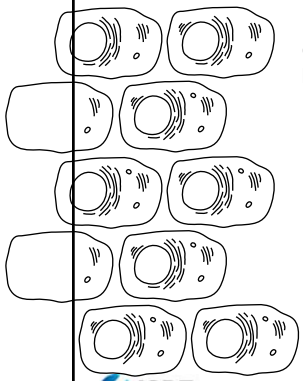
Cell & Gene Therapy Basics

An Introduction

Why?



Gene therapy is important because it may allow us to address a broad range of diseases **at the genetic level.**

We are all made of cells—they are the building blocks of life. Our cells contain our DNA, which is divided up into segments called genes.

First Day of a 'New Life' for a Boy With Sickle Cell

https://www.nytimes.com/2024/09/16/health/sickle-cell-gene-therapy-kendric-cromer.html

Kendric Cromer, 12, is among the first patients to be treated with gene therapy for sickle cell disease in the commercial setting

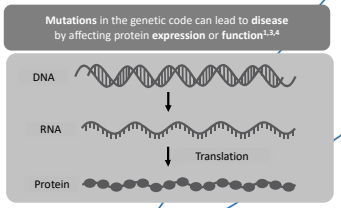
"I can't wait to start my new life"

What is gene therapy?

Gene therapy is an intervention that uses **genetic material** to treat or prevent diseases[1]

Gene therapy approaches include:

- **Addition** of new genetic material[2]
- **Disruption** or **editing** of existing genetic material[2]
- A combination of the two[2]



DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

1. US Food and Drug Administration. How Gene Therapy Can Cure or Treat Diseases. (2022). Available at: https://www.fda.gov/consumers/consumer-updates/how-gene-therapy-can-cure-or-treat-diseases. Accessed Mar 26, 2024. 2. Morgan RA, Gray D, Lomova A, Kohn DB. *Cell Stem Cell*. 2017;21(5):574-590. doi:10.1016/j.stem.2017.10.010. 3. Genetic Alliance; the New England Public Health Genetics Education Collaborative. Understanding Genetics: a New England Guide for Patients and Health Professionals. Washington (DC): Genetic Alliance; 2010. Available from: https://www.ncbi.nlm.nih.gov/books/NBK132180/
Accessed Mar 26, 2024. 4. Mercadante AA, et al. Biochemistry, Replication and Transcription. 2020 Aug 28. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. PMID: 30986011.

Types of Gene Therapy

There are three major types of gene therapy

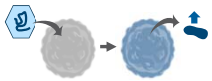
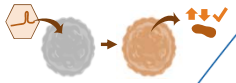
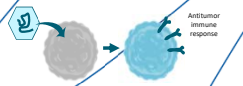
GENE ADDITION THERAPY is the insertion of a functional genetic material into the cell, with the goal of enabling the body to make a functional protein it could not adequately make before

GENE-BASED CELLULAR IMMUNOTHERAPY involves inserting genetic material into immune cells with the goal of enabling them to attack cancer cells such as in chimeric antigen receptor (CAR) T cell therapy

GENE EDITING is the addition, deletion, or alteration of DNA at specific locations in the genome using a system which consists of a "guide" genetic material and an enzyme

[NIH GT 2019/p1/para1/bullet3]
[NIH IMMUNO 2017/p2/para5]
[NIH GE 2019/p1/para1]

Overview of gene therapy approaches: distinctions from CAR-T

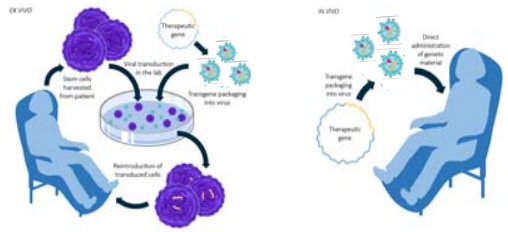
Gene Addition	Gene Editing	CAR-T
Insertion of genetic material to ***increase*** production of a functional protein[6,7]	Targeted modification of existing genetic material to ***increase*** or ***decrease*** production of a functional protein or to ***correct*** protein function[6,7]	Insertion of genetic material to produce a ***chimeric antigen receptor*** to enhance the ***antitumor immune response***[10,11]
		
Are approved[12] and are being studied for use as a one-time or repeated therapy for patients with an ***underlying genetic disease***[8]	Is approved[12] and are being studied for use as a one-time therapy for patients with an ***underlying genetic disease***[9]	Are approved[12] and currently being studied for use in the ***oncology*** setting for ***relapsed/refractory malignancy,***[3] ***and are being evaluated for use in autoimmune diseases***[13,14]

CAR-T, chimeric antigen receptor T cell.

1. Dong AC, *et al. Adv Exp Med Biol.* 2017;1013:155-176. 2. Hartmann J, *et al. EMBO Mol Med.* 2017;9(9):1183-1197. 3. Kalos M, *et al. Cell Metab.* 2017;26(1):94-109. 4. Alapati D, *et al. Am J Respir Cell Mol Biol.* 2017;56(3):283-290. 5. Zhao L, *et al. Front Immunol.* 2019;10:2250. 6. Morgan RA, *et al. Cell Stem Cell.* 2017;21(5):574-590. 7. **Cavazzana M, et al.** *Mol Ther.* 2017;25(5):1142-1154. 8. Prieto J, *et al. Gut.* 2003;52 Suppl 2(Suppl 2):ii49-54. 9. Babačić H, *et al. PLoS One.* 2019;14(2):e0212198. 10. David ML, *et al. Int J Hematol.* 2014;99(4):361-371. 11. Wang D, *et al. Discov Med.* 2014;18(97):67-77. 12. FDA. Accessed Apr 29, 2024. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products. 13. Müller F, *et al. N Engl J Med.* 2024;390(8):687-700. 14. Granit V, *et al. Lancet Neurol.* 2023;22(7):578-590.

Gene Addition Therapy

When "gene therapy" is used without specifying a sub-type, it typically refers to gene addition therapy.



- *Ex vivo* gene addition therapy
  - genetically altering a person's cells outside of the body then reintroducing the cells back into the person
- *In vivo* gene addition therapy
  - direct administration of genetic material systemically or locally to a specific organ of interest (e.g., eye, muscle)

[KUMAR 2016/p2/Figure1]

Gene Addition Therapy: Vectors

How does the new genetic material get inside the cell?


VIRAL VECTOR


NON-VIRAL VECTOR

Viral Vectors are synthetic vectors derived from naturally-occurring viruses (e.g., lentivirus, adeno-associated virus (AAV)) that **transport and deliver** genetic material into cells

Non-Viral Vectors involve **direct administration** of genetic material (e.g., via chemical disruption, electroporation, lipid nanoparticle) into cells

Gene addition therapy can also be used to insert genetic material that produces functional non-coding RNA, such as shRNA, rather than a functional protein.

[SHERIDAN 2011/p126/Figure1; p121-123/Viral vectors/para1; p127/Box2/Nonviral vectors]

Viral Vector Types for Gene Delivery

Different Viral Vectors Work in Different Cell Types

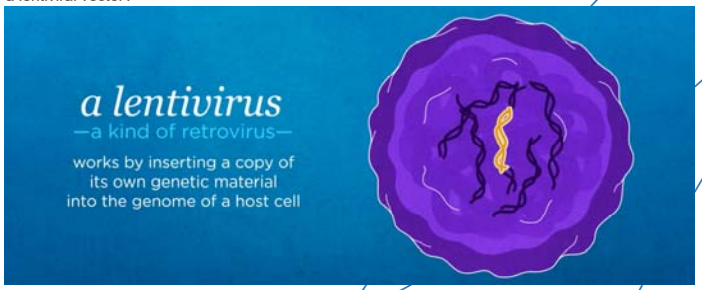
VECTOR (Genome)	PACKAGING CAPACITY	TYPES OF CELLS TARGETED
Retrovirus (RNA)	8 kb	Dividing cells only
Lentivirus (RNA)	8 kb	Broad: non-dividing cells (e.g., hematopoietic cells) and dividing cells
HSV-1 (dsDNA)	>40 kb	Efficient in neurons
Adeno-Associated Virus (AAV) (ssDNA)	~5 kb	Broad, with variable transduction efficiency in hematopoietic cells
Adenovirus (dsDNA)	8 kb	Broad

kb is a unit of measure of the length of a nucleic-acid chain that equals 1000 base pairs

[LUNDSTROM 2018/p2/Table1/rows4-5] [GOINS 1999/p520/col1/para2] [CHAMBERLAIN 2016/p1/Abstract/lines5-6; col1/para1/lines8-12; BROWN 2017/p451/col2/para2] [LEE 2017/p47/col2/para3]

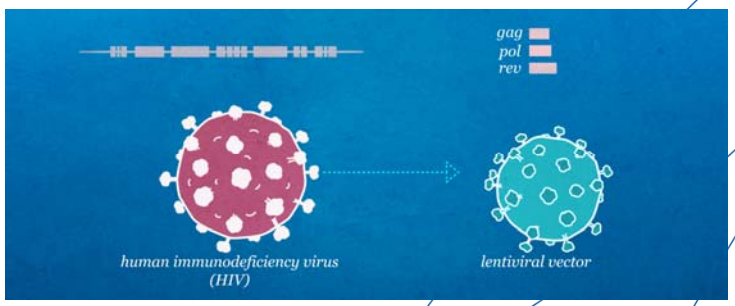
Gene Therapy in HSCs Using Lentiviral Vectors

What is a lentiviral vector?



Lentiviral vectors are based on the blueprint of the human immunodeficiency virus (HIV), a type of **lentivirus**

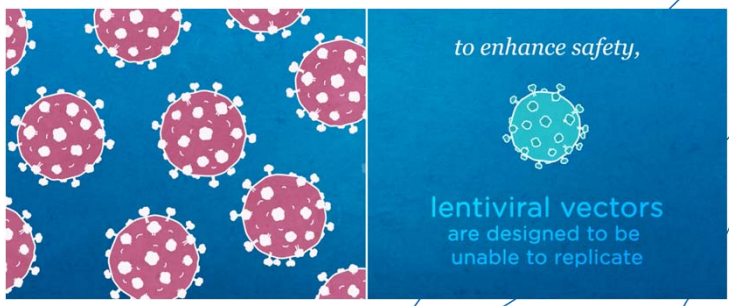
[DURAND 2011/p134/para4; DONG 2017/p4/para1]



Only the genes **required for certain essential functions** are used as blueprints to make **lentiviral vectors**

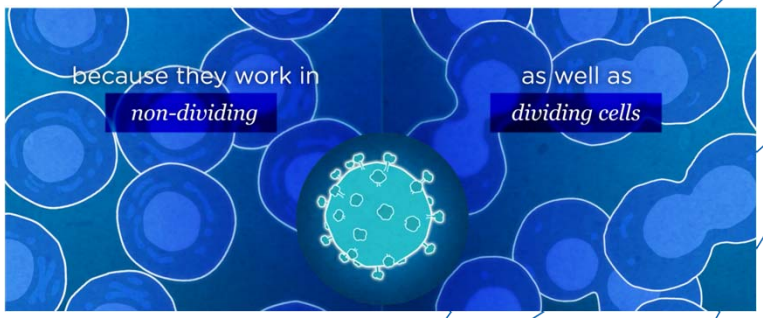
- These functions include **reverse transcription** and **integration**

[SEGURA 2013/ p4/col1/para2-3; col2/para1-2; p5/col1/para1/lines5-11; MILONE 2017/p1530/col2/para2]



- Each lentiviral vector can only deliver its genetic material to **one cell**

[DONG 2017/p4/para1]



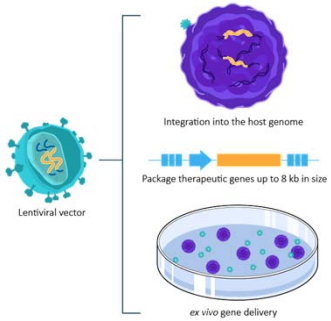
Lentiviral vectors work in both **non-dividing** and **dividing cells**, expanding the range of diseases they can be used to treat.

[ESCORS 2010/p3/para5]

- Lentiviral vectors have additional features that allow them to be used in a **variety of clinical therapeutic applications**:
  - **Low risk of immunogenicity (or producing an immune response)**
  - Ability to accommodate **large transgenes** (up to 8 kb)
  - Potential to provide **long-term, stable expression of a therapeutic gene** delivered to the host cell

[FROELICH 2010/p3/para1]
[LUNDSTROM 2018/p2/Table1/row6]
[SEGURA 2013/p2/col2/para3; BONCI 2003/p631/col1/para2; MAO 2015/p410/col2/para1]

Lentiviral Vectors: Key Differences From Other Vectors



Lentiviral vectors may **enable durable gene expression** via **integration into the host genome**, while other viral vectors typically rely on non-integrating methods

Lentiviral vectors can **package larger therapeutic genes (8 kb)** than AAV (~5 kb)

Lentiviral vectors are primarily used for ***ex vivo* gene delivery**, while adenovirus has traditionally been used for ***in vivo* gene delivery**

[SEGURA 2013/p2/col2/para3]

Key Features of Today's Third-Generation Lentiviral Vectors

- Deliver genetic material into both **dividing** and **non-dividing** cells
- **Integrate stably** into the host cell genome which is thought to **maintain long-term expression**
- Deliver **larger amounts of genetic material** than other commonly used vector systems
- Are thought to be **non-pathogenic** (including non-oncogenic) and **non-immunogenic**
- Can be **replication-incompetent** and **self-inactivating**

[ESCORS 2010/p3/para5]
[SEGURA 2013/p2/col2/para3; BONCI 2003/p631/col1/para2; MAO 2015/p410/col2/para1]
[LUNDSTROM 2018/p2/Table1]
[FROELICH 2010/p3/para1]
[CAVAZZANA 2015/p112/col2/para3-4]

Making a Lentiviral Vector

Lentiviral vectors are made in **packaging cells** transfected with plasmids containing the component genes.

The packaging cells are able to produce **lentiviral vectors carrying the therapeutic genetic material.**

The vectors are then used to **add a functioning gene** into patients' hematopoietic stem cells.

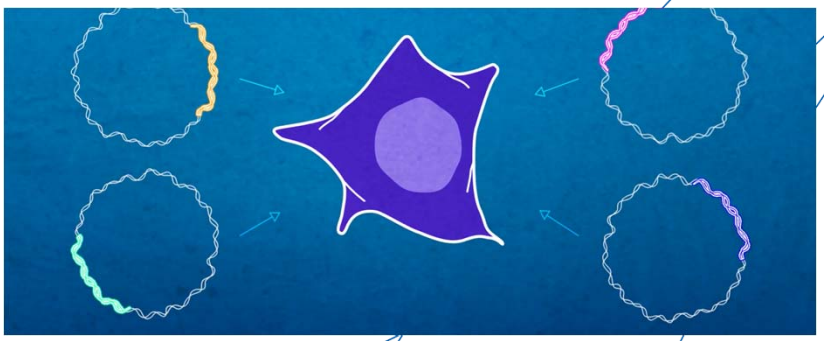
[GANDARA 2018/p4/col1/para2; p1/col2/para2]
[MILLINGTON 2009/p1/col2/para1/lines11-22]

Viral **packaging functions are kept separate** in **different plasmids** to **prevent unwanted recombination** during vector production

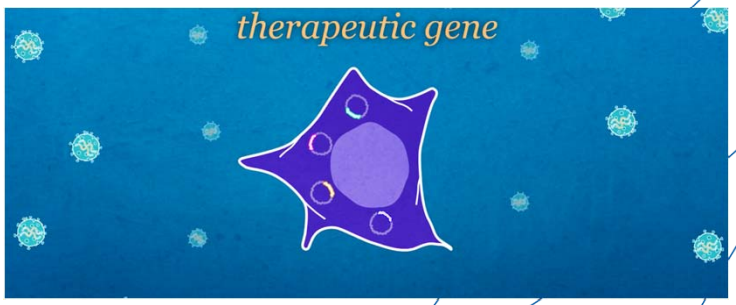
[GANDARA 2018/p4/col1/para2; p1/col2/para2]

These plasmids include:

- A **transfer plasmid** with the therapeutic gene
- **Packaging plasmids** with genes for structural proteins, enzymes, and regulation
- An **envelope plasmid** with the gene for an envelope protein



[MILONE 2017/p1432/Fig2; col2/para3,5; SEGURA 2013/p4/col1/para1; col2/para3; p5/col1/para1, p2/col1/para3]



Packaging cells transfected with these plasmids are able to produce **lentiviral vectors carrying the therapeutic gene**

[GANDARA 2018/p4/col1/para2]

[MILLINGTON 2009/p1/col2/para1/lines11-22]

Collecting Patient HSCs for Gene Therapy

Hematopoietic stem cells are stimulated to move from bone marrow to blood using a **mobilizing agent.**

Cells are collected directly from **peripheral blood** using **apheresis**.

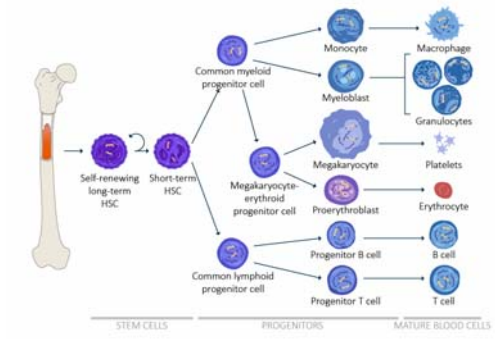
CD34⁺ cells are **isolated** at a central processing facility.

[MORGAN 2017/p575/col1/para3; col2/para2/lines2-4]
[MILLINGTON 2009/p2/col1/para3,4; col2/para1]

HEMATOPOIETIC STEM CELLS (HSCs)

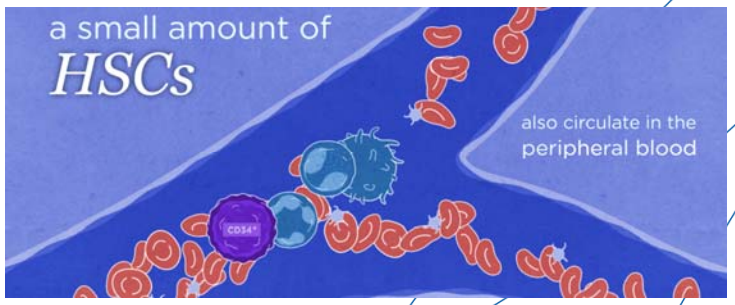
HSCs are self-renewing cells
Allows for hematopoiesis to occur indefinitely

Lentiviral Vectors Are Used for Gene Addition in Hematopoietic Cells (HSCs)



[NIH STEM CELLS 2001/p2/para1; Figure5-1]
[Ferrari G, et al. *Nat Rev Genet.* 2020. https://doi.org/10.1038/s41576-020-00298-5.]
[Fraoqua E, et al. *Proc Natl Acad Sci USA.* 2003;100(Suppl 1):11842-11849.]

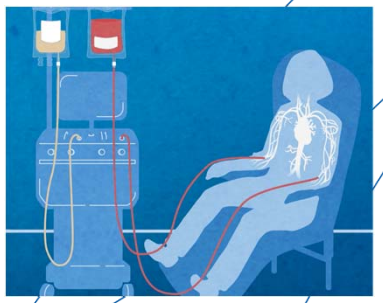
[MORGAN 2017/p575/col1/para3]



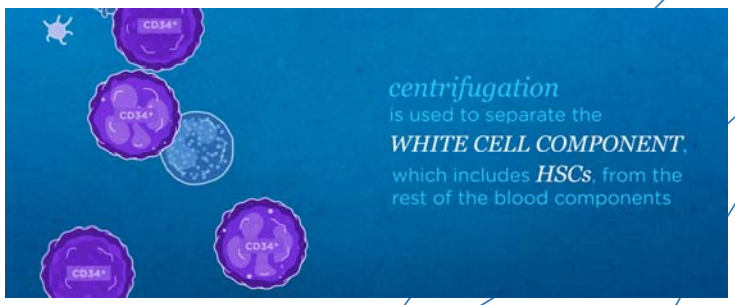
HSCs primarily reside in the **bone marrow,** but a small amount also **circulate in the peripheral blood**.

[HOGGATT 2011/p1/col2/para1]
[NIH 2001/p3/Peripheral Blood]

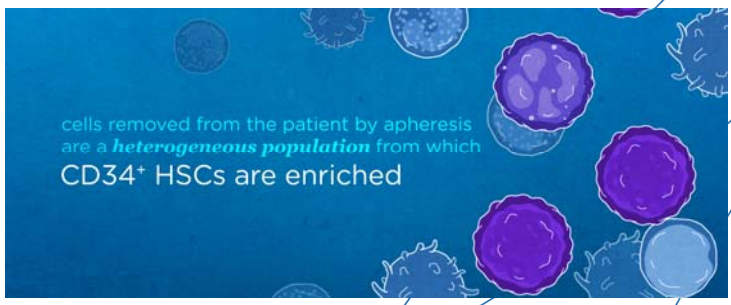
A **heterogeneous cell population containing HSCs** is collected from the **peripheral blood** by **apheresis**



[MORGAN 2017/p575/col1/para3; col2/para2/lines2-4]



[TURA 2007/p2/col2/para3; p3/col1/para1]



[TURA 2007/p2/col2/para3; p3/col1/para1]

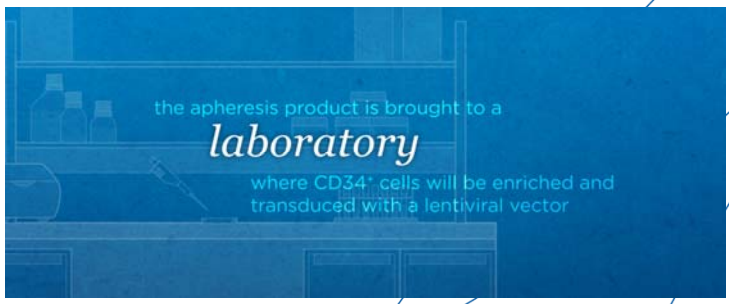
Construction the Ex Vivo Gene Therapy Product

Once **CD34⁺ cells** are **isolated** from the apheresis product, **lentiviral vectors** are used to **transduce** them.

The **therapeutic gene** carried by the lentiviral vectors is **integrated into the HSC genome.**

The transduced HSCs are **washed**, **frozen**, and **tested for safety and quality**.

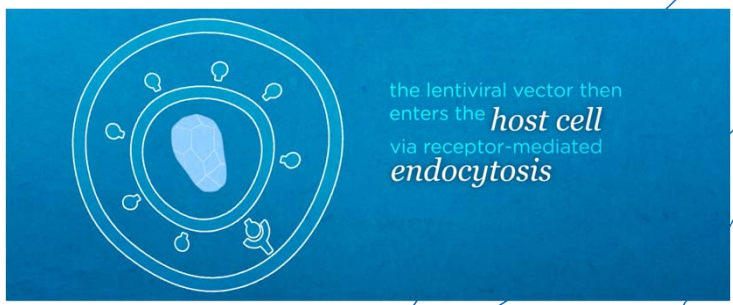
[TURA 2007/p2/col2/para3; p3/col1/para1] [MILLINGTON 2009/p2/col1/para3,4; col2/para1] [MILONE 2017/p1530/col1/para1/l12-18] [MILONE 2017/p1533/Fig3; NEGRE 2016/p158/col1/para1-2; col2/para1]



[MILLINGTON 2009/p2/col1/para3,4; col2/para1]

The lentiviral vectors are **added to the patient's CD34⁺ cells,** along with other components (e.g., cytokines) to **help the vector transduce** them

[MILLINGTON 2009/p2/col1/para3,4; col2/para1; Figure1B; p3/col1/para2] [DELSHE 2003/p326/col1/para2; p330/col1/para2]



Receptor-mediated endocytosis is a process that allows cells to absorb specific substances from outside the cell

[MILONE 2017/p1530/col1/para1; ZHENG 2015/p8/Figure5; DONG 2017/p4/para1]

the genetic material
—containing the therapeutic gene—
is released into the host HSC

[MILONE 2017/p1530/col2/para2; p1531/col1/para1]

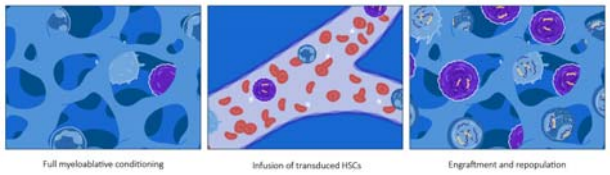
The **viral RNA** is **reverse-transcribed into DNA** by reverse transcriptase and transported to the nucleus, where it is **integrated into the host genome**

[MILONE 2017/p1530/col2/para2; col1/para1/li12-18; p1531/col1/para1]

After **successful transduction** of the therapeutic gene **into HSCs,** the transduced cells are **washed, cryopreserved,** and **rigorously tested** for **safety** and **quality**

[MILONE 2017/p1533/Fig3; NEGRE 2016/p158/col1/para1-2; col2/para1]

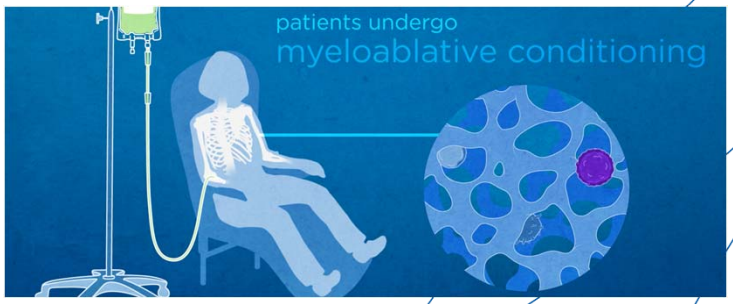
Delivering the Ex Vivo Lentiviral Gene Therapy to a Patient



Genetically modified HSCs are delivered to a patient via standard autologous hematopoietic stem cell transplant, which involves:

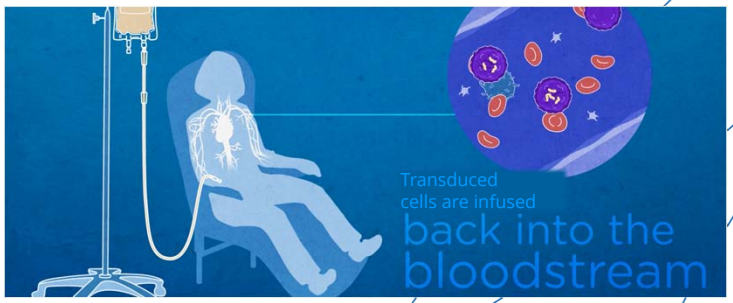
- **Full myeloablative conditioning** to clear space in the bone marrow
- **Infusion of transduced HSCs** into the bloodstream
- **Engraftment** and **repopulation** of the bone marrow

[NEGRE 2016/p158/col2/para1]
[MORGAN 2017/p577/Figure2; NEGRE 2016/p158/col2/para1; p159/col1/para2/lines23-34]
[CAOCCI 2 2017/p8/col2/para3; p1/Abstract; HATZIMICHAEL 2010/p106/col1/para4]



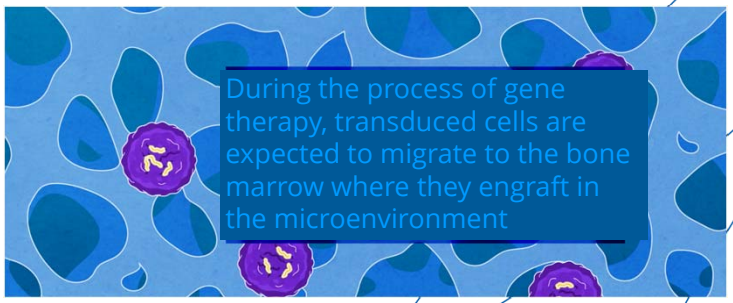
In preparation to receive their **transduced cells,** patients undergo **myeloablative conditioning to clear space in the bone marrow**, typically via high-dose chemotherapy

[NEGRE 2016/p158/col2/para1, p159/col1/para2/ln23-34]



Cryopreserved transduced cells are **thawed and infused back into the bloodstream** after washout of conditioning agents

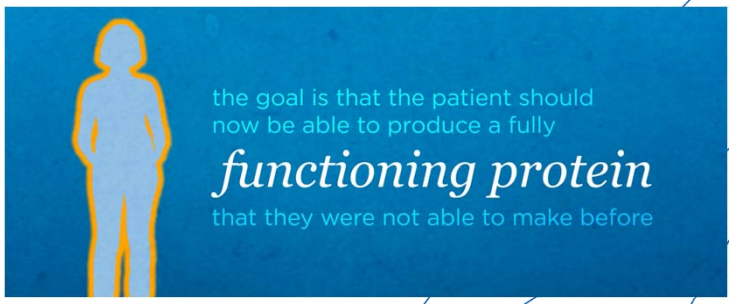
[MORGAN 2017/p577/Figure2; NEGRE 2016/p158/col2/para1; p159/col1/para2/lines23-34]



[CAOCCI 2 2017/p8/col2/para3; p1/Abstract]

[CAOCCI 2 2017/p8/col2/para3; p1/Abstract; HATZIMICHAEL 2010/p106/col1/para4]

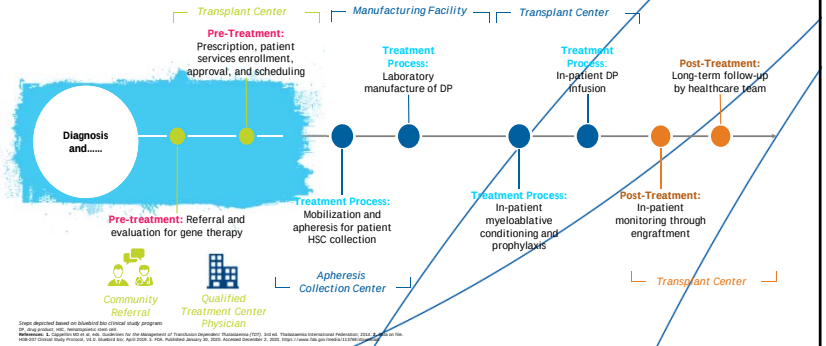
[SEGURA 2013/p2/col2/para3; BONCI 2003/p631/col1/para2; MAO 2015/p410/col2/para1]



The body should now be able to use this protein to **supplement the protein deficiency** causing the disease

[NIH GHR 2018/p1/para1,3]

Patient journey overview: The gene therapy treatment process is a structured collaboration[1-3]



Steps depicted based on bluebird bio clinical study program.
DP, drug product; HSC, hematopoietic stem cell.

References: 1. Cappellini MD et al, eds. *Guidelines for the Management of Transfusion Dependent Thalassemia (TDT)*. 3rd ed. Thalassemia International Federation; 2014. Available on file. **2.** HGB-207 Clinical Study Protocol, Vo.0. bluebird bio. April 2016. **3.** FDA. Published January 30, 2020. Accessed December 2, 2020. https://www.fda.gov/media/113796/download

Questions?