GENE THERAPY AND DUCHENNE

An Overview of Micro-Dystrophin Gene Therapy Research for Duchenne Muscular Dystrophy (DMD)



Duchenne muscular dystrophy, sometimes called DMD or Duchenne, is a rare genetic disease. Duchenne is caused by a change (called a *mutation*) in the *DMD* gene, which prevents the body from making dystrophin, a protein found in muscle cells. Dystrophin protects muscles from injury. If the body can't make dystrophin, or can't make enough of it, muscle cells become damaged.

Duchenne is an irreversible condition that gets worse over time. As the disease progresses, it affects the muscles in the shoulders, arms, legs, and trunk, which leads to functional difficulties raising the arms or walking. In later stages, Duchenne affects the muscles that affect breathing and allow the heart to function.

Researchers are looking for ways to slow or stop the progression of Duchenne. One approach is called *gene therapy*. Gene therapy adds a new gene to a person's body, with the hope of treating the disease. Sarepta Therapeutics is leveraging its platform approach in gene therapy to research a potential treatment for Duchenne. The information in this guide provides an overview and answers some frequently asked questions about micro-dystrophin gene therapy.

What is micro-dystrophin gene therapy?

The aim of micro-dystrophin gene therapy is to deliver micro-dystrophin, a shorter form of the dystrophin gene, to muscle cells affected by Duchenne muscular dystrophy. Once the micro-dystrophin gene is inside the muscle cells, researchers believe it may help the cells make micro-dystrophin protein.

What are the key building blocks of micro-dystrophin gene therapy platform?

1.

The **VECTOR**, which carries the micro-dystrophin transgene through the body and delivers it into the muscle cells

2.

The micro-dystrophin **TRANSGENE**, a gene made of DNA that tells the muscle cells to make micro-dystrophin protein

3.

The **PROMOTER**, which tells the body to produce micro-dystrophin protein in muscle cells





Understanding Micro-dystrophin Gene Therapy

DNA

Healthy DNA contains the DMD gene that gives cells the instructions needed for making dystrophin.

MICRO-DYSTROPHIN TRANSGENE

The gene for dystrophin is very long, so portions of the gene believed to be most relevant to DMD are selected.

3.

GENE INSERTED IN THE VECTOR

To deliver the transgene to target cells, the transgene must be inserted into a protein shell, called a vector.

GENE THERAPY IS GIVEN

In a single treatment, many copies of the vector containing the transgene are introduced into the patient's body via intravenous (IV) infusion.

5.

VECTOR ENTERS CELL AND DELIVERS TRANSGENE

The vector has an affinity to muscle cells that are affected by Duchenne muscular dystrophy.

target cell

6.

PROMOTER TELLS THE CELL TO MAKE MICRO-DYSTROPHIN

The promoter tells the cells to "turn on" the transgene and begin making micro-dystrophin protein.

Why is micro-dystrophin used in gene therapy instead of dystrophin?

The dystrophin gene is one of the largest genes in the body. AAV vectors are not large enough to carry the entire dystrophin gene, so a shorter version of the gene must be made to fit inside the vector.

To create the micro-dystrophin transgene, scientists at Sarepta researched and carefully selected the parts of the dystrophin gene to use in micro-dystrophin. These sections were combined to create a micro-dystrophin transgene that fits inside AAV vectors.



The gene that codes for micro-dystrophin fits inside the vector. And while it doesn't contain all the parts of dystrophin, the parts that were included in micro-dystrophin were specifically chosen because it is believed that they are critical for muscle function.

What controls the production of micro-dystrophin in muscle cells?

The *promoter* is the region of the gene that determines where and when that gene is turned on. When designing a gene therapy for Duchenne, Sarepta researchers identified a muscle-specific promoter. A muscle-specific promoter enables micro-dystrophin protein production when the gene is delivered to skeletal, diaphragm, and heart muscle cells.

The promoter can also recognize when a gene is delivered into a non-muscle cell, and will not activate the gene.



What is a vector and why is it used in micro-dystrophin gene therapy research?

To deliver the micro-dystrophin transgene to muscle cells, the transgene needs to be protected as it travels throughout the body. The transgene also needs to be able to be delivered to the correct location within cells.

Viruses are naturally able to enter cells, and they can be used to provide a protective covering for the transgene. Scientists working on gene therapy are able to modify inactive viruses to allow them to carry genes into target cells. These modified viruses are referred to as *vectors*.

Scientists choose specific vectors with qualities that are important for their research. Sarepta scientists chose an adeno-associated virus (AAV) for Duchenne research.

How does a vector target muscle cells?

Different types of AAVs (e.g., AAV3, AAV8) are used as vectors in gene therapy. Each type of AAV has a preference for a specific type or types of cell. This is called *tropism*. Researchers in Duchenne choose vectors that are attracted to the muscle cells affected by Duchenne.

What are antibodies and why do they matter to gene therapy?

Antibodies are proteins in the blood that are produced in response to a specific viral infection. After a person recovers from a viral infection, the antibodies specific to that virus stay in his or her body to quickly recognize that virus if they are exposed to it again in the future.

The vectors used in gene therapy are created from modified viruses. If a person has already been exposed to the same virus as the virus used in the vector, there is a chance that he or she may already have antibodies to that virus. These preexisting antibodies could potentially make someone ineligible for gene therapy because the antibodies would recognize the vector when it entered the body, much in the same way that antibodies recognize viruses from previous infections.

How long will gene therapy last?

It is not yet known how long a one-time infusion of micro-dystrophin gene therapy for Duchenne will last. Each gene therapy, condition, and person are different. This makes it difficult to predict *durability*, or how long the gene therapy will remain active. If a gene therapy is approved by the FDA, it will still need to be studied over years (or even decades) for scientists to truly understand its effects.

Can someone receive gene therapy more than once?

Currently, no. When someone receives gene therapy, his or her immune system will develop antibodies to the vector used. If the same or similar vector were to be used again for another dose of gene therapy, the antibodies in that person's immune system would recognize the vector and it would be removed from the body. Because of this, it is believed the gene therapies in development today can only be given once.

GLOSSARY OF TERMS

TERM	DEFINITION
ADENO- ASSOCIATED VIRUS (AAV)	A small virus that infects humans and some other primate species, and is not known to cause disease. AAV vectors may be used to carry genes into host cells as part of gene therapy. The various distinct types of AAV differ in the types of tissue they target.
CELL	Considered the building blocks of life, cells are the basic unit of all living things. Most cells contain DNA. The human body contains trillions of individual cells that vary in type and function, such as muscle cells.
DNA	DNA is a molecule contained in most types of human cells that provides the cell with instructions for building and maintaining itself. DNA is inherited from your parents, and in most cases, it is unique to only you. Nearly all your cells contain the same unique DNA.
DURABILITY	Durability is the ability of a physical product to remain functional. In gene therapy, it refers to the amount of time a gene can functionally produce protein following the administration of gene therapy. The durability of gene therapies is currently unknown.
DYSTROPHIN	Dystrophin is a protein that connects to muscle cells and serves to protect muscle cells from injury. The genetic instructions for producing dystrophin are contained in the DMD gene, which is one of the largest human genes known. Duchenne muscular dystrophy is the result of an error in the DMD gene which makes the body unable to produce a working dystrophin protein.
GENE	A sequence of DNA that contains cellular instructions for making a protein with a specific function. For example, the DMD gene contains the instructions for producing the protein dystrophin. Humans are estimated to have around 20,000 protein-coding genes.
GENE THERAPY	The delivery of foreign genetic material (DNA) into cells for therapeutic purposes. Gene therapy is an approach that seeks to fix genetic problems at its source by correcting genetic errors.
MUTATION	An alteration or error in the genetic material contained in DNA. Some genetic mutations have no effect, while others may result in a modified product of a gene. Duchenne muscular dystrophy is caused by a mutation that alters the production of dystrophin.
PROMOTER	A region of DNA that activates a gene, initiating the production of a corresponding protein. Promoters may be specific to cell types, and can tell different cell types which genes to activate or silence.
TRANSGENE	A gene that has been isolated from one organism and transferred to another. Like all genes, a transgene is made of a segment of DNA and contains instructions for producing a specific functional protein.
TROPISM	In the context of gene therapy, tropism refers to the biological phenomenon where a virus preferentially targets specific cell types. Naturally occurring tropism in AAV can be used to select vectors based on target cell types.
VECTOR	A carrier used to artificially deliver genetic material into cells. Gene therapies often use an inactive virus, such as AAV, as a vector.

To learn more about our research into potential gene therapies for Duchenne muscular dystrophy, visit us at Sarepta.com.

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SAREPTA THERAPEUTICS IS A BIOTECHNOLOGY COMPANY DEVELOPING POTENTIALLY LIFE-CHANGING PRECISION GENETIC MEDICINE.

Our long-term goal is to provide therapies to 100% of individuals with Duchenne muscular dystrophy by unlocking the full potential of RNA technologies, gene therapy, and gene editing.



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