GENE THERAPY INDUSTRY REPORT 2021

A GROWING MARKET, CURRENT CHALLENGES, AND AN EXCITING FUTURE

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THE GLOBAL GENE THERAPY LANDSCAPE

An Introduction

A SHORT HISTORY OF GENE THERAPY

Although hailed as one of the greatest scientific success stories of the 21st century, the gene therapy field is relatively young and has been marred by major setbacks in its short life. In 1972, US researchers Theodore Friedmann and Richard Roblin published a paper in *Science* titled *'Gene therapy for human genetic disease?'*. In this paper, they discussed the huge potential of introducing healthy DNA sequences into the cells of people with genetic disorders. They also pointed out the lack of knowledge and understanding around this technology and urged the scientific community to further study gene therapy. They had good reason.

At first, the gene therapy field saw a massive surge in popularity. In 1990, 18 years after Friedmann and Roblin published their paper, the first gene therapy trial began. The first test subject was a four-year-old girl born with a rare genetic condition called severe combined immunodeficiency; she lacked the enzyme adenosine deaminase, which made her immune system weak and susceptible to constant infection. The girl received an injection of a viral vector carrying a functional copy of the gene encoding for the adenosine deaminase enzyme. After the treatment, her immune system improved and she was able to live a normal life.

The success of this first gene therapy trial led to a surge of similar trials throughout the 1990s. Most of these trials deployed adenoviruses to deliver healthy genes into patients, based on the research of Canadian scientist Frank Graham in the 1970s. In 1973, he developed a cell line for viral vector production, which is still the most widely used cell line for adenovirus development today: the human embryonic kidney cell line, (HEK)293.

In 1999, the gene therapy field went from boom to bust. 18-year-old Jesse Gelsinger had a genetic condition called ornithine transcarbamylase deficiency, a disease that prevented his liver from breaking down toxic ammonia that accumulated in his blood. Gelsinger had signed up for an experimental gene therapy trial at the University of Pennsylvania, where he received the treatment: an adenovirus carrying a healthy copy of the mutated gene into his liver cells. But four days after the treatment, Gelsinger suffered a severe immune reaction and died.

The gene therapy field collapsed practically overnight. An investigation was launched by the FDA into the trial, as well as 69 other gene therapy trials across the US. The overall consensus was that research had moved too fast and safety had not been put first. From then on, researchers continued more cautiously and progress in the gene therapy field slowed.

In 2003, China approved the very first gene therapy for head and neck cancer, Gendicine. That same year, the complete human genome sequence was published for the first time by the Human Genome Project, the result of a 15-year global research collaboration.

The second gene therapy to be marketed was Russia's Neovasculgen for peripheral artery disease in 2011. One year later, Europe approved its first gene therapy against the rare genetic disorder lipoprotein lipase deficiency: uniQure's Glybera. As the most expensive treatment in the world at the time, with a price of €1M ($1.2M) per patient, Glybera was a commercial failure and withdrawn from the market in 2017.

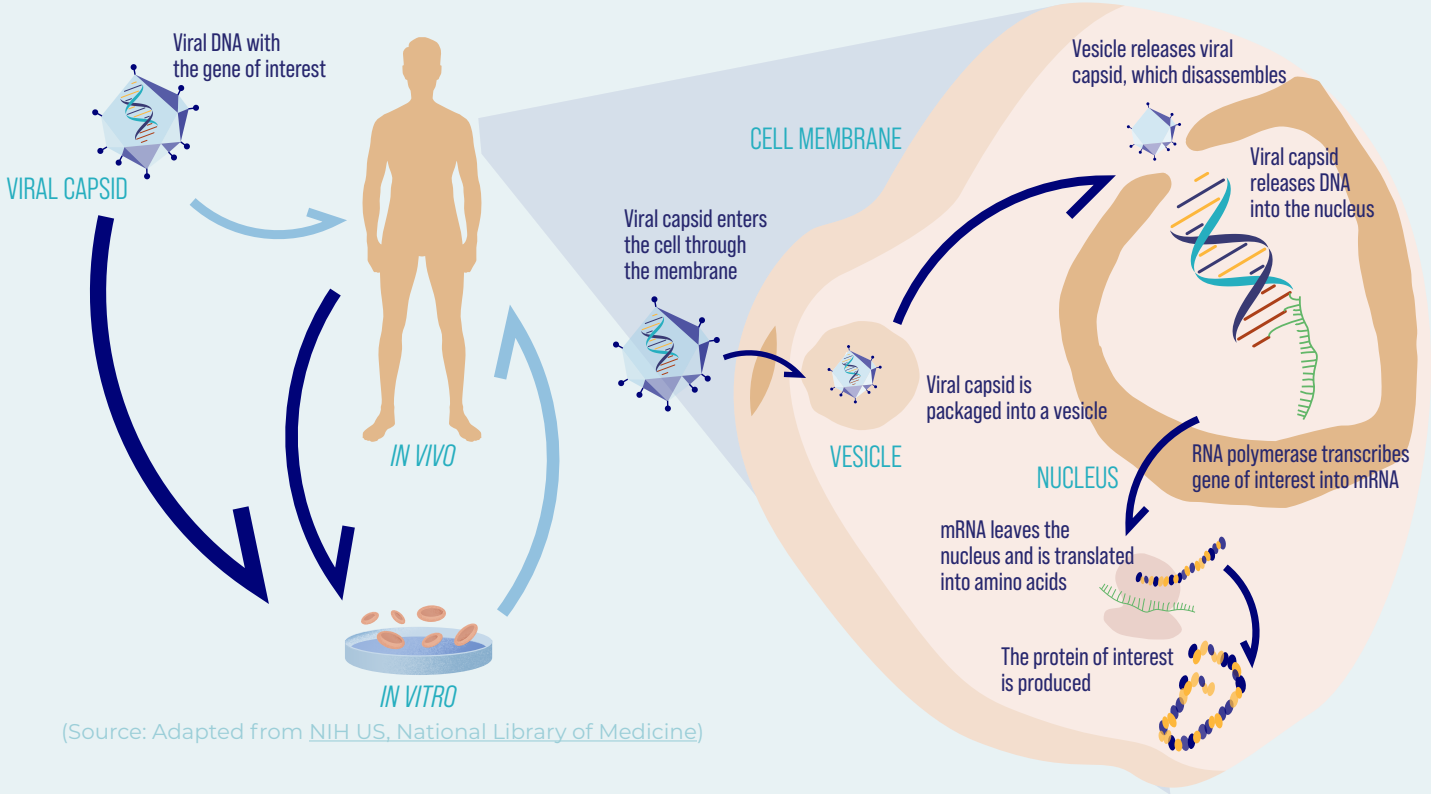
Since these early beginnings, a lot has changed in the gene therapy field. Today, there are over 20 gene therapies on the market, an increasing number of therapies in the global pipeline, and several advances in gene therapy manufacturing.

HOW DO GENE THERAPIES WORK?

The basic idea of gene therapy is to introduce genetic material into cells to replace mutated genes or to provide the instructions to produce an advantageous protein. For example, in some genetic diseases a certain protein is lacking or faulty. In this case, a gene therapy can introduce a healthy copy of a gene into cells, which can then be transcribed by the cell's own machinery and translated into a functioning version of the missing or faulty protein.

In order for the gene of interest to reach the inside of the cell, it has to be packaged into a carrier vehicle. Mostly, these vehicles are viral vectors, but there are also non-viral carriers. Viruses are often used as carriers because they can deliver the gene of interest by naturally infecting cells. However, the viruses used are engineered so they are incapable of causing disease in humans. In gene therapy, the vehicle is composed of the virus' capsid, the protein shell of the virus that carries the genetic material.

HOW A GENE THERAPY WORKS



(Source: Adapted from NIH US, National Library of Medicine)

The viral vector carrying the viral DNA and the gene of interest enters the cell through the cell membrane. At this stage, the viral vector is packaged into a vesicle and transported to the cell's nucleus. Here, the vesicle disintegrates and the viral capsid disassembles, releasing the viral DNA and gene of interest into the cell's nucleus through a nuclear pore.

Some viruses, like retroviruses, integrate their DNA directly into a chromosome once they enter the human cell's nucleus. Other viruses, such as adenoviruses, deliver their DNA into the nucleus but do not integrate it into the cell's genetic material.

Inside the nucleus, the gene of interest is transcribed into messenger RNA (mRNA) by the enzyme RNA polymerase. The mRNA then travels through a nuclear pore into the cell's cytoplasm where it is translated into an amino acid chain by a ribosome, and folded into a functioning version of the missing or faulty protein.

Gene therapies can be delivered either *in vivo* – where the viral vector is injected or administered intravenously into the body – or *in vitro*, where a patient's cells are removed, the viral vector is then inserted into these cells outside of the body, and the cells containing the vector are then reintroduced into the patient.

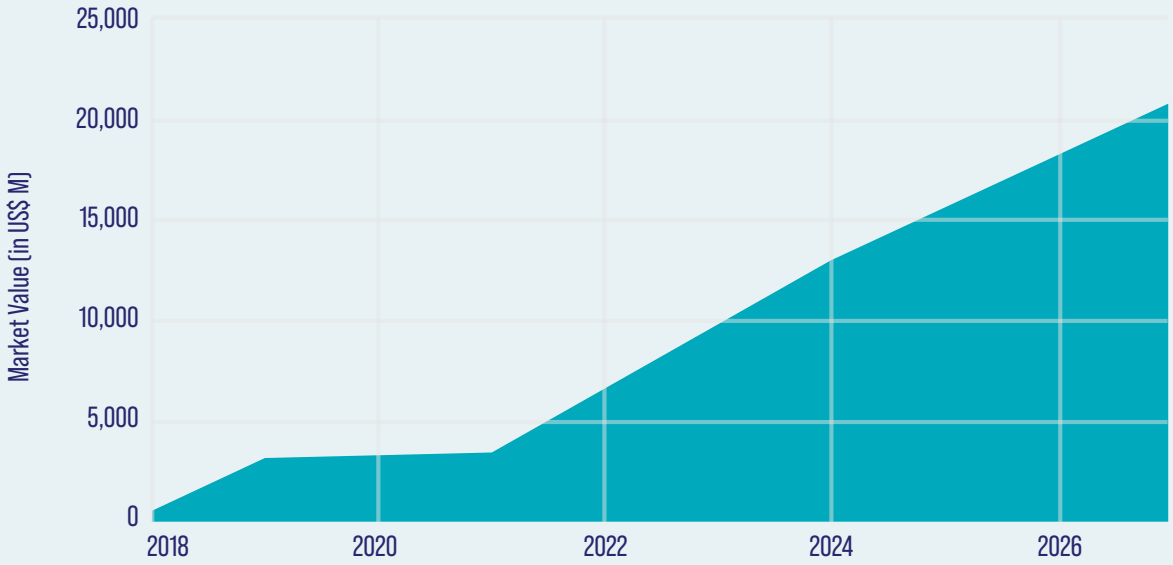
A Growing Market

DISCUSSING CURRENT MARKET TRENDS

In 2018, the global gene therapy market was on a trajectory to skyrocket if key therapeutics reached commercialization. Following the successful launch of Akcea Therapeutics' Tegsedi in 2018 and the approval of Novartis' Zolgensma in 2019 – among others – the global gene therapy market began to grow substantially. By the end of 2019, it had reached a market value of €2.74B ($3.22B).

But then the Covid-19 pandemic swept across the globe and the gene therapy market took a big hit. As investors and global governments began to shift their focus to Covid-19, money and support that could have gone to the gene therapy sector were invested in vaccine and treatment solutions for the global health crisis. The three main areas affected by the pandemic were the manufacture and delivery of gene therapies, research and clinical development, and commercial operations. Overall, a projection from November 2020 estimated that the gene therapy market would decline by 13.6% in 2020 compared to the previous year.

ESTIMATED GLOBAL GENE THERAPY MARKET VALUE



(Source: Data retrieved from Global Newswire, Grandview Research, and PR Newswire)

Despite this setback, the global gene therapy market is expected to recover and then grow steadily over the next few years. By 2027, it is estimated to reach a market value of €17.8B ($20.9B), and by 2028, it is forecast to rake in €8.5B ($10B) in revenues. Overall, by 2026, sales of gene therapy products are estimated to reach €10.8B ($13B) worldwide.

While the gene therapy market saw a slump in value in 2020 due to the coronavirus pandemic, investor interest continued to be strong throughout the year after initial losses in March. In fact, publicly traded gene therapy companies saw a 70% increase in stock performance compared to the previous year. A number of notable financings also occurred globally, including initial public offerings (IPOs), follow-on financings, venture capital financings, and private partnerships.

In Europe, the largest IPO in 2020 by a gene therapy company was that of UK firm Freeline Therapeutics, which closed a whopping €130M ($159M) Nasdaq IPO in August, closely following its €106M ($120M) Series C financing round from late June. Both financings will help bring the company's gene therapy for hemophilia B to a pivotal phase IIb/III trial.

The largest 2020 IPO in the US of a gene therapy company was Passage Bio's, which raised €181M ($216M) on the Nasdaq in June. Other notable IPOs by gene therapy companies in 2020 included Akouos, Generation Bio, Beam Therapeutics, and Taysha Gene Therapies. 2021 IPOs by gene therapy companies were kicked off by Boston-based Decibel Therapeutics – which develops gene therapies to combat hearing loss – with a €105M ($127M) IPO in February.

Overall in the regenerative medicine field, gene therapy companies fell behind cell therapy companies, which raised larger IPOs in 2020. For example, the GenScript subsidiary Legend Biotech raised a massive €408M ($487M) with its Nasdaq IPO in June to bring its autologous cell therapy against multiple myeloma to market.

The gene therapy field also saw a number of exciting follow-on financings in 2020. In Europe, Swiss company CRISPR Therapeutics, which takes a gene-editing approach to develop therapeutics, raked in €400M ($450M) through a public share offering on the Nasdaq global market. The company will use the money to further develop therapies using the gene-editing tool CRISPR-Cas9 for genetic disorders and cancer.

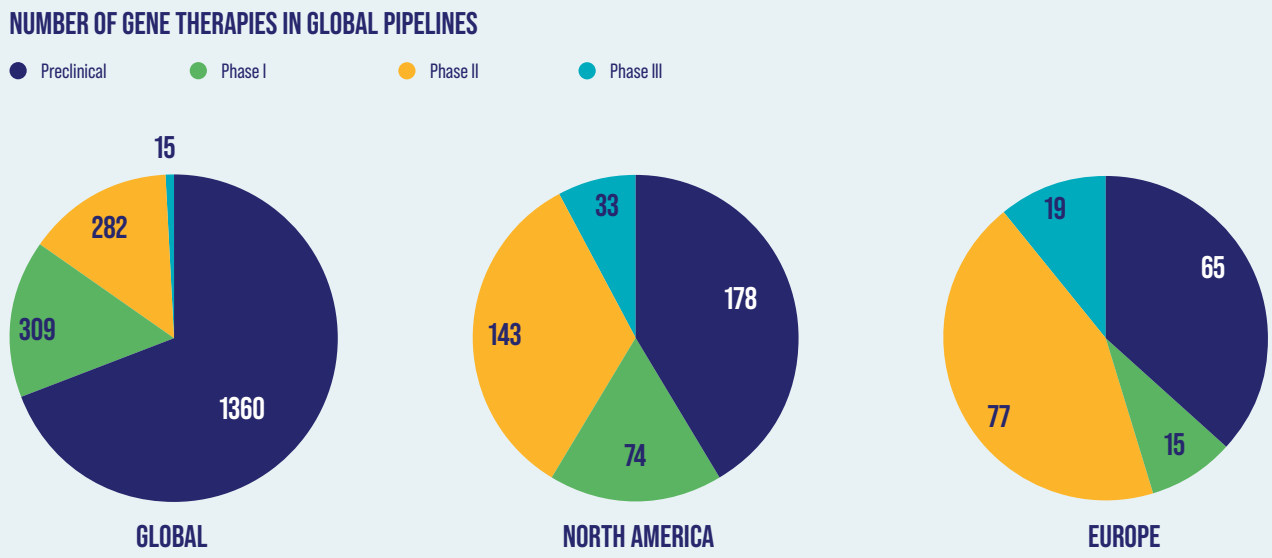
US gene therapy companies saw many more follow-on financings than European firms. Examples include Bluebird Bio's €482M ($575M) public offering in May 2020 and Adverum Biotechnologies' €182M ($217M) public offering of common stock in August.

In the follow-on financing area, cell therapy companies also did better than gene therapy companies in 2020. For instance, US cell therapy firm Allogene Therapeutics raised a whopping €530M ($632M) and UK Adaptimmune Therapeutics saw €217M ($259M) coming in from its public offering of its American Depositary Shares.

The gene therapy market has also benefited from a number of significant acquisitions in recent years, with particular focus from big pharma companies. German big pharma Bayer made headlines in 2020 with the acquisition of the US gene therapy specialist AskBio for a massive €3.5B ($4B). In 2018, Novartis acquired AveXis, which developed the gene therapy Zolgensma, and Roche acquired Spark Therapeutics, the company behind Luxturna, a gene therapy for inherited blindness, for €3.6B ($4.3B) in 2019. And in January 2020, Japanese pharma giant Astellas acquired US gene therapy biotech Audentes Therapeutics.

GENE THERAPIES IN THE GLOBAL PIPELINE

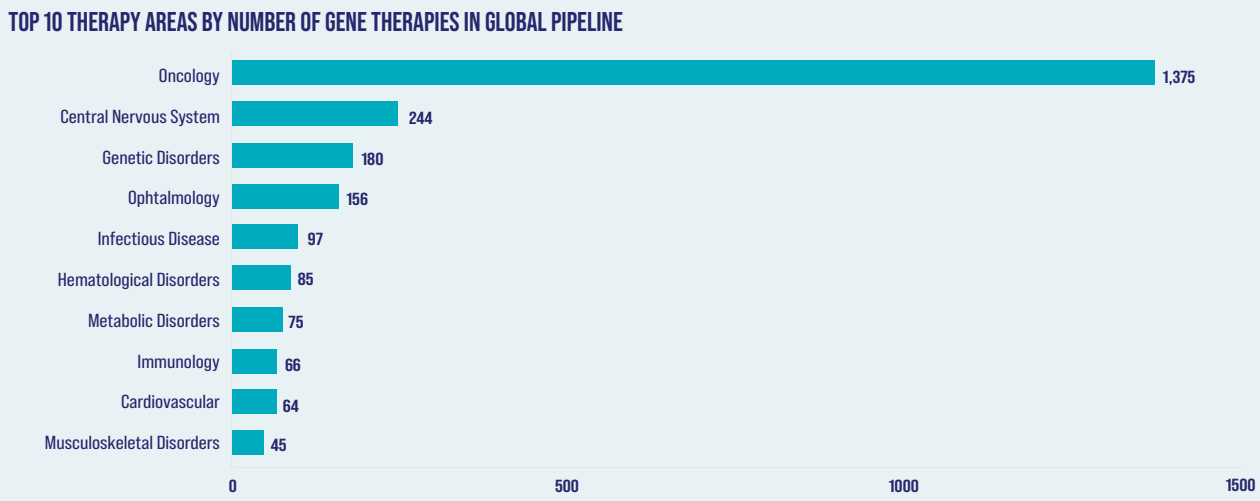
As of March 2021, there were over 2,400 gene therapy drugs in the global pipeline, according to GlobalData. Of these, over 1,300 were in preclinical development with just over 600 in phase I, II, or III trials, and the majority of them in phase I clinical development. A handful of the gene therapy drugs in phase III were already marketed and were being studied for use in other indications.



(Source: Adapted from GlobalData, data as of April 2021)

In Europe, there were 176 gene therapy drugs in the pipeline, most of which targeted genetic disorders. Most gene therapies in Europe's drug development pipeline are currently in phase II (77) and at preclinical stage (65). In North America, there were 414 gene therapy drugs in the pipeline with the main focus on oncology. Similar to Europe, most gene therapies in North America's pipeline are in phase II (143) and preclinical development (178).

Most of the gene therapy drugs in the global pipeline are targeting cancer, while other 'popular' therapy areas include central nervous system disorders, genetic disorders, and ophthalmic diseases.



(Source: Adapted from GlobalData, data as of April 2021)

Moreover, the Covid-19 pandemic has boosted the number of gene therapies in the global pipeline for infectious diseases. This is because gene therapy companies often use viral vectors to introduce genes into cells, and these vectors could also be used to carry genetic material from SARS-CoV-2, the virus causing Covid-19, to trigger an immune response. Hence, gene therapy companies are theoretically well-positioned to research, develop, and manufacture Covid-19 vaccines.

APPROVED GENE THERAPIES TO DATE

There are over 20 gene therapies on the global market today. Of these, the majority target either cancer or genetic disorders, such as Duchenne muscular dystrophy. Despite their relatively short time on the market, a handful of gene therapies have already been withdrawn. The main reason for these withdrawals is commercial failure, rather than safety or efficacy issues.

The most well-known is probably uniQure's Glybera, which was celebrated in the EU as a breakthrough moment for gene therapy but turned out to be a commercial failure. Priced at €1M ($1.2M), it was one of the most expensive treatments in the world and was withdrawn in 2017 after it had only been prescribed to a single patient in five years.

More recently, Orchard Therapeutics' Strimvelis made headlines after a patient, who had received the gene therapy for the rare genetic condition adenosine deaminase deficiency, was diagnosed with leukemia. It is thought that the leukemia is linked to the gene therapy, resulting in Strimvelis being taken off the market in November 2020.

In February 2021, Bluebird Bio had to halt late-stage clinical trials and stop marketing Zynteglo (LentiGlobin) in the EU after two participants of a trial testing Zynteglo were diagnosed with different blood cancers. The next setback came in early May 2021, when the company announced that it would abandon the sale of Zynteglo in Germany after pricing negotiations with the country's health authority failed.

The safety concerns raised for Strimvelis and Zynteglo represent the novelty of the gene therapy field. The cause of harmful mutations and the longevity of these therapies are still not fully understood and will need more research in the future.

MARKETED GENE THERAPIES WORLDWIDE BETWEEN 2003 AND 2020

DRUG NAME	VECTOR TYPE	COMPANY	INDICATION	APPROVAL DATE
Gendicine	Adenovirus	Shenzhen SiBiono	Head and neck cancer squamous cell carcinoma; nasopharyngeal cancer	2003, China
Oncorine	Adenovirus	Shanghai Sunway Biotech	Rhinopharyngeal cancer	2006, China
DeltaRex-G	Retroviral	Epeius Biotechnologies	Solid tumors	2007, Philippines
Neovasculgen	DNA plasmid	Human Stem Cells Institute	Peripheral arterial/ vascular disease	2011, Russia
Glybera	Adeno-associated virus	UniQure	Familial lipoprotein lipase deficiency	2012, EMA (withdrawn, 2017)
Imlygic	Herpes simplex virus 1	Amgen	Metastatic melanoma	2015, FDA & EMA
Strimvelis	Retroviral	Orchard Therapeutics	Adenosine deaminase deficiency	2016, EMA
Spinraza	No Vector	Biogen	Spinal muscular atrophy	2016, FDA 2017, EMA
Invossa	Retroviral	Kolon Life Science	Knee osteoarthritis	2017, Korea (withdrawn, 2019)
Zalmoxis	Retroviral	MolMed	Hematologic blood cancer	2017, EMA (withdrawn, 2019)
Luxturna	Adeno-associated virus	Spark Therapeutics	Retinal dystrophy	2017, FDA 2018, EMA
Zolgensma	Adeno-associated virus	AveXis	Spinal muscular atrophy type 1	2019, FDA 2020, EMA
Collategene	DNA Plasmid	AnGes	Critical limb ischemia	2019, Japan
Zynteglo/ LentiGlobin	Lentiviral	Bluebird Bio	Beta thalassemia	2020, FDA & EMA
Libmeldy	Lentiviral	Orchard Therapeutics	Metachromatic leukodystrophy	2020, FDA & EMA

(Source: Adapted from Nature Biotechnology & GlobalData)

MAJOR PLAYERS AND STARTUPS IN THE GENE THERAPY FIELD

In Europe, there are more than 300 biotech and pharma companies working in the gene therapy field, while there are over 600 in North America, according to GlobalData. A large number of small- and medium-sized biotechs are developing gene therapies and many big pharma companies are also working in the field.

Examples of European pharma giants working in gene therapy include the French company Sanofi, Swiss firms Roche and Lonza, UK company GlaxoSmithKline, German firm Merck, and Italian firm Chiesi. The Swiss pharma giant Novartis, for example, made headlines in Europe last year after the EMA approved Zolgensma, a gene therapy for the treatment of spinal muscular atrophy.

Other big pharma players outside of Europe include the US companies Bristol-Myers Squibb and CSL Behring, as well as the Japanese multinationals Astellas Pharma and Takeda. In April 2020, Takeda signed a collaboration agreement with the German biotech giant Evotec to develop gene therapies for the treatment of cancer, rare diseases, and neurological and gastroenterological disorders. And in June 2020, CSL Behring snatched up uniQure's phase III-stage hemophilia B gene therapy for up to €1.8B ($2.2B).

The table below provides a list of major gene therapy players in Europe and North America, sourced from the company watchlists by Grand View Research and Research and Markets. Companies are listed in alphabetical order.

MAJOR GENE THERAPY PLAYERS IN EUROPE AND NORTH AMERICA

NAME	HQ LOCATION	ANNUAL REVENUE IN 2019/2020	TYPE OF VEHICLE DELIVERY	NR. OF GENE THERAPIES IN PIPELINE	HIGHEST DEVELOPMENT STAGE OF GENE THERAPY
Abeona Therapeutics	Dallas, Texas, US	€8.3M ($10M)	AVV	7	Phase III
Adverum Biotechnologies	Redwood City, California, US	n/a	AAV	5	Phase II
Alnylam Pharmaceuticals	Cambridge, Massachusetts, US	€299M ($362M)	Lipid nanoparticles	4	Marketed
American Gene Technologies	Rockville, Maryland, US	€3.9M ($4.7M)	LV	1	IND-Enabling
Applied Genetic Technologies Corporation	Alachua, Florida, US	€2M ($2.5M)	AAV	8	Phase II
Biogen	Cambridge, Massachusetts, US	€11B ($13.4B)	AAV	n/a	Marketed
BioMarin Pharmaceuticals	Novato, California, US	€1.53B ($1.86B)	AAV	3	Phase III

Bluebird Bio	Cambridge, Massachusetts, US	€206M ($250M)	LV, CRISPR	5	Marketed
CRISPR Therapeutics	Zug, Switzerland	€239M ($291M) in 2019, €413K ($502K) in 2020	CRISPR	9	Phase III
Editas Medicine	Cambridge, Massachusetts, US	€423M ($512M)	CRISPR	9	Phase I
Eyevensys	Paris, France	n/a	DNA plasmids, electro transfection	4	Phase II
GenSight Biologics	Paris, France	€5.6M ($6.8M)	AAV	3	Pending marketing authorization
Gene Biotherapeutics	San Diego, California, US	n/a	AV	1	Phase III
Gyroscope Therapeutics	Herefordshire, UK	n/a	AAV	1	Phase II
Horama	Paris, France	n/a	AAV	3	Phase I/II
Intellia Therapeutics	Cambridge, Massachusetts, US	€9M ($10.9M)	CRISPR	9	Phase I
Orchard Therapeutics	London, UK	€2.1M ($2.6M)	LV, RV	12	Marketed
Oxford BioMedica	Oxford, UK	€100M (£87.7M)	LV	8	Phase II
REGENXBIO	Rockville, Maryland, US	€127.8M ($154.6M)	AAV	10	Phase III
Sangamo Therapeutics	Richmond, California, US	€97.7M ($118.2M)	AAV	3	Phase III
Sarepta Therapeutics	Cambridge, Massachusetts, US	€377M ($456M)	AAV	26	Marketed
Ultragenyx Pharmaceutical	Novato, California, US	€224M ($271M)	AAV	2	Phase II
UniQure	Amsterdam, Netherlands	€31M ($37.5M)	AAV	4	Marketed (withdrawn)
Vertex Pharmaceuticals	Boston, Massachusetts, US	€5.1B ($6.2B)	CRISPR	n/a	Phase I/II
Voyager Therapeutics	Cambridge, Massachusetts, US	€141M ($171M)	AAV	7	Phase II/III

(Sources: information collected from the company watchlists by Grand View Research and Research and Markets, and the company websites. Data as of May 2021. AAV: adeno-associated virus, LV: lentivirus, AV: adenovirus, CRISPR: clustered regularly interspaced short palindromic repeats)

A number of startups are also working in the gene therapy field. Founded in early 2020, Italian biotech Genespire, for example, received €16M ($19.5M) in funding from French VC firm Sofinnova Partners in April 2020. Genespire will use lentiviral vectors and gene-editing to develop gene therapies for people with inherited metabolic disorders and primary immunodeficiencies, respectively.

Epsilen Bio is another Italian gene therapy startup that received funding from Sofinnova Partners in 2020. Founded in late 2019, Epsilen Bio is developing gene therapies that can silence specific genes linked to disease.

German firm GeneQuine, on the other hand, is developing gene therapies for the treatment of osteoarthritis, a joint disorder characterized by inflammation and cartilage loss. The company's gene therapy can be injected directly into the inflamed joint where it triggers the production of a therapeutic protein by the joint cells over a long period of time. In January 2021, GeneQuine raised more than €9M ($11M) in a Series A funding round to boost the development of its gene therapy pipeline.

Innoskel is a French biotech that develops gene therapies for type 2 collagenopathies, a group of rare skeletal diseases that affect the structure of the body's collagen. The firm launched in early 2020 with a €20M ($24M) Series A financing co-led by Jeito Capital and Vida Ventures, with support from Turenne Group and Région Sud Investissement.

UK company Ori Biotech is addressing the manufacturing bottlenecks in cell and gene therapy manufacturing by developing an automated robotics system that takes over labor-intensive tasks, such as gene transduction and cell expansion. In October 2020, Ori Biotech raised €25M ($30M) in a Series A funding round to help get its platform to market.

Germany biotech ViGeneron is addressing a number of limitations that adeno-associated viral vectors usually have, such as the fact that they can only carry short DNA sequences or their inability to cross certain physical barriers in the body like the blood-brain barrier. The company is developing its preclinical gene therapy technology for the treatment of two blindness conditions. In January 2021, the company signed a collaboration agreement with Biogen, which will involve using its proprietary adeno-associated viral vector platform to develop gene therapy products to treat inherited eye diseases.

DEVELOPING GENE THERAPIES

Gene Therapy Delivery Vehicles

VIRAL VECTORS

The inability of viruses to self-replicate unless they infect a living cell is the fundamental reason they have become so valuable for gene therapy development. In essence, viral vectors are modified viruses that contain the viruses' gene delivery skills, while their pathogenic characteristics have been removed.

A number of viral vectors have been developed to introduce genetic material into target cells. In gene therapy, the cargo can either replace a mutated, disease-causing gene with a healthy gene, inactivate an improperly functioning gene, or introduce a new gene into a patient's body to help fight a disease. There are four main viruses used as biotherapeutic vectors today: retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses.

Each of these viral vectors comes with its own limitations and advantages. For instance, lentiviruses are commonly used for *ex vivo* therapies, like CAR-T cell therapies. Novartis' leukemia therapy Kymriah is one example where a lentivirus is used to deliver a cancer-seeking chimeric antigen receptor to T cells outside of the patient body, after which the modified T cells are introduced back into the patient.

A lentiviral vector is also used in Bluebird Bio's gene therapy Zynteglo. However, unlike other viral vectors, lentiviruses insert their DNA into the target cell genome, which can result in off-target integration of DNA into the host genome. This can trigger dangerous cancer-causing mutations. For example, in February 2021, marketing of Zynteglo in Europe was suspended after two patients in a clinical trial for the gene therapy were diagnosed with different blood cancers. This incident has raised concerns over the safety of lentiviral vectors for gene therapy delivery.

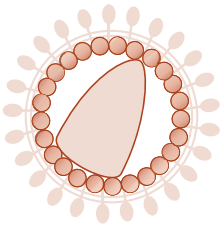
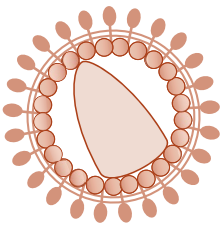
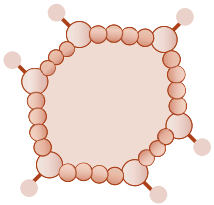
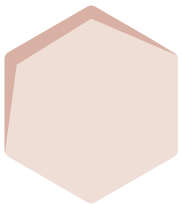
Red flags also went up after a patient treated with Orchard Therapeutics' gene therapy Strimvelis was diagnosed with leukemia. Strimvelis uses a retrovirus and concerns that the gene therapy's vector might have caused insertional mutagenesis are currently being investigated. Use of Strimvelis is on hold for now and no additional patients will be treated before the investigation is completed.

Most recently, adenoviral vectors have come under scrutiny as part of investigations into rare blood clots which may be related to the AstraZeneca/Oxford University and Johnson & Johnson Covid-19 vaccines. Both Covid-19 vaccines use different types of adenoviral vectors, so researchers are still unsure as to whether the vectors are really the cause of the clotting events.

Although all of the four main viral vectors – retroviral, lentiviral, adenoviral, and adeno-associated viral vectors – are in use for gene therapy research, adeno-associated viral vectors are currently seen as the most promising for gene therapy approaches. One example of a marketed gene therapy using an adeno-associated viral vector is Spark Therapeutics' Luxturna, which was approved in the EU in 2018. The *in vivo* gene therapy uses the vector to deliver a functional copy of a mutated gene causing vision loss into a patient's retinal cells, which can restore the patient's vision.

Examples of plasmid and viral vector developers are the German companies Cevec Pharmaceuticals and Sirion Biotech, the French company GEG-Tech, Belgian biotech Univercells, the Italian firm Anemocyte, and the US company Brammer Bio.

THE FOUR MAIN TYPES OF VIRAL VECTORS FOR GENE THERAPY

VIRUS	CHARACTERISTICS	ADVANTAGES	LIMITATIONS
Retrovirus (RV) 	(1) Genetic material carried in the form of RNA, which is retro-transcribed into linear double-stranded DNA upon entry into the target cell (2) Several viruses of the RV family are used for gene therapy, including oncoretroviruses, lentiviruses, and spumaviruses	(1) Simple preparation of the recombinant RV and easy subsequent insertion of the therapeutic gene (2) Stable and efficient integration into the genome of the target cell (3) Low immunogenicity in host organisms	(1) *In vivo* application limited, as the vector can only integrate during cell division (except lentiviruses) (2) Possibility of insertional mutagenesis or oncogene activation through random integration into the host genome (3) Can trigger a moderate immune response in patients
Lentivirus (LV) 	(1) Part of the RV family and includes well-known viruses, like HIV (2) Like other RVs, LVs carry their genetic material in the form of RNA, which requires the enzyme reverse transcriptase to be converted into DNA	(1) Unlike other viruses, LVs can enter non-dividing cells, making them attractive for *in vivo* gene therapy applications (2) Stable expression of the gene of interest due to direct integration into the host genome (3) Low immune response in patients	(1) Limited capacity for genetic cargo (2) High possibility of insertional mutagenesis through random insertion into the host genome
Adenovirus (AV) 	(1) AVs carry their genetic information in the form of double-stranded, linear DNA	(1) AVs can introduce many genome copies into the host cell, resulting in high readouts of the gene of interest (2) The AV's DNA cargo is not inserted into the host genome, but remains free within the host cell nucleus where it is transcribed, lowering the risk of unwanted integration (3) AV vectors can transduce dividing and non-dividing cells	(1) Because AVs do not insert their DNA cargo into the host cell genome directly, when the cell replicates, the virus' genetic information is not replicated, meaning further administration will be required if additional gene expression is needed (2) AVs are known to trigger severe immune reactions, limiting their *in vivo* use
Adeno-associated virus (AAV) 	(1) Originally, AAVs are human parvoviruses that need an adenovirus or another helper virus to initiate an infection (2) AAVs carry their genetic information in the form of single-stranded, linear DNA	1) AAV vectors can transduce both dividing and non-dividing cells (2) AAV vectors are known to have a good safety profile with low immunogenicity, making them ideal for *in vivo* gene therapy applications (3) AAVs have a long-term gene expression	(1) AAVs have a much smaller genetic cargo capacity than other viral vectors (2) Upscaling production of AAVs remains challenging

(Source: Adapted from Nature Gene Therapy, Nature Medicine, addgene)

NON-VIRAL GENE THERAPY DELIVERY

A number of drawbacks associated with viral vectors have led to the study and development of non-viral vector solutions for gene therapy delivery into target cells or tissues. These drawbacks include manufacturing bottlenecks, upscaling challenges, cancer-causing mutations, and immunogenicity of viral vectors.

Non-viral administration methods can address a number of these limitations, especially those associated with safety. Synthetic gene therapy delivery vehicles, for example, usually have lower immunogenicity than viral vectors because patients will not have pre-existing immunity, which is the case with some viral vectors. Many non-viral vectors are also easier to manufacture and can deliver much larger genetic cargoes than some viral vectors.

Despite these advantages, very few of these non-viral vector solutions are actually used in the clinic, as they come with their own limitations. The main challenge currently facing non-viral vector solutions is the effective delivery of genetic material into mammalian cells, due to a number of barriers, such as potential degradation of the vehicle before it can even reach the target cell.

Nevertheless, research is advancing. A diverse range of non-viral delivery methods have been developed so far. They can be classified into physical gene delivery systems and chemical gene delivery systems. Examples of physical gene therapy delivery vehicles include sonoporation, magnetofection, hydroporation, needles, microprojectile gene transfer, photoporation, and electroporation.

Electroporation is seen as one of the most promising physical delivery methods for gene therapies because it is known to increase transfection efficiency in a variety of tissues. During electroporation, a high-voltage electrical field is applied to the cell membrane, which results in the formation of pores that allow the plasmid carrying the genetic material to pass into the cell. French gene therapy developer Eyevensys is one example of a company using electrical currents to deliver gene therapy into the muscle of the eye.

Chemical gene therapy delivery systems have emerged as a promising alternative to viral vectors. They include liposomes, polymers, and engineered nanoparticles. Polymeric vectors have been studied since the 1960s and are seen as an attractive alternative to viral vectors because they have an immense chemical diversity and can promote gene transfection *in vitro* and *in vivo*. However, traditional polymer-based vectors face challenges regarding transfection activity and cytotoxicity. Hence, researchers are currently studying novel variations of polymeric vectors.

Lipid-based vectors are among the most commonly used non-viral gene therapy vehicles. As early as 1980, research revealed that liposomes could encircle and deliver DNA to primate kidney cells. A few years later, researchers showed that synthetic lipids could spontaneously form liposomes, which were able to efficiently deliver DNA in a number of different mammalian cell lines. But lipid-based vectors also come with their own limitations, including low efficacy due to the fact that they can be broken down before reaching the target cells, as well as triggering inflammatory and anti-inflammatory responses.

Nanoparticles are engineered to encapsulate DNA, mRNA, or siRNA. A number of different nanoparticle types have been developed as carriers of genetic material, such as lipid-based nanoparticles, polymer-based nanoparticles, and inorganic nanoparticles.

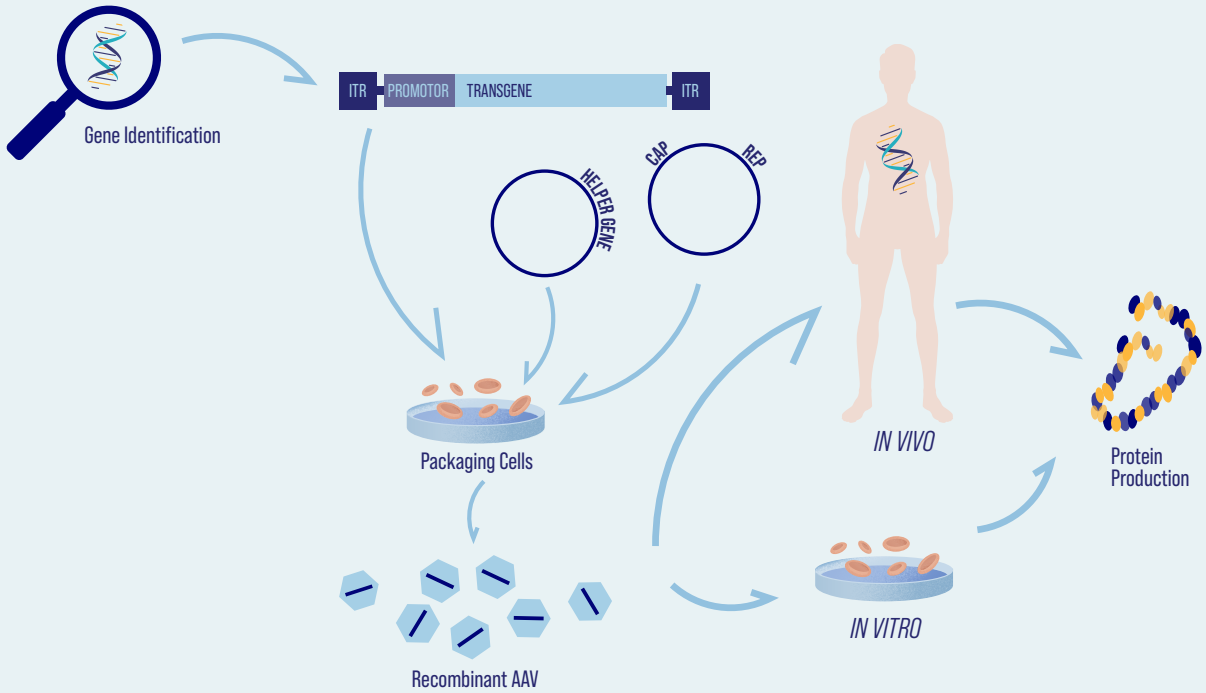
The Gene Therapy Development Process

A STEP BY STEP GUIDE TO GENE THERAPY DEVELOPMENT

The development of a gene therapy begins with the identification of the gene that needs to be replaced, inactivated, or introduced into the human body. Next, researchers will work on the development of a cassette – a component of vector DNA that holds the gene of interest and the regulatory sequences controlling the gene's expression. This cassette is then introduced into a specific cell line, which consists of cells containing all the viral proteins needed to produce the capsid – the part of the virus holding the DNA. These cells then act as viral vector factories, producing the viral vector carrying the gene of interest.

Here's a closer look at the production workflow of a gene therapy using an adeno-associated virus.

PRODUCTION WORKFLOW OF AN AAV GENE THERAPY



(Source: Adapted from PerkinElmer | Cisbio & abm)

First, the mutated gene has to be identified. If we take Spark Therapeutics' gene therapy Luxturna as an example, the mutation lies on a gene called RPE65, which codes for a retinal protein that is needed for the eye to be able to respond to light.

Once the gene has been identified, a cassette for the therapeutic gene has to be developed. In the case of Luxturna, this cassette carries a healthy copy of RPE65. The cassette also holds two so-called inverted terminal repeats, which are needed so the gene can later be packaged into the viral vector.

Next, the production of an adeno-associated viral vector requires a packaging plasmid containing the two genes REP and CAP, which are required for the viral genome replication and packaging, and its encapsulation, respectively. And lastly, a plasmid carrying the adenovirus helper gene is needed, since the adeno-associated virus requires a helper virus to replicate.

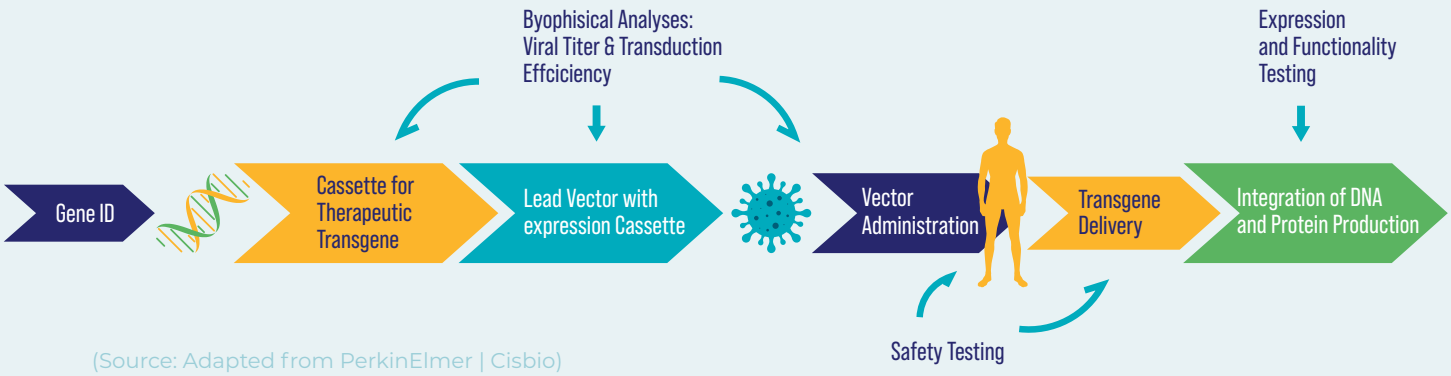
Together, the cassette carrying the therapeutic gene, the packaging plasmid, and the plasmid carrying the helper gene are all added to a packaging cell line, resulting in the production of the adeno-associated viral vector within the packaging cells. The packaging cells are then collected and subjected to three freeze-thaw cycles, after which the recombinant adeno-associated viral vector can be collected.

Once the adeno-associated viral vector carrying the gene of interest has been produced, it can be administered *in vivo* or *in vitro*. Luxturna, for example, is an *in vivo* gene therapy, which is directly injected beneath the retina of the eye. Once there, the gene of interest is integrated into the target cells, where the production of the missing protein takes place. In the case of Luxturna, the protein that is needed to absorb light is produced, improving vision in blind patients with a mutation in the RPE65 gene.

ENSURING THE SAFETY AND EFFICACY OF A GENE THERAPY

Each step of the gene therapy production workflow has to be carefully monitored to ensure a number of criteria are met. First and foremost, rigorous testing and monitoring needs to occur frequently to ensure that the benefits of the gene therapy outweigh the risks, as well as continuous safety and efficacy.

OVERVIEW OF VIRAL VECTOR DEVELOPMENT AND OPTIMIZATION FOR GENE THERAPY DELIVERY



(Source: Adapted from PerkinElmer | Cisbio)

The viral vectors need to undergo biophysical analyses from early stages, including monitoring of the viral titer and the integrity of the vector. The goal of these analyses is to check that the viral vector has been manufactured in consistent high quantities and purity without contamination. Moreover, once the viral vector is administered *in vitro* or *in vivo*, the transduction efficiency has to be checked thoroughly. This means ensuring that the integration of the gene of interest into the target cell has occurred and that enough genetic material has reached the target.

At this stage, safety testing is of utmost importance. To ensure the criteria for the correct safety profile are met, tests for purity, sterility, replication incompetence, and cytotoxicity have to be carried out. The main goal of these tests is to make sure that components of the gene therapy, such as the viral vector or possible contaminants, do not trigger dangerous immune responses.

Also, some viral vectors, like lentiviral vectors, have the ability to integrate into other parts of the target genome other than the target site, which can cause mutations elsewhere in the genome or activate cancer genes. For these viral vectors, it is important to check that no off-target insertions have occurred. Safety testing has to be continued for several years after the administration of the gene therapy, as shown by the current safety concerns regarding cancer diagnoses in patients who have received the gene therapies Strimvelis and Zyntelgo.

Lastly, upon administration of the gene therapy, the expression and functionality of the inserted gene has to be tested. This step is necessary to confirm that therapeutic levels of the protein have been produced and can treat or reverse the genetic disorder.

ADDRESSING GENE THERAPY MANUFACTURING CHALLENGES

As discussed above, the early stages of gene therapy development involve continuous biophysical analyses and safety testing of the therapy's viral and genetic components to ensure the safety and efficacy of the therapy when used in humans. However, challenges also pop up throughout different stages of the gene therapy manufacturing process.

A major challenge faced by manufacturers are the compressed timelines of gene therapy development. While the average development of a conventional biologic takes between eight and ten years, gene therapy development takes between three to five years. These compressed timelines also result in a number of other problems, including an increasing demand for plasmids – the key building blocks for viral vector development – and growing bottlenecks in plasmid production; one of the reasons researchers are studying non-viral delivery methods for gene therapies.

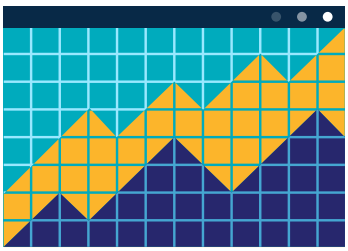
The increasing challenge of producing viral vectors at a large scale has forced many companies to think outside the box. One example is the German company Cevec Pharmaceuticals, which has developed a production platform for the large-scale production of adeno-associated viral vectors. Traditionally, adeno-associated viral vectors were produced using adherently growing cells, which meant that the packaging cells needed a substrate to grow and survive in a dish. But growing adherent cells at a large scale is extremely challenging.

Cevec's solution was to develop a stable producer cell line that is able to grow in suspension, allowing the production of viral vectors in huge bioreactors instead. Moreover, the company's cell line does not require plasmids and transfection reagents, and actually skips the transfection step altogether. This circumvents the challenge of finding plasmids in an increasingly competitive environment. Stable producer cell lines are now seen as a breakthrough in viral vector manufacturing for large-scale gene therapy production.

Another challenge related to process development is the fact that each disease and each target tissue requires a different dosage. This complicates the production of standardized gene therapy development protocols. At the same time, the growing popularity of gene therapy means the demand is outstripping supply of expertise and manufacturing capacity. Here, gene therapy developers are advocating to move manufacturing processes towards more automation and software solutions, and to hand the entire process to contract manufacturing organizations. However, this would require further education in this respect.

Moreover, a lack of optimized processes and fast testing is seen as one of the main drivers for the high costs of gene therapies. To address this problem, researchers are working on the development of new analytical methods like robust, fast, easy-to-use, and reproducible assays. These should be developed only for gene therapy assessment rather than being borrowed from traditional antibody development processes, as is currently the case.

The fact that gene therapy is a relatively new field has resulted in a lack of historical data, which could otherwise be referred to during regulatory processes. There is also a lack of standardized regulatory guidelines that is currently increasing the risk of failure for novel gene therapies. However, regulatory guidelines regarding gene therapy development and manufacturing are evolving continuously, and gene therapy developers have to make sure they stay up to date with the changes in order to align with the regulatory authorities and prevent setbacks in later development stages.



THE EVOLVING GENE THERAPY LANDSCAPE

Fixing Current Issues

The fact that gene therapies are designed as long term treatments, means that the effects of clinically tested and marketed therapies in humans have to be observed carefully over years. The recent cancer diagnoses in patients who had received Strimvelis or Zynteglo, respectively, show how crucial long-term observation is.

Related to this is the challenge that some gene therapies seem to lose effectiveness over time, although they are designed as one-time treatments. One example is Pfizer and Sangamo's hemophilia A gene therapy candidate, giroctocogene fitelparvovec, which is designed to replace the missing clotting factor VIII in patients with the bleeding disorder. However, data presented at the ASH conference in December 2020 showed that factor VIII levels seemed to drop over time in patients who had received the gene therapy, raising concerns over its lack of durability.

Similar concerns hit Biomarin's rival hemophilia A drug candidate valoctocogene roxaparvovec, which was rejected by the FDA in August 2020 due to durability worries. In January 2021, Biomarin published phase III results showing its gene therapy had reduced bleeding rates in patients with hemophilia A. However, factor VIII levels dropped in patients who were followed for two years, which means the FDA's durability concerns remain.

The key problem is that these gene therapies use a viral vector, which can work once but may have problems entering the body a second time. This is because the body's immune system will have built up defenses against it, which take the form of neutralizing antibodies. Moreover, many people already have neutralizing antibodies from previous viral infections that prevent the viral vectors from carrying the gene of interest to the target cells in the first place. For example, up to 70% of people already carry neutralizing antibodies against adeno-associated viruses.

To solve these issues, researchers are building an in-depth understanding of the immune responses that lead to the destruction of viral vectors. Based on this knowledge, vector designs can be improved and specific immune modulators can be co-introduced into the body. For example, AAV gene therapies are often administered together with steroids to counterbalance inflammatory and antiviral T cell responses. Knowledge can also be taken from CAR-T cell therapy, where the immunotoxic side effect known as cytokine release syndrome can be prevented through the inclusion of monoclonal antibodies that block interleukin signaling.

The Swedish company Hansa Biopharma is taking another approach to treat virus-resistant patients with gene therapy. It is developing a pretreatment called imlifidase, an enzyme that can eliminate the neutralizing antibodies prior to the administration of gene therapy treatments. Imlifidase is already approved to treat highly sensitized organ transplant patients. Hansa Biopharma recently signed a deal worth €350M ($427M) with Sarepta Therapeutics to further develop imlifidase, which Sarepta will then use in AAV-resistant patients before administration of its gene therapies for Duchenne muscular dystrophy and Limb-girdle muscular dystrophy.

The current complexity of gene therapy manufacturing and the small number of therapies on the market are causing prices for marketed gene therapies to skyrocket. These high prices are seen as controversial because many worry that they make treatments inaccessible for many patients, especially for people in poor countries.

Spark Therapeutics' Luxturna, for example, has received considerable criticism after being priced at €349,000 ($425,000) per eye in the US, resulting in costs of €700,000 ($850,000) for the complete one-off treatment. In Germany, Luxturna has been priced at €345,000 ($421,000) per eye. Other expensive gene therapies include Novartis' Zolgensma – which is the most expensive drug in the world and is priced at €1.9M ($2.3M) per patient in the US – and Bluebird Bio's Zynteglo, which was recently withdrawn from the German market after pricing negotiations with the health authority failed. Zynteglo would have cost €1.6M ($1.9M) per patient in Germany.

So while governments, healthcare providers, insurers, and patients are looking for solutions to make gene therapies more accessible, gene therapy developers are trying to optimize workflows to make the production process cheaper. The Belgian biotech Univercells, for example, has developed fully automated manufacturing technologies, which require fewer steps and materials to produce viral vectors. The company's manufacturing facilities are also designed to reduce the space and costs required to produce viral vectors. Other companies working to cut the costs of gene therapy manufacturing are Lonza and Merck.

Ultimately, the goal for gene therapy developers will be to develop a platform technology that will enable the scalable, measurable, and reproducible production of viral vectors at lower costs, similar to what has been developed for the production of monoclonal antibodies. This, in turn, will allow for the development of more affordable gene therapies, accessible to more patients worldwide.

Developments to look out for in the future

Due to technical limitations, gene therapies are currently applied to diseases caused by only one genetic mutation, and to more accessible areas of the body, like the liver or eyes. But what if gene therapy could tackle more complex diseases and target organs that are currently out of reach, such as the brain?

This is the goal of a new generation of gene therapy, which aims to use a number of different technologies to introduce genetic material into a patient that could then be switched on or off, or dialled down or up in intensity.

For example, a research group at University College London led by Dimitry Kullmann is studying different ways in which gene therapy could help patients with epilepsy. Currently, epilepsy treatments affect all parts of the body, not only the epileptic zones of the brain. Kullmann and his team are developing a gene therapy that can be injected directly into the seizure-causing brain area. But it is only effective in the excitatory nerve cells that cause seizures without affecting healthy nerve cells. The group is now getting ready to start research in humans.

A similar approach is being used by the Swedish biotech company CombiGene, which is developing an adeno-associated virus-based gene therapy that delivers a specific neurotransmitter and its receptor into overexcitable brain cells, where they can reduce the number of epileptic seizures. In 2021, CombiGene is finalizing its preclinical studies and aims to start first in-human studies in 2022.

Another approach Kullmann and his colleagues are researching is known as chemogenetics. Using gene therapy, they can introduce a specific receptor into brain cells, which will respond to a drug that can reduce the activity of excitatory neurons and suppress seizures as a result. Ultimately, this approach means that the therapeutic effect can be switched on or off, depending on whether the drug is administered or not.

Although this research is at an early stage, it could enable the treatment of a large range of conditions affecting the central nervous system, including multiple forms of epilepsy, as well as Parkinson's, amyotrophic lateral sclerosis, or pain.

Other novel gene therapy approaches involve optogenetics, in which a protein that reacts to light can be introduced into cells to reduce vision loss, or thermogenetics, where cells are engineered to make proteins that react to the heat of infrared light.

In the early 2000s, researchers began studying different genome editing techniques to make gene therapies safer and more precise. In recent years, the CRISPR gene-editing technology has made headlines around the world. One of the main advantages of genome editing is the fact that it can target polygenic diseases – diseases caused by more than one gene defect. Examples include cancer, blood disorders, blindness, AIDS, cystic fibrosis, and muscular dystrophy. Companies such as CRISPR Therapeutics, Vertex Pharmaceuticals, and Editas Medicine are currently testing their CRISPR-based therapies in clinical trials.

As its popularity grew, the gene therapy field saw a shift in stakeholders. While early stages of gene therapy research mainly involved universities and small biotechs, today, large pharma companies, contract development and manufacturing organizations (CDMOs), and patient organizations have joined the quest to develop suitable gene therapies.

The involvement of CDMOs in drug development in general has grown rapidly in recent years. In gene therapy development, CDMOs are often responsible for the production of essential viral vectors and plasmids. One example is the UK CDMO Touchlight DNA Services, which has developed a form of synthetic DNA designed to simplify gene therapy and DNA vaccine manufacturing.

Another example is AskBio, which was acquired by Bayer in 2020. The biotech opened a CDMO branch, which focuses on gene therapy manufacturing for ultra rare diseases and the production of therapies for poorer countries. It invested in storage technologies that can extend the shelf life of fragile therapies and uphold cold chains while they are delivered to difficult-to-reach patients.

Increasingly, patient organizations are also becoming more involved in gene therapy development. In a recent panel discussion at Bio-Europe Spring 2021, leaders in the gene therapy field highlighted how the role of patients in gene therapy development is changing: Today, patients discuss the challenges related to a disease openly with biotechs and guide companies to explore possible solutions. This is especially important because patients who have received a gene therapy treatment need to be monitored for years to ensure the therapy is still working and there have been no adverse events. Here, regulators, biotechs, and patients have to come together and build a relationship based on trust, which will allow the monitoring of patients over time.

Patients are also a strong voice in terms of clinical trial design. In fact, increasingly, patient organizations actually finance early- and late-stage clinical trials, as well as manufacturing facilities. And, in order to get as many patients involved in a trial as possible, patient organizations also lead worldwide genotyping programs to help patient enrollment, which carries importance in the rare disease field where very few patients are actually diagnosed with the disease.

One of the main issues pointed out by the panelists was the lack of data sharing in gene therapy development. When the field was in its infancy, collaborations and sharing of data were frequent, but as the field progressed and became commercialized, data was siloed and accessing information grew more difficult. In the future, data sharing would be something to work on, the panelists agreed.

If, for example, patients in a trial experienced adverse events, the company conducting the trial should share this information openly to prevent others from making the same ‘mistakes’. Moreover, as the technology itself is still evolving and technical and scientific questions remain unanswered, data sharing within the industry could help advance the field faster and make gene therapies as safe and effective as possible.

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