Gene Therapy for Rare Diseases: Manufacturing

Gene therapy* has emerged in recent decades as a potentially ground-breaking approach to the treatment of many serious diseases, including rare and hereditary diseases.[1] Producing these therapies involves the use of living **cells** and other biological materials, as well as advanced (and often brand-new) technologies.[2,3]

Creating a new gene therapy is a complex process that happens in stages. First, small quantities of a potential new therapy are made in a laboratory. These small batches are used in early **preclinical research**. But to help ensure safety, lab-created batches of potential new gene therapies cannot be used in **clinical trials** (studies done with human beings). This means that before a potential new gene therapy can be tested in people, a safe and reliable larger-scale manufacturing process must be developed.[3,6] This manufacturing process must be capable of producing the therapy in large quantities with consistent potency (strength) and purity, batch after batch. To meet these considerable logistical and technological demands, scientists who work on developing new gene therapies consult and collaborate with potential manufacturers starting from the very early stages of the research process.[6]

This brochure describes some of the steps and processes that may be involved in a large-scale manufacturing process for a gene therapy. Although one specific type of gene therapy is used as an example, it's important to note that gene therapies can involve a wide range of different components and technologies. Every gene therapy is unique, and requires an individualized approach to manufacturing.

Note: some words that may be unfamiliar are highlighted and are defined in the glossary at the end of this brochure.

*Defined as modifying a person's genetic material, or introducing new genetic material, to treat a disease.

Amicus Therapeutics

Amicus Therapeutics has developed this educational resource in collaboration with the rare disease community and thought leaders.

Understanding important elements of gene therapy manufacturing

What are the main components of a gene therapy?

- A gene therapy is typically made up of two main components: **genetic material** and a **vector**
  - the genetic material provides genetic information designed to help the targeted cells function correctly[7]
  - the vector is the delivery system that brings the genetic material to the targeted cells.[7] Many different types of vectors exist[8]

What is a plasmid?

- **Plasmids** are circular pieces of **DNA** that are found in almost all types of bacteria. Plasmids replicate within bacteria independently of the bacteria's own DNA
- Plasmids may be used for a variety of different tasks in the development and manufacturing of gene therapies[9-11]

What is a virus?[12]

- A **virus** is a very small biological agent that can enter cells
- Viruses are made up of genetic material enclosed in an outside shell called a **capsid**
- When a naturally occurring virus—for example, an influenza virus—enters and infects a cell, the following steps occur[13]:
  - the virus's capsid attaches to the cell, allowing the virus to insert its genetic material into the cell
  - the virus's genetic material takes over the cell's machinery, causing the cell to create copies of the virus rather than performing its usual functions

How are viruses used as vectors?

- When a virus is used as a vector in a gene therapy, it is modified so that it can safely and effectively deliver the genetic material to targeted cells[11,14]:
  - the virus's own genetic material is disabled or removed, and is replaced with genetic material designed to treat the disease (this also prevents the virus from causing disease)
  - the virus's capsid is modified to help the virus 1) target the correct cells within the body, and 2) successfully insert the new genetic material into the cells

Overview:
the challenges of scaling up from lab to manufacturing facility[4-6]

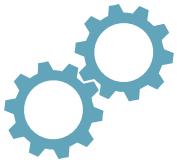
Creating a scalable process that makes it possible to *safely, efficiently,* and *consistently* reproduce a lab-created gene therapy in large quantities is one of the most important—and most challenging—steps in gene therapy development.

The reasons for this are complex. However, most of them stem from one basic fact: processes and equipment used in the lab may be impossible—or simply not practical—to use for larger-scale manufacturing.

Think of it like cooking or baking. When you multiply a recipe by 10 (or 100 or 1000), a lot of things may have to change! Different equipment will be needed, of course, but other aspects of the process can change as well.

Laboratory scale:
small batches of a gene therapy made in a lab; used in early preclinical research.

Baking bread is a simple example. The larger the loaf, the greater the difference in temperature from its outside surface to its center—which means that larger loaves are generally baked at lower temperatures, and for longer periods of time, to compensate for their greater surface area.



Scientists who are developing a potential new gene therapy typically work closely with a manufacturing organization to move through each scale of production—from laboratory scale to clinical scale, and then, finally, to commercial scale (see definitions in call-out boxes at left).

Clinical scale:
larger batches of the gene therapy created in a manufacturing facility; used in later preclinical research and in clinical trials.

The shift from laboratory-scale batches to clinical-scale manufacturing is especially important. This is the stage in the development process when the "recipe" for producing the gene therapy in large quantities—that is, a well-defined manufacturing process with reliably consistent results—is first established.

Commercial scale:
even larger batches of the gene therapy produced in a manufacturing facility; created for commercial use.

The following pages describe some of the main steps and processes that may be involved in creating, using, and quality-testing a recipe for the large-scale manufacturing of a gene therapy.

1. Plasmid production: creating the genetic material[9,10]

In order for a gene therapy to be manufactured at a large (clinical or commercial) scale, the genetic material developed to treat the disease must first be produced in sufficient quantity. A fragment of DNA called a plasmid is often used for this task (see page 2, *Understanding important elements of gene therapy manufacturing*).

Using plasmids to create large quantities of genetic material for a gene therapy

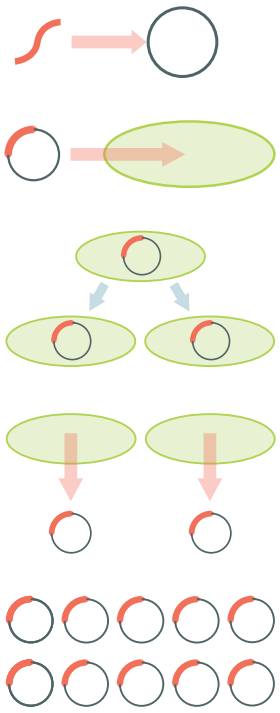
The genetic material and the plasmid are structurally altered, allowing them to be combined

The plasmid (which now includes the genetic material) is inserted into a bacterium

The bacterium reproduces itself, creating multiple identical copies of the modified plasmid

The modified plasmid is then removed from the bacteria

Large amounts of plasmid that contains the desired genetic material are obtained



2. Creating a "recipe" for large-scale manufacturing[2-4,6]

Creating a scalable recipe that allows for safe and reliable large-scale manufacturing of a gene therapy involves several complex steps.

- Key attributes of the therapy (sometimes called critical quality attributes, or CQAs) are defined
- Technical challenges are addressed (see page 3, *Overview: the challenges of scaling up from lab to manufacturing facility*):
  - additional research may be done to further evaluate materials and technologies
  - if necessary, new manufacturing tools and platforms are created to meet specific needs
  - standardized processes are developed to ensure quality and safety
- Reliable testing methods are developed to confirm that the therapy's CQAs are met consistently

3. Vector production: creating the delivery system[5,11,14]

After sufficient genetic material has been produced and a safe and reliable large-scale manufacturing process has been developed, the vector that will deliver the genetic material can be created at the desired quantity. Steps that may be involved in manufacturing a viral vector* are described below.

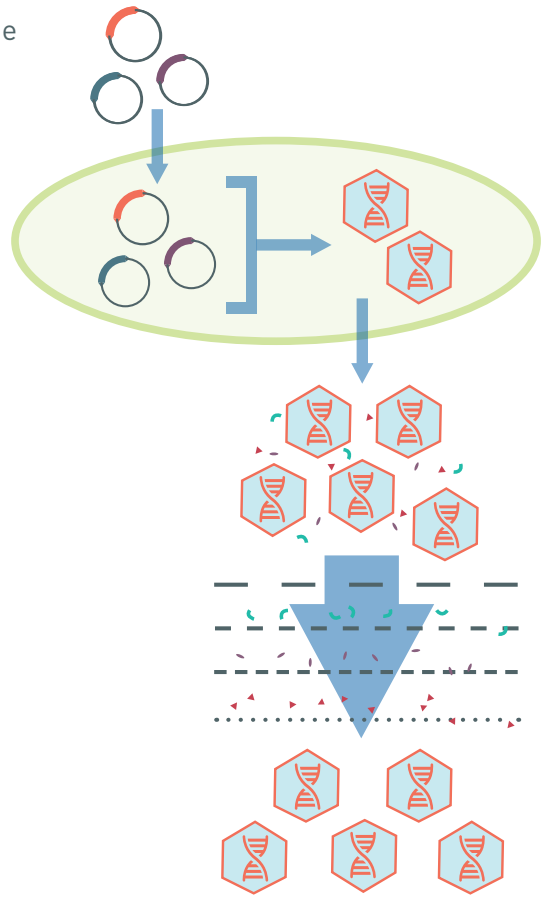
Manufacturing a viral vector for a gene therapy

In a manufacturing facility, plasmids that contain the genetic material developed to treat the disease are inserted—along with helper plasmids—into large numbers of host cells.

Working together within the cells, the plasmids use the cell's machinery to create the vectors (viral vectors are made up of modified virus capsids that contain genetic material developed to treat disease) (see page 2, *How are viruses used as vectors?*).

The viral vectors are then collected from the host cells. At this stage, impurities—such as unwanted fragments of plasmid DNA or proteins—are still present in the solution.

Finally, the solution is thoroughly cleaned and purified. This process includes the use of multiple filters to remove the impurities remaining from the manufacturing process.



*This is just one example; other types of vectors can be used in gene therapies.

4. Quality review[6,15]

As a final and very important step, the gene therapy is carefully assessed to ensure that it is safe for use by patients. Every batch is extensively tested to confirm that all requirements for quality and proper manufacturing procedures were met. Many aspects of the therapy are evaluated, including

- Potency
- Presence of the desired genetic material
- Viral titer (ie, the number of viral vectors present in a given amount of the solution)
- Purity
- Sterility

Glossary

Capsid: the outside shell of a virus that allows it to target specific cell types

Cell: basic building block of all living things

Clinical trial: voluntary research studies conducted in people and designed to answer specific questions about the safety or effectiveness of drugs, vaccines, other therapies, or new ways of using existing treatments

DNA (deoxyribonucleic acid): substance within genes that contains instructions, or code, for making proteins

Genetic material: DNA (or sometimes RNA [ribonucleic acid], a biochemical that helps DNA send its biological instructions) provided by a gene therapy to treat a disease

Plasmid: circular pieces of DNA found in almost all types of bacteria that replicate independently of the bacteria's own DNA

Preclinical research: laboratory studies done in animals or in laboratory equipment such as test tubes

Vector: a biological agent (virus) or biochemical agent (liposome or polymer) used to carry and transfer genetic material into a cell

Virus: a very small biological agent that can enter cells; in gene therapy, the virus used is modified so it will not cause disease

To learn more

Amicus offers additional resources that provide families with information about gene therapies; please contact us at patientadvocacy@amicusrx.com to request copies.

References:

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