



# Investigational Gene Therapies for Lysosomal Storage Disorders

*All AVROBIO gene therapies are investigational medicines. Investigational medicines have not yet been approved by the U.S. Food and Drug Administration (FDA) or any other regulatory agency. The safety and efficacy of AVROBIO gene therapies have not yet been established.*



*A strand of deoxyribonucleic acid (DNA)*



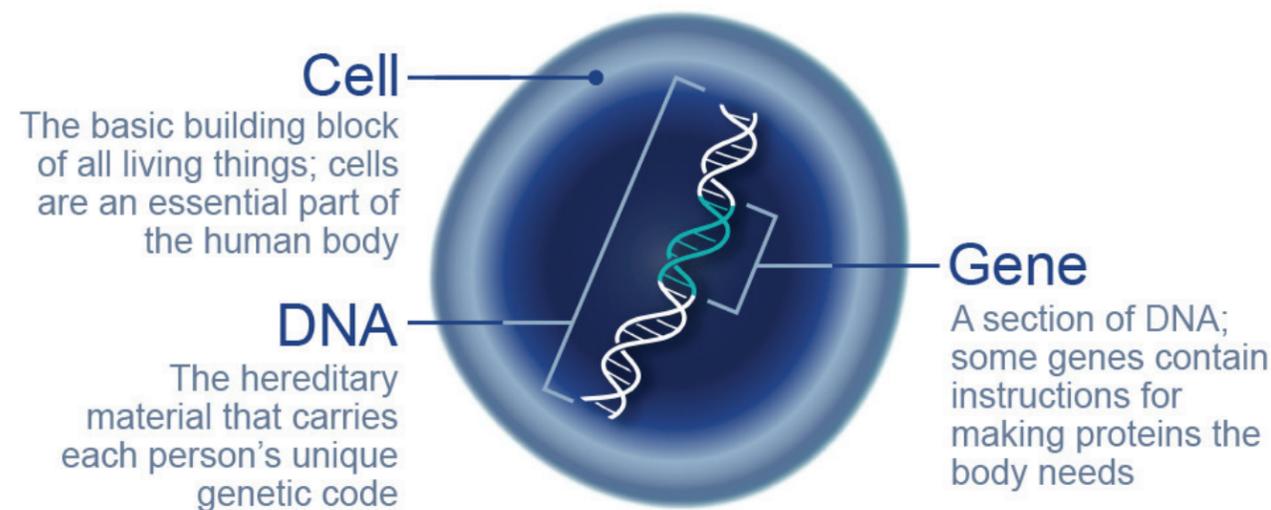
**AVROBIO** is committed to developing gene therapies with the potential to transform lives.

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## What is a Gene?<sup>1</sup>

- A gene is the basic unit of heredity.
- Genes pass on certain traits, like eye color, from a parent to a child.
- Faulty (mutated) genes cause genetic diseases.
- There are about 25,000 genes in almost every cell in the body.
- Genes are made up of DNA, or deoxyribonucleic acid.
- DNA contains instructions to make proteins the human body needs to grow, function, and survive.



## What is a Genetic Mutation?<sup>2</sup>

A genetic mutation is a change in the DNA of a specific gene, which makes it different from what is found in most people. Mutations can be inherited from a person's parents or occur spontaneously at some point before they are born (while in the womb) or during their life. Some mutations are harmless, but others can cause serious health problems. For example, Gaucher disease is caused by mutations in the *GBA* gene:

- The *GBA* gene is responsible for telling cells how to make a certain enzyme.
- When the *GBA* gene has a mutation, the enzyme it produces may not work properly in the body.
- Without an adequately produced enzyme in the body, there will be a buildup of a substance that the enzyme normally helps break down.
- The buildup of this substance damages the cells in tissues and organs and causes the symptoms of Gaucher disease.

## What is Gene Therapy?<sup>3,4</sup>

Gene therapy is a new approach to medicine, in that it uses genetic material instead of drug therapy or surgery to treat or prevent disease. Most current treatments for life-threatening diseases address symptoms rather than the underlying cause of the disease. They may also prevent the disease from getting worse. Gene therapies are designed to address the cause of genetic disease—the faulty gene and its consequences.

### How is gene therapy delivered?

There are different ways to deliver gene therapy to a patient. All types of gene therapy use a vector to deliver genetic material to a patient's cells. *Ex vivo* means the vector is supplied outside the body, and *in vivo* means the vector is supplied inside the body.

- In an *ex vivo* gene therapy procedure, cells are taken from a patient and target cells are separated in a laboratory. In the lab, the target cells are then introduced to a vector carrying the new genetic material, which it inserts into the cells. The treated cells containing the therapeutic, or corrected, gene are then inserted back into the patient.
- During *in vivo* gene therapy, a vector carrying the genetic material is administered directly into the body. Once in the body, the vector will spread to the target cells and inserts its genetic material. The procedure to insert the gene can be different and depends on which organ or cells doctors are trying to reach.

### What are the different methods of gene therapy?<sup>5</sup>

#### Gene editing:

- Corrects a non-functioning gene using molecular scissors, or enzymes that are able to cut specific DNA sequences (*in vivo*)
- Can be used to alter DNA and change the characteristics of a cell (e.g., CRISPR)

#### Gene silencing:

- Inactivates, or "knocks out," a faulty gene that is not working properly
- Can be performed *in vivo* or *ex vivo*

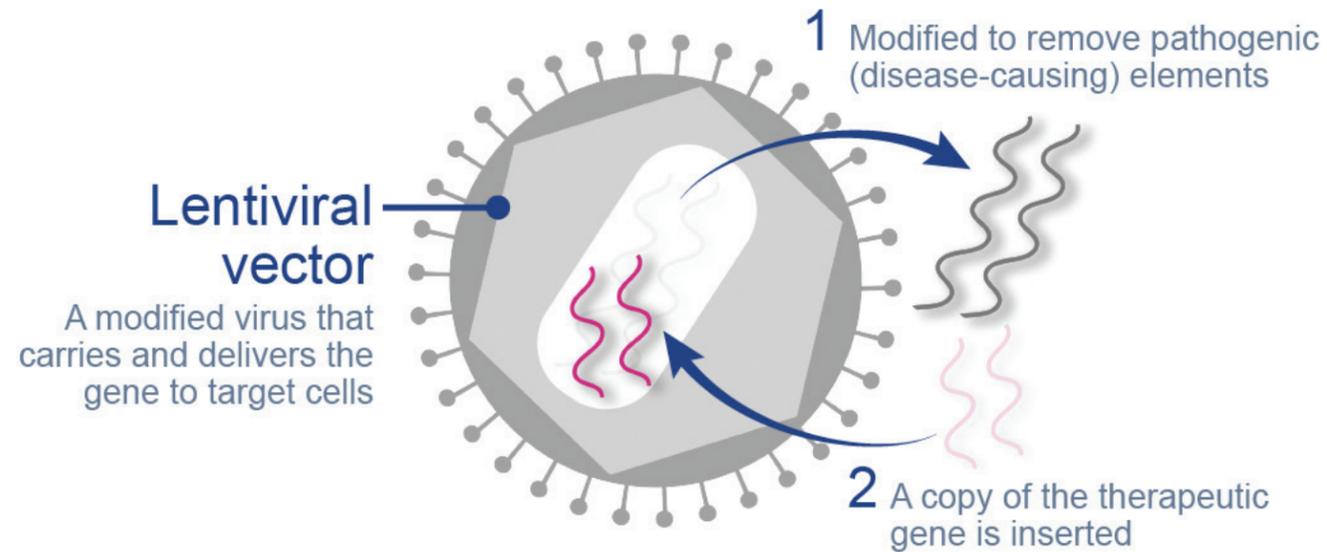
#### Gene addition:

- Introduces a therapeutic gene that helps the cell produce a normal, functioning protein or enzyme

### Where does the gene used for gene therapy come from?

The genetic material used for gene therapy is made in a laboratory by recombinant DNA technology, which combines strands of DNA that wouldn't otherwise be found together. Genes cannot be donated the way someone would donate a kidney or liver.

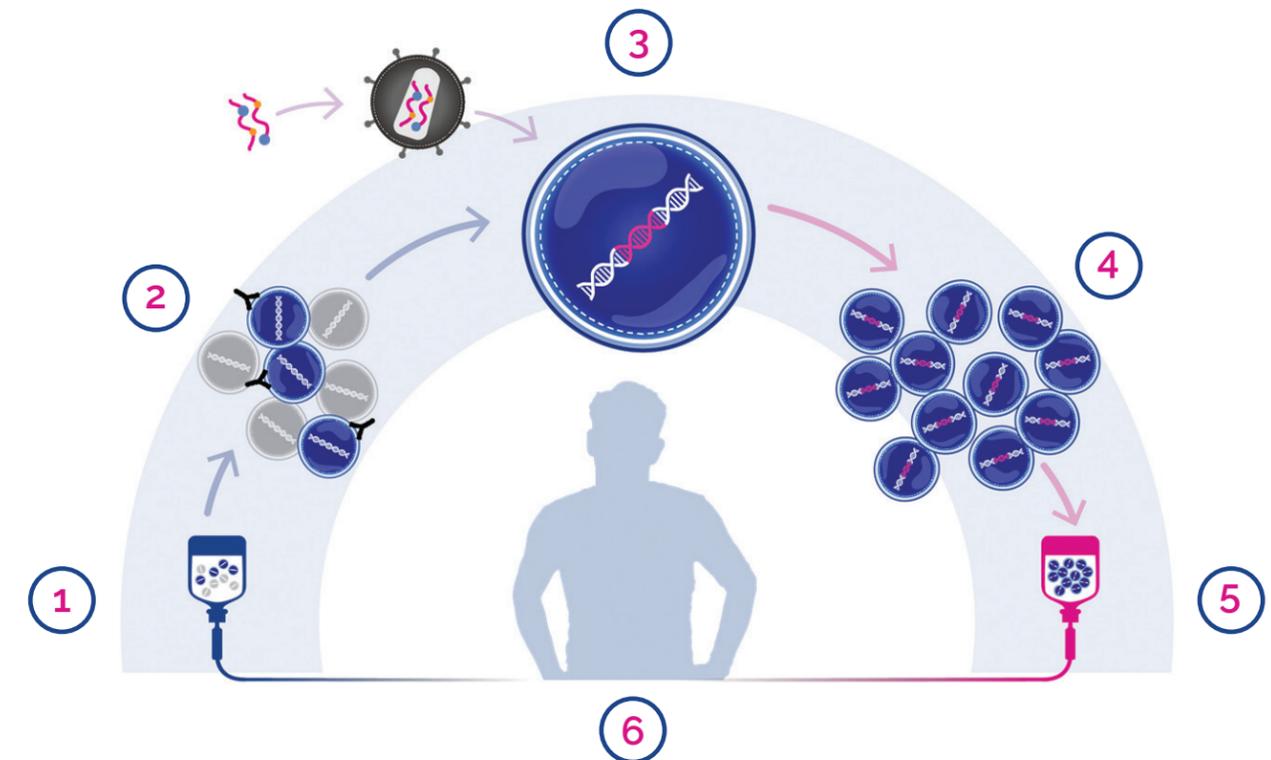
## The Lentiviral Approach to Gene Therapy



*Lentiviral gene therapy is a novel approach with two recent approvals, for transfusion-dependent  $\beta$ -thalassemia (TDT) in Europe and acute lymphoblastic leukemia (ALL) in the U.S., Europe, and Japan. Lentiviral gene therapy for lysosomal storage disorders (LSDs) is still investigational and has not been approved by the U.S. Food and Drug Administration (FDA).*

A vector is an inactivated virus that is unable to reproduce or spread. The vector acts like an envelope that carries the therapeutic gene into cells. There are different types of vectors such as adeno-associated viral vector (AAV) and lentiviral vector (LV).

The aim of *ex vivo* lentiviral gene therapy is to provide a single-dose therapy with the potential to provide life-long therapeutic benefits.



1. An investigational *ex vivo* gene therapy procedure for LSDs begins when the patient undergoes "mobilization," a procedure which uses medication that stimulates stem cells to move out of the bone marrow and into the bloodstream.
2. The patient's mobilized blood is collected and taken to a lab. Once in the lab, certain stem cells, called CD34<sup>+</sup> cells, found in the blood are selected and separated out.
3. The separated stem cells are then combined with a lentiviral vector. The vector carries and delivers the new genetic material to the stem cells.
4. The stem cells with the new therapeutic gene are then collected and frozen in the lab or facility.
5. The patient goes through a conditioning process to prepare their body to receive back their own cells with the therapeutic gene through a one-time intravenous (IV) infusion. The patient may be hospitalized during this process.
6. Once in the body, the stem cells containing the therapeutic gene will begin to move into the space created in the bone marrow during conditioning. These cells will then divide to form more stem cells, as well as different types of blood cells, which will make the functioning protein that was missing. Stem cells that settle into the bone marrow enable a long-lasting production of the previously faulty protein/enzyme.

*There are risks associated with an investigational gene therapy procedure. A person interested in participating in an investigational gene therapy trial should discuss whether they are a candidate for the trial, as well as the possible risks with their doctor and/or a stem cell transplant specialist.*



## Frequently Asked Questions

### What is a lentivirus?

A lentivirus is a virus from the *Retroviridae* family of viruses, which means it contains an enzyme that allows it to produce DNA from RNA, which processes proteins and carries genetic information. The lentivirus used in gene therapy is modified to remove the pathogenic elements.

### Why does gene therapy use a virus?

Viruses are used as gene therapy vectors because they are good at getting genetic material into our cells. Viruses used in gene therapy have been modified to remove pathogenic elements. Lentiviruses are feasible for gene therapy because they can deliver a lot of genetic information into the DNA of cells where it is permanently integrated.

### How safe is lentiviral gene therapy?

Several lentiviral gene therapies, including AVROBIO's, are investigational products, which means they are being studied in the clinical trial setting and their safety and effectiveness have not been established by the FDA. Gene therapy procedures can cause side effects. Patients should discuss any potential side effects with their doctor.



**Clinical trials** are research studies involving human volunteers to gain medical knowledge about a disease or potential treatment.

**For investigational gene therapies, the risks and benefits are currently unknown. It's important to talk to a doctor about whether a gene therapy clinical trial is the right option for you.**

### If a person receives gene therapy, can they pass along the therapeutic gene to their children?

Since gene therapy does not involve reproductive cells, a person who receives it will not be able to pass along the therapeutic gene to future children.

### How will gene therapy affect my fertility?

There may be risks to your fertility and these risks may vary by individual.

### What is conditioning and what is its purpose?

Conditioning is an important process in lentiviral gene therapy. The process helps the patient's body prepare to receive their modified cells. During conditioning, the patient receives a chemotherapy medication that works by getting rid of some existing cells in their bone marrow to make space for their new cells. When used for conditioning, patients receive this medication in one cycle. It is not given in multiple cycles or used in combination with other agents, as it is when used to treat cancer. It is also used for a shorter period of time.



### What is a CD34+ cell?

- CD34+ cells are stem cells, or cells that can develop into many different types of cells. They are found in bone marrow, umbilical cord blood, and in circulating blood (in very small amounts).
- CD34+ stem cells are capable of becoming all different types of blood cells, including white blood cells, which help the body fight infection and disease.
- CD34+ cells play a key role in lentiviral gene therapy because, once back in the patient's body, they survive for a long time in the bone marrow. Once engrafted, they divide to produce billions of cells in the blood that can deliver the therapeutic protein/enzyme throughout the body.

### How are the effects of gene therapy measured?

Researchers measure the effects of gene therapy in different ways, depending on the type of disease and the clinical trial design.

For its investigational gene therapy, AVROBIO assesses the safety, enzyme or protein activity, substrate (a substance upon which an enzyme acts) levels, vector copy number (VCN), among other measures.<sup>6</sup> Vector copy number (VCN) is the average number of copies of the therapeutic gene in blood or bone marrow cells that have a nucleus. The nucleus of a cell controls all its activities.

### How do modified cells help correct the "regular cells" in the body?

The modified, mature cells can help in the treatment of genetic disease in two ways:

- In some cases, modified, mature cells that result from gene therapy can produce the adequate and functional protein that is otherwise missing. Once that active protein is in the bloodstream, it can be taken up by cells in different organs and tissues and help correct those cells' underlying problems due to the mutated gene and related lack of produced and/or functional protein.
- In other cases, the modified stem cells develop and produce mature cells that carry the therapeutic gene and produce the functional protein. These modified, mature cells can directly replace the disease-causing cells that had the faulty gene.

## Can the modified cells in the bloodstream and bone marrow reach other organs in the body?

The mature cells that develop from the gene-modified stem cells can enter into the bloodstream. Once in the circulation they can reach many organs in the body and deliver the active protein/enzyme to that tissue.

## Is gene therapy a cure?

Because gene therapy for LSDs is still investigational, and there are currently no long-term data, researchers cannot yet determine if it is a cure.

## If a person receives gene therapy, will they still need continued care for their disease?

Yes, AVROBIO's gene therapy is an investigational medicine and a doctor will continue to monitor a person's health in similar ways to before treatment. It is currently unknown if gene therapy will have a lasting benefit.

## How long does an *ex vivo* lentiviral gene therapy process typically take?

This can depend on several factors. The process could take weeks, during which patients could spend several days in the hospital.

## Is investigational gene therapy a one-time procedure?

Researchers are still in the process of studying how long the effects of gene therapy may last.



### Gene Therapy Resources

American Society of Gene  
& Cell Therapy (ASGCT)  
[asgct.org/education](https://www.asgct.org/education)

National Institutes of Health (NIH)  
Genetics Home Reference  
[ghr.nlm.nih.gov](https://ghr.nlm.nih.gov)

Alliance for Regenerative  
Medicine (ARM)  
[alliancerm.org](https://www.alliancerm.org)

### References

<sup>1</sup>What is a gene? NIH Genetic Home Reference Website. <https://ghr.nlm.nih.gov/primer/basics/gene>. Published October 16, 2018. Accessed October 23, 2018. <sup>2</sup>What is a gene mutation and how do mutations occur? NIH Genetic Home Reference Website. <https://ghr.nlm.nih.gov/primer/mutationsanddisorders/genemutation>. Published October 9, 2018. Accessed October 11, 2018. <sup>3</sup>Gene therapy. Mayo Clinic Website. <https://www.mayoclinic.org/tests-procedures/gene-therapy/about/pac-20384619>. Published December 29, 2017. Accessed October 2, 2018. <sup>4</sup>What Is Gene Therapy? How Does It Work? U.S. Food & Drug Administration Website. <https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm589197.htm>. Published December 19, 2017. Updated December 22, 2017. Accessed October 2, 2018. <sup>5</sup>What is gene therapy? Genetics Home Reference. <https://ghr.nlm.nih.gov/primer/therapy/genetherapy>. Published December 18, 2018. Accessed December 20, 2018. <sup>6</sup>Miller-Keane Encyclopedia and Dictionary of Medicine, Nursing, and Allied Health, Seventh Edition, 2003: <http://medical-dictionary.thefreedictionary.com/substrate>.

## AVROBIO's Current Clinical Development Programs

### Fabry Disease (AVR-RD-01)

AVROBIO is currently enrolling patients in a Phase 2 clinical trial. The primary objective is to evaluate the safety, effectiveness, and tolerability of infusing the patient's own genetically modified stem cells that express the functional enzyme  $\alpha$ -galactosidase A (AGA) in previously untreated classic Fabry male patients.

### Gaucher Disease (AVR-RD-02)

AVROBIO is currently enrolling patients in a Phase 1/2 trial in patients with Type I Gaucher disease. In this trial, the therapy is designed to genetically modify stem cells, so when they mature to produce macrophages (a type of immune cell), they express glucocerebrosidase (GCase), the enzyme that is deficient in Gaucher disease.

### Cystinosis (AVR-RD-04)

AVROBIO expects to treat the first patient in a planned academic-sponsored Phase 1/2 trial. The therapy is designed to genetically modify stem cells so when they mature to produce specialized blood cells (which include macrophages), the blood cells will express cystinosis (CTNS), a lysosomal transporter protein that prevents the abnormal buildup of toxic crystals in the cells.

### Pompe Disease (AVR-RD-03)

AVROBIO's gene therapy platform combined with proprietary lysosomal targeting tag system is advancing pre-clinical studies.

*Safety and efficacy have not been established for any of our product candidates. All are investigational, and none have been approved by the FDA or any other regulatory agency.*

Clinical development programs for new potential therapies are done in phases of clinical trials. If the goals of each phase are met, the therapy will go onto the next phase. In rare disease trials, these phases may be combined due to small patient populations and the urgent need for treatments.

### Gene Therapy Clinical Trials

**Phase 1:** Evaluates safety of treatment in patients

**Phase 2:** Evaluates safety and efficacy in as large of a patient population as possible, no placebo

**Phase 3 and above:** Confirms safety and efficacy of treatment in patients, evaluates different patient sub-populations (e.g., pediatric)

**To learn more about AVROBIO, our gene therapy programs, or our clinical trials, contact us at [info@avrobio.com](mailto:info@avrobio.com).**

**AVROBIO, Inc.** is a leading, Phase 2 gene therapy company focused on the development of its investigational gene therapy, AVR-RD-01, in Fabry disease, as well as additional gene therapy programs in other lysosomal storage disorders including Gaucher disease, cystinosis and Pompe disease.

The Company's plato™ platform includes a proprietary vector system, automated cell manufacturing solution and refined conditioning regimen deploying therapeutic drug monitoring. AVROBIO is headquartered in Cambridge, Mass. and has offices in Toronto, Ont. For additional information, visit [www.avrobio.com](http://www.avrobio.com).