation and patients usually have borderline microcytic, hypochromic anemia and they are **usually asymptomatic** or **have mild symptoms**.

Some mutations in the HBB gene prevent the production of any beta-globin. The absence of beta-globin is referred to as **beta-zero (β0) thalassemia**. Other HBB gene mutations allow some beta-globin to be produced but in reduced amounts. A reduced amount of beta-globin is called **beta-plus (β+) thalassemia**. **Having either β0 or β+ thalassemia does not necessarily predict disease severity**, however; people with both types have been diagnosed with thalassemia major and thalassemia intermedia. In summary, it is a genetic disease characterized by anemia, a condition which results from a shortage of healthy red blood cells (RBCs). Without sufficient healthy RBCs, cells and tissues throughout **the body do not get the oxygen they need to function**. Anemia causes people to **feel tired, weak or short of breath**.

In **the most severe form of beta-thalassemia**, also referred to as **transfusion dependent thalassemia (TDT)**, patients require **lifelong regular red blood cell transfusions to survive**. If left untreated, the disease can damage organs and potentially lead to death. **TDT** is an **inherited genetic disorder** that is typically diagnosed within the first two years of life. It is characterized by **impaired production of the beta chains of hemoglobin** and **underproduction of hemoglobin**. Patients are subsequently oxygen deficient and suffer from **chronic anemia**.

Individuals with Beta-thalassemia who require regular transfusions spend an average of **10 hours**, **every 3-4 weeks in a hospital** to receive the blood transfusions **necessary for survival**. Chronic transfusions are associated with unavoidable **iron overload**. This means that the treatment itself can cause serious complications – including **progressive multi-organ damage** and **organ failure** – and requires chronic treatment with chelation therapy to remove iron. **Transfusions** and **iron chelation therapy** only provide **temporary relief**, and despite intense treatment regimens patients may still experience a wide range of symptoms, including fatigue and weakness to heart failure, diabetes or organ malfunction. **Allogeneic hematopoietic stem cell transplantation** (allo-HSCT) is the only potentially curative option that is currently available for TDT, but it has serious limitations. It is recommended only for **younger patients**, and its use is limited by a need for **matched donors** as well as **risks of severe complications** such as **transplant-related mortality**, **graft rejection**, and **graft-versus-host disease**.



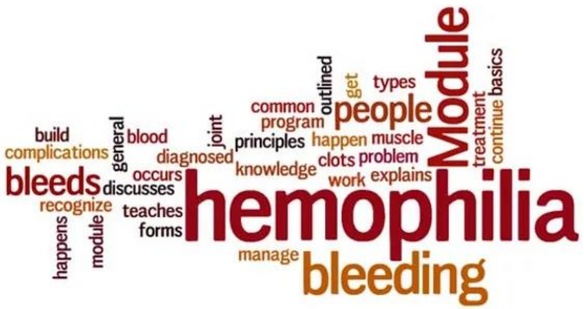


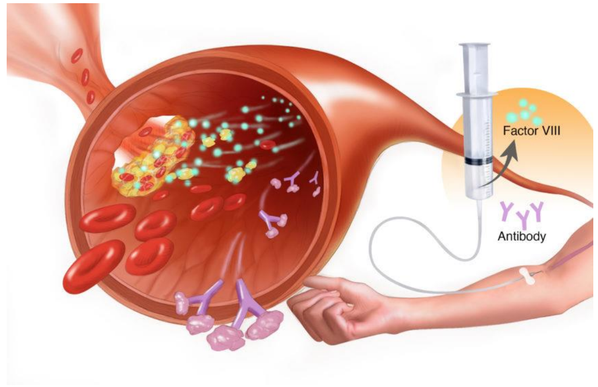
Lifelong transfusions treat the symptoms of beta-thalassemia but do not address the underlying genetic cause. For patients who **lack a suitable HLA-matched donor**, **ex vivo gene therapy** using **autologous HSCs** brings hope as a potential curative treatment option. **ZYNTEGLO** (betibeglogene autotemcel) is **a gene therapy** that uses **gene addition** to help your body produce **functional adult hemoglobin**, potentially eliminating the need for regular transfusions. Active substance of Zynteglo is **autologous CD34+ cell enriched population** that contains hematopoietic stem cells transduced with **lentiglobin** BB305 **lentiviral vector** encoding the **beta-A-T87Q-globin gene.** The procedure involves **ex vivo lentiviral transfer of a therapeutic β-globin gene derivative** (βAT87Q-globin) to **hematopoietic stem cells**, driven by cis-regulatory elements that confer high, **erythroid-specific expression**. Specifically made for each person, **ZYNTEGLO uses a person's own blood stem cells** and adds working copies of the beta-globin gene (needed to create functional adult hemoglobin) to the person's cells.

ZYNTEGLO uses a type of vector called **a lentiviral vector (LVV).** A gene delivery **vector** is like an envelope that delivers the working copies of the gene directly to the "address" of your blood stem cells. LVV uses HIV's natural ability to deliver genes into a cell but does not include the genes that cause HIV infection. The LVV is built using only the parts of HIV that are good at delivering the working copies of the beta-globin gene to your stem cells. The working copies of the modified beta-globin gene are delivered by the vector directly into your blood stem cells. Although ZYNTEGLO will not give you HIV infection, **treatment with ZYNTEGLO may cause a false-positive HIV test** result by some commercial tests.

In two phase 3 studies, ZYNTEGLO was studied in **41 patients**. All studies of ZYNTEGLO were **open label**, meaning that study participants and their doctors knew they were receiving ZYNTEGLO. Each study ran for an initial **24-month period** to assess **transfusion independence**. Clinical trial patients in **the long-term follow-up** study and patients who enroll in the registry will be followed for a total of **15 years** after receiving ZYNTEGLO. **About 9 out of 10** (89%) patients treated with ZYNTEGLO **stopped transfusions**. Transfusion independence (TI) meant that patients achieved a weighted average hemoglobin of ≥9 g/dL without any transfusions for a continuous period of ≥12 months at any time during the study after infusion of ZYNTEGLO. **The majority of patients achieved transfusion independence** (89%; 32/36 patients) and **had a normal or near-normal median total hemoglobin of 11.5 g/dL**. The majority of patients who achieved transfusion independence **were not receiving chelation as of last follow-up**. The most common side effects of ZYNTEGLO following treatment for up to 6 months: **Low level of platelets**, which may reduce the ability of blood to clot and may cause bleeding**, low level of white blood cells**, which may make you more susceptible to infection, pain in arms or legs.

It was developed by **Bluebird Bio** and was given breakthrough therapy designation by the U.S. Food and Drug Administration in February 2015. Zynteglo with the price tag of **$2.8 million** is the third gene therapy for an inherited disease to be **approved by FDA on Aug 17, 2022** in the U.S. following a treatment for a type of genetic blindness and another for spinal muscular atrophy. The other two are also expensive, with list prices of $850,000 and $2.1 million, respectively.



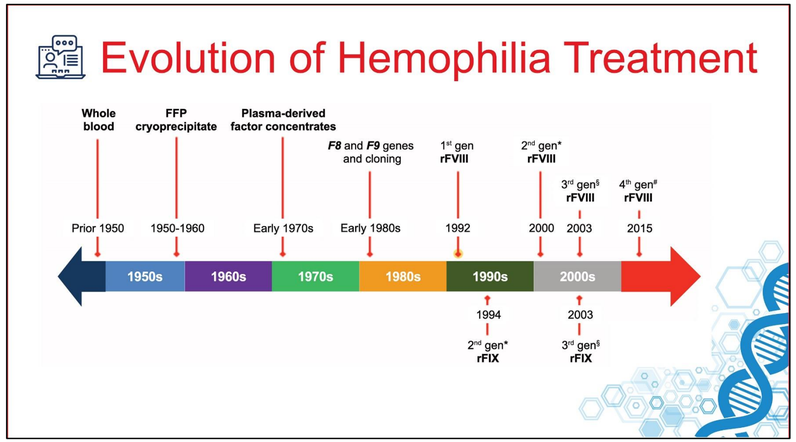


Hemophilia is a mostly **inherited genetic disorder** that **impairs** the body's ability **to make blood clots**, a process needed to **stop bleeding**. This results in people **bleeding for a longer time after an injury**, easy bruising, and an increased risk of bleeding **inside joints** or the **brain**. Those with a mild case of the disease may have symptoms only after an accident or during surgery. Bleeding into a joint can result in **permanent damage** while bleeding in the brain can result in long term **headaches**, **seizures**, or a **decreased level of consciousness**. There are two main types of hemophilia: **hemophilia A**, which occurs due to low amounts of **clotting factor VIII**, and **hemophilia B**, which occurs due to low levels of **clotting factor IX**. They are typically inherited from one's parents through an **X chromosome** carrying a nonfunctional gene. **Rarely a new mutation** may occur during early development or hemophilia may develop later in life due to **antibodies** forming against a clotting factor. Other types include **hemophilia C**, which occurs due to low levels of **factor XI**, **Von Willebrand disease**, which occur due to low level of a substance called von Willebrand factor in their blood, and **parahemophilia**, which occurs due to low levels **of factor V**. Hemophilia A, B, and C prevent **the intrinsic pathway from functioning properly**; this clotting pathway is necessary when there is damage to the endothelium of a blood vessel. **Acquired hemophilia** is associated with **cancers**, **autoimmune disorders**, and **pregnancy**. **Diagnosis** is by **testing the blood for its ability to clot** and **its levels of clotting factors**.

Hemophilia A affects about **1 in 5,000–10,000**, while **hemophilia B** affects about **1 in 40,000**, **males** at birth. As hemophilia A and B are both **X-linked recessive disorders**, females are rarely severely affected. Typically, females possess two X-chromosomes, and males have one X and one Y-chromosome. Since the mutations causing the disease are X-linked recessive, a female carrying the defect on one of her X-chromosomes may not be affected by it, as the equivalent dominant allele on her other chromosome should express itself to produce the necessary clotting factors, due to X inactivation. Therefore, **heterozygous females** are **just carriers** of this genetic disposition. If the genes responsible for production of factor VIII or factor IX present on a male's X-chromosome are deficient there is no equivalent on the Y-chromosome to cancel it out, so the deficient gene is not masked and the disorder will develop. **Since Hemophilia C** is an **autosomal genetic disorder** involving a lack of functional clotting Factor XI, it **occurs equally in both sexes** and is mostly found in Ashkenazi Jews.

Prevention may occur by removing an egg, fertilizing it, and testing the embryo (**Preimplantation genetic diagnosis-PGD**) before transferring it to the uterus. Human embryos in research can be regarded as the technical object/process. **Missing blood clotting factors are replaced to treat hemophilia**. This may be done on a regular basis or during bleeding episodes. The best way to treat hemophilia is **to replace the missing blood clotting factor** so that the blood can clot properly. This is accomplished by infusing (administering through a vein) **commercially prepared factor concentrates**. The clotting factors are made either from **human blood** or by **recombinant methods**. Replacement may take place at home or in hospital. Clinicians typically prescribe treatment products for **episodic care** or **prophylactic care**. Episodic care is used to stop a patient’s bleeding episodes; prophylactic care is used to prevent bleeding episodes from occurring. Today, it’s possible for people with hemophilia, and their families, **to learn how to give their own clotting factor treatment products at home**. Giving factor treatment products at home means that bleeds can be treated quicker, resulting in less serious bleeding and fewer side effects. About 15-20 percent of people with hemophilia develop **an antibody (called an inhibitor)** that stops the clotting factors from being able to clot the blood and stop bleeding. Treatment of bleeding episodes becomes extremely difficult, and the cost of care for a person with an inhibitor can skyrocket because **more clotting factor** or **a different type of clotting factor** is needed. People with inhibitors often experience more joint disease and other problems from bleeding that result in a reduced quality of life.

HEMGENIX®
etranacogene dezaparvovec -XXXX



US FDA Approves World's Costliest Gene Therapy Drug for Hemophilia B

CSL Behring

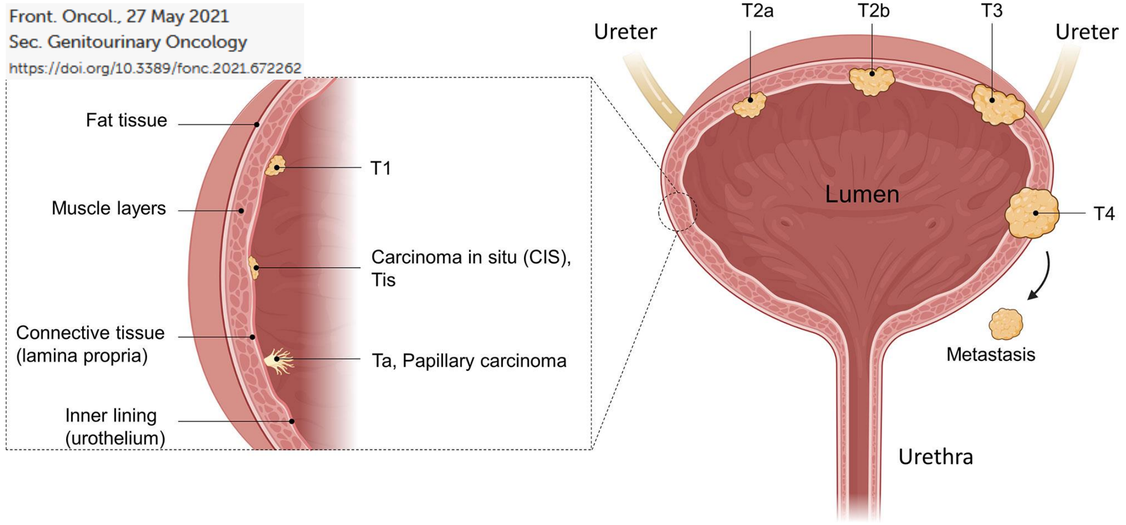
The approval was granted to CSL Behring LLC, a global rare disease biotech company

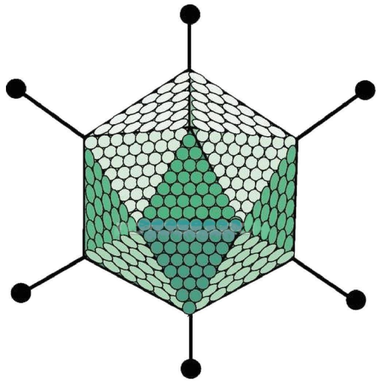
Hemgenix (etranacogene dezaparvovec) is a gene therapy that **reduces the rate of abnormal bleeding** in eligible people with **hemophilia B** by enabling the body to continuously produce **factor IX**, the deficient protein in hemophilia B. It uses **AAV5**, a non-infectious viral vector, called **an adeno-associated virus (AAV)**. **The AAV5 vector** carries the **Padua gene variant of Factor IX (FIX-Padua)** under the control of **a liver-specific promoter** to the target cells **in the liver**, **generating factor IX proteins** that are **5x-8x more active** than normal. These genetic instructions remain in the target cells, but generally do not become a part of a person's own DNA. Once delivered, the new genetic instructions allow the cellular machinery to produce stable levels of factor IX. Treatment with Hemgenix should be initiated under the supervision of **a physician experienced in the treatment of Hemophilia and/or bleeding disorders**. This medicinal product should be administered in a setting where personnel and equipment are immediately available to treat infusion-related reactions.

Hemgenix is indicated for the treatment of **severe and moderately severe Hemophilia B** (congenital Factor IX deficiency) in adult patients **without a history of Factor IX inhibitors**. The safety and effectiveness of Hemgenix were evaluated **in two studies of 57 adult men (**18 to 75 years of age) with **severe or moderately severe Hemophilia B. Effectiveness was established** based **on decreases in the men's annualized bleeding rate (ABR).** The latest clinical trial of Hemgenix, which included **54 people with hemophilia B**, reported a **54% reduction in the number of bleeding episodes per year**, and **94% of participants discontinued any prophylactic therapy** within two years of receiving the single dose. In summary, the benefits of Hemgenix are **the induction of relevant plasma levels of factor IX** and **the reduction of bleeding episodes**. The most common side effects reported in more than 5% of patients were **liver enzyme elevations** (increased ALT and AST), headache, elevated levels of a certain blood enzyme, **flu-like symptoms**, infusion-related reactions, fatigue, nausea, and feeling unwell.

On 22 November 2022, **the US Food and Drug Administration** (**FDA**) **approved the first gene therapy for the genetic blood-clotting disorder hemophilia B — a one-time treatment** that costs **US$3.5 million**. Hemgenix is developed by the pharmaceutical company **CSL Behring**, based in King of Prussia, Pennsylvania. Clinical trial data suggest that **the single dose of Hemgenix** will provide **people with moderate to severe hemophilia** with adequate **protection from uncontrolled bleeding for eight years**, and **potentially longer**. But the treatment's hefty price tag makes it **the most expensive drug in the world**. People with hemophilia B (who account for 15% of hemophilia cases) are currently given factor IX once or twice a week. **In the US, the treatment of an adult with hemophilia B averages $700,000–800,000 per year**. The company claims that **even at a cost of $3.5 million**, Hemgenix could **save the US health-care system $5 million to $5.8 million per person treated**, because of its proven effectiveness at decreasing or eliminating the need for regular injections of factor IX. But scientists worry that the price would not be affordable in low- and middle-income countries, where most people with hemophilia live and where supplies of treatments and factor IX are often insufficient. As new technologies such as gene therapy emerge on the scene, those who would benefit most can least afford to pay.

The FDA's approval highlights difficulties in the quest to develop gene therapies for hemophilia more generally. **Only 15% of people with hemophilia have hemophilia B, most have hemophilia A**. **Finding an effective gene therapy for hemophilia A has proved challenging**, because a greater increase in factor VIII production is needed to get a good therapeutic effect, and some clinical trial participants have shown **strong immune responses to the viral vector (AAV)** used to deliver the gene. Once you have adeno-associated viral gene therapy, **you make antibodies to the AAV vector, so you cannot have it again**.



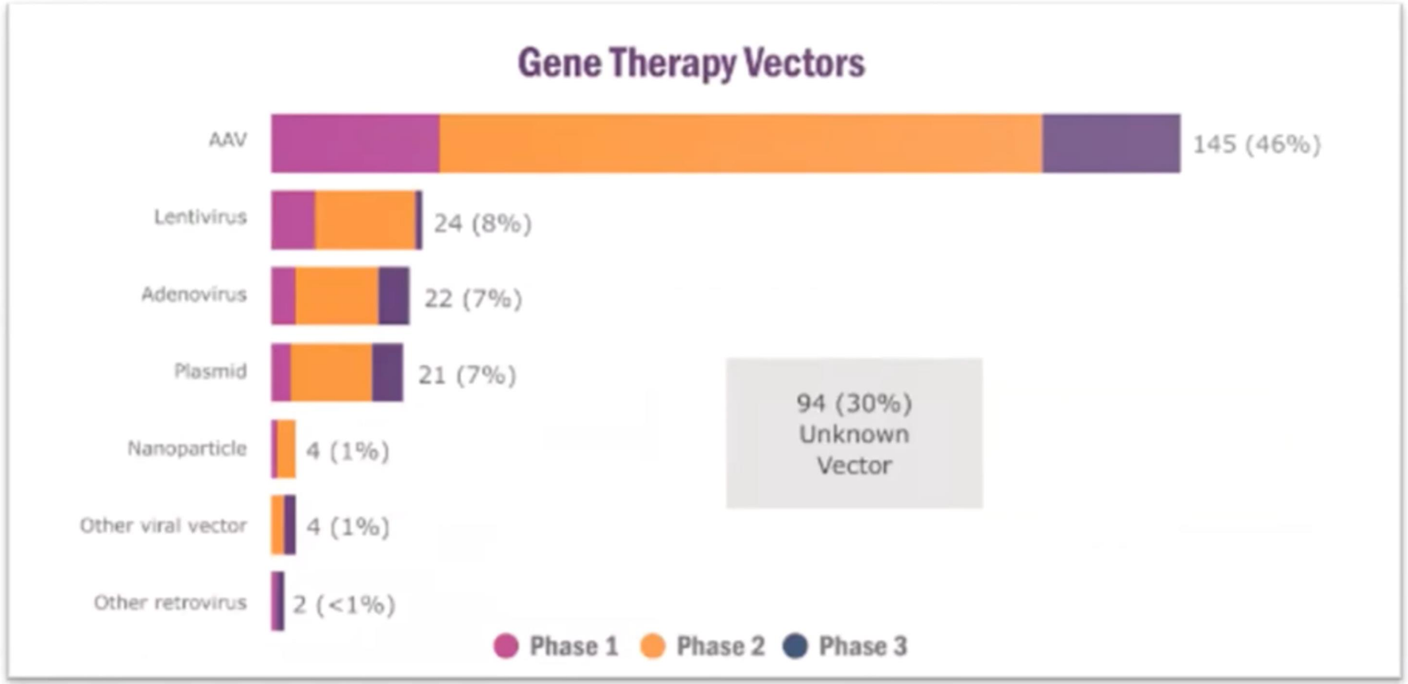


Bladder cancer is cancer that starts **in the inner lining of the bladder**. Bladder cancer can be classified **by how far it has spread**. If the cancerous cells are contained inside **the inner lining of the bladder**, it is described as **non-muscle-invasive**, **early** or **superficial**, i.e. **NMIBC**. This is **the most common type** of bladder cancer. At diagnosis, **70–80 % of bladder cancers** are NMIBC. Early bladder cancer usually appears as small growths, shaped like **mushrooms**, which grow out of the bladder lining. This is called **papillary bladder cancer**. These growths can be **surgically removed** and they may never come back. However, some types of NMIBC are **more likely to come back**, including **carcinoma in situ (CIS)** and **high-grade T1 tumors**, both of which can grow quickly. **CIS** are **flat cancers** that do not grow out of the bladder wall, but the cancer cells look very abnormal; this is known as **high-grade cancer**. **High-grade T1 tumors** are **superficial cancers** that have grown from the bladder lining into a layer underneath, called the **lamina propria**. **High grade means the cancer is more likely to grow spread and come back after treatment**. The treatment option for patients with high-risk NMIBC includes either **a course of BCG treatment** (using a variant of the BCG vaccine), or an operation to **remove the bladder** (**cystectomy**). BCG is a well-established treatment for preventing or delaying tumor recurrence following NMIBC resection. However, many patients will experience **recurrence or progression** during or following BCG.

Adstiladrin (Nadofaragene firadenovec; rAd-IFN/Syn3; Instiladrin) is **a non-replicating adenoviral vector-based** gene therapy designed to deliver a copy of a gene encoding **a human interferon-alfa 2b (IFNα2b)** to **the bladder urothelium**. It is **an advanced gene therapy treatment** option indicated for the treatment of **adult patients** with **high-risk** BCG-unresponsive **NMIBC** with **carcinoma in situ (CIS)** with or without **papillary tumors**. Intravesical instillation of Adstiladrin results in **cell transduction** and **transient local expression of the IFNα2b protein** that is anticipated **to have anti-tumor effects**. This in turn **enhances the body's natural defenses against the cancer**. Adstiladrin is a suspension for intravesical instillation, supplied as single-use vials. The recommended dose of Adstiladrin is 75 mL at a concentration of 3 x 10e11 viral particles (vp)/mL instilled **once every three (3) months** into the **bladder** via a **urinary catheter**. Adstiladrin is not for intravenous use, topical use, or oral administration.

The U.S. Food and Drug Administration (**FDA**) **approved** Adstiladrin® developed by Ferring Pharmaceuticals **on 16 December 2022**. Approval was based on results from a multicenter clinical study that included **157 patients** with **high-risk BCG-unresponsive NMIBC**, 98 of whom had **BCG-unresponsive CIS** with or without papillary tumors and could be evaluated for response. In the study, patients received Adstiladrin **once every 3 months for up to 12 months**, or until unacceptable toxicity to therapy or recurrent high-grade NMIBC. **The complete response (CR) rate was 51% at 3 months**, with **about half of these responding patients** remaining in **CR for at least 1 year**. The median duration of response was 9.7 months. The gene therapy is not indicated for individuals who are immunosuppressed or immune-deficient.

AAV is most prevalent delivery vehicle in clinical gene therapy research

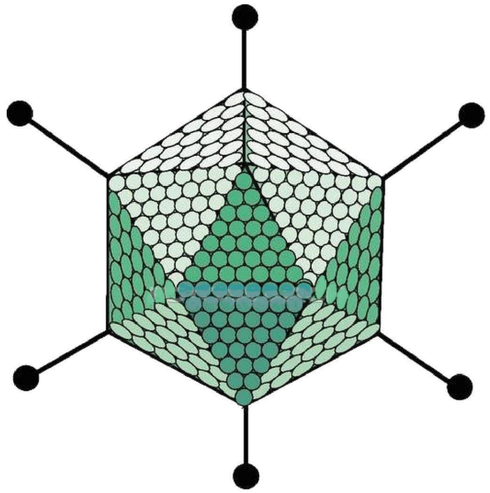


Source: https://alliancerm.org/sector-report/2021-annual-report/

Approved marketed products

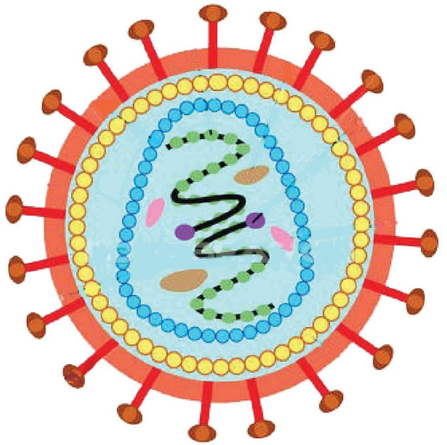
- LUXTURNA
  - Spark Therapeutics
  - AAV2/RPE65
  - Dec. 2017
- ZOLGENSMA
  - Novartis
  - AAV9/SMN1
  - May 2019

American Society of Gene + Cell Therapy



Adenovirus

AAV



Lentivirus

Viral vector	Packaging capacity	Length of expression	Relative viral titer	Transduction efficiency	Infect both dividing and non-dividing cells	Immunogenicity
Adenovirus	7.5 kb	Transient	+++	+++	Yes	High
Adeno-associated virus	4.5 kb	Transient and Stable	++	++	Yes	Low
Oncoretrovirus	8 kb	Stable	+	+	No	Moderate
Lentivirus	8 kb	Stable	+	++	Yes	Low

FDA STATEMENT

Statement from FDA Commissioner Scott Gottlieb, M.D. and Peter Marks, M.D., Ph.D., Director of the Center for Biologics Evaluation and Research on new policies to advance development of safe and effective cell and gene therapies

For Immediate Release: January 15, 2019

Statement From: Scott Gottlieb, M.D.

Content current as of:
01/15/2019

"By 2025, we predict that the FDA will be approving 10 to 20 cell and gene therapy products a year based on an assessment of the current pipeline and the clinical success rates of these products."

Summary

Take home message:

Over 3 decades, gene therapy has advanced from a logical idea to becoming a clinical reality for devastating pandemic diseases, as well as other inherited disorders. Several gene therapy medicines have been licensed for marketing and many more are advancing toward that goal to make them widely available, beyond clinical trials.

Next-generation gene therapy protocols (CRISPR) are already being developed, which will also help expand the spectrum of diseases that can be treated by gene therapy.

Thanks for your patience

The Department of Gene and Cell Therapy,
Akdeniz University

http://genetherapy.akdeniz.edu.tr