



ADVANCEMENTS IN GENE THERAPY

USAGE OF AAV VECTORS

Whitepaper by Innoplexus AG

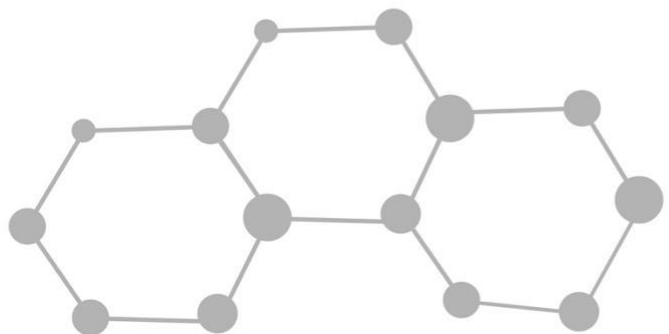


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1. Context

Gene therapy, like a magic bullet, holds the promise to “cure” a wide range of diseases which have been incurable till date, e.g. genetic blindness, muscular dystrophy, blood cancer and many other diseases. Gene therapy can fundamentally address the root cause(s) for these diseases, which are different kinds of errors in their genes¹. By replacing a faulty gene in a patient's DNA or repairing a defective gene by gene editing, gene therapy can provide a one-time treatment option with no requirement for prolonged treatment of symptoms. In this series of white papers, we attempt to address the potential of various types of gene therapies along with the challenges plaguing its development. In the first report, we evaluate the usage of viral vectors, especially adeno-associated virus (AAV) vectors in clinical development.

The recent focus on gene therapy has been augmented by multiple factors:

1. Several recent biotechnological advances including chimeric antigen receptor T-cell immunotherapy (first approved by FDA in 2017), genomic editing by CRISPR/Cas9 (evidence of first-in-human results in ATTR amyloidosis patients by Intellia Therapeutics and Regeneron Pharmaceuticals), viral vector platforms and advanced imaging techniques for target specific vector delivery²
2. FDA approval of mRNA-based vaccines against SARS-COV-2 virus (2020) has helped overcome barriers for such therapy, especially in oncology (also because of promising therapeutic outcomes of mRNA cancer vaccines achieved in several clinical trials against multiple aggressive solid tumors), and highlights a novel mechanism of action
3. Rapid upsurge in R&D - since 2016, ~1350 trials have started in gene therapy compared to ~535 trials in previous years (2010-15) (Figure 9)
4. An increase in approvals has resulted in increased commercial interest in gene therapy. Only 7 gene therapies had been approved till 2015, whereas 18 gene and CAR-T cell therapies have reached the market in the last five years
Additional support also provided by approval of drugs using oligonucleotide-based therapies (7 therapies in the last 5 years), cell based and *in vivo* gene therapies (7 therapies since 2017)³
5. With gradual advances in gene therapeutics, investors’ interest also increased significantly in companies that are dealing in gene therapeutics and is evident from the followings:
 - a. Rising M&A deals
 - b. Traction in fund raising via IPO route

Rising M&A deals: Gene therapy focused companies were in the sweet spot for M&A recently. There were over 100 deals during the last 5 years amounting to ~\$130 billion (Figure 1). Few notable deals being Celgene acquiring Juno Therapeutics for \$9 billion in 2018, Bristol-Myers Squibb (BMS)

acquiring Celgene for \$74 billion in 2019, Novartis acquiring AveXis for \$8.7 billion in 2018 and Bayer acquiring Asklepios BioPharmaceutical for \$4 billion in 2020.



Figure 1: M&A activity (count & deal value) across years in gene therapy

Traction in fund raising via IPO route: Number of companies that raised funds via IPO route has increased significantly since 2018. Major traction is visible in 2021, until the first quarter of 2021, the amount raised is ~80% of the total fund raised during the entire 2020. Moreover, there was a significant jump in funds raised in 2020 over 2019.

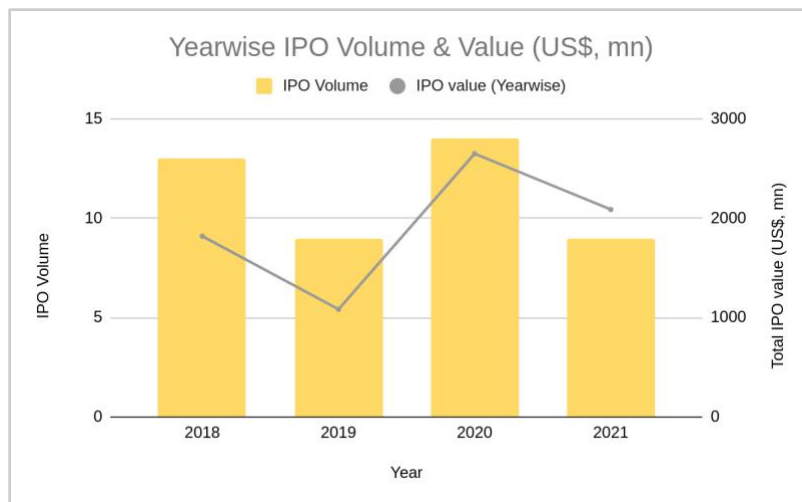


Figure 2: Traction in fund raising via IPO route

2. Introduction and evolution of gene therapy

After years of clinical development and addressing hurdles of safety and efficacy issues, gene therapy has finally arrived. With a slew of approvals and advancements in techniques, this therapy is finally living up to its potential. Though a lot still remains to be improved and evolved, it is interesting to follow the developmental journey of this once elusive technique.

2.1 - What is gene therapy?

Gene therapy technologies utilize therapeutic delivery of gene/nucleic acids/genetically modified cells as drugs to treat or prevent disease. This technology targets genes that are defective from birth or are mutated during one's lifetime and lead to faulty protein expression and thereby cause disease⁴. Gene therapies can work by several mechanisms⁵:

- a. **Gene addition:** introduce a new or modified gene into the body to treat a disease e.g. Roctavian, SPK-8011 etc. for the treatment of hemophilia in which a modified virus is used to introduce a copy of the gene that encodes for the clotting factor missing in patients
- b. **Gene replacement:** replace a mutated disease causing gene by a healthy copy e.g. Luxturna works by delivering a normal copy of the RPE65 gene directly to retinal cells of patients where they were originally mutated
- c. **Gene inhibition:** inactivate the function of a disease causing gene e.g. Oxlumo (lumasiran) is a HAO1-directed small interfering ribonucleic acid used to treat primary hyperoxaluria type 1 by lowering increased urinary oxalate levels in pediatric and adult patients.

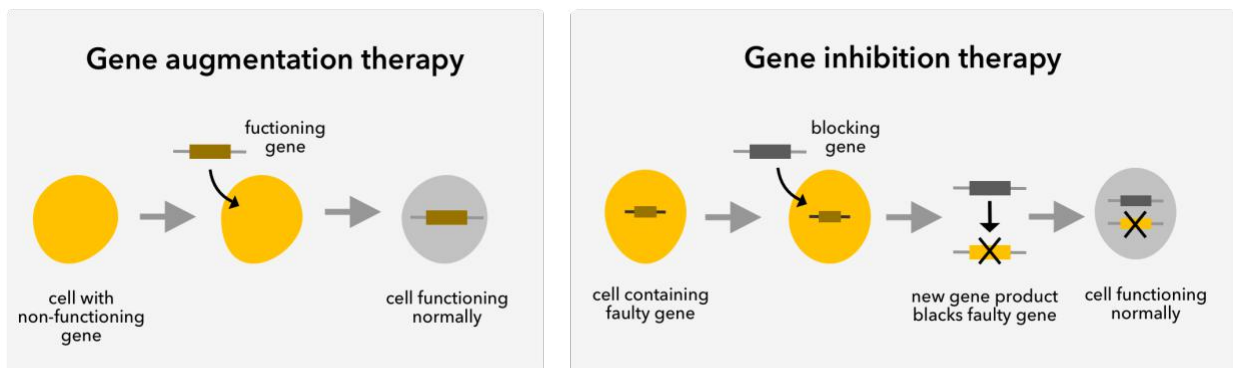


Figure 3: Gene therapy mechanisms

In its early days, the main focus of gene therapy was on special genetic disorders, but now diverse diseases, such as cancer, cystic fibrosis, heart disease, diabetes, hemophilia and AIDS, with different

patterns of inheritance and acquired diseases are targeted. Broadly there are two major categories of gene therapy⁶:

- a. Somatic cell gene therapy where therapeutic genes are introduced into any cell other than a gamete, germ cell or undifferentiated stem cell.
- b. Germ line gene therapy where genetic modifications are introduced into germ cells (sperm/egg cells) and can be inherited. Due to ethical reasons, this type is prohibited/closely regulated in multiple countries.

To administer gene therapy, certain vectors (vehicles for gene transfer) may be used, e.g. genetically modified viruses, bacteria etc^{3,7}. In some cases, instead of full replacement of a defective gene, gene editing techniques like CRISPR can correct a specific genetic defect.

Gene therapy can modify cells either inside (*in vivo*) or outside (*ex vivo*) the body⁵.

- a. For *in vivo* therapies, vectors containing the modified gene are directly injected into the target organ e.g. by using viral vectors as in Zolgensma.
- b. In *ex vivo* therapies, specific cell types from blood or tissue from a patient are extracted and the target gene is introduced in a laboratory e.g. CAR-T cell therapies. These cells are grown, often expanded, and then injected back into the patient where they eventually show the desired effect.

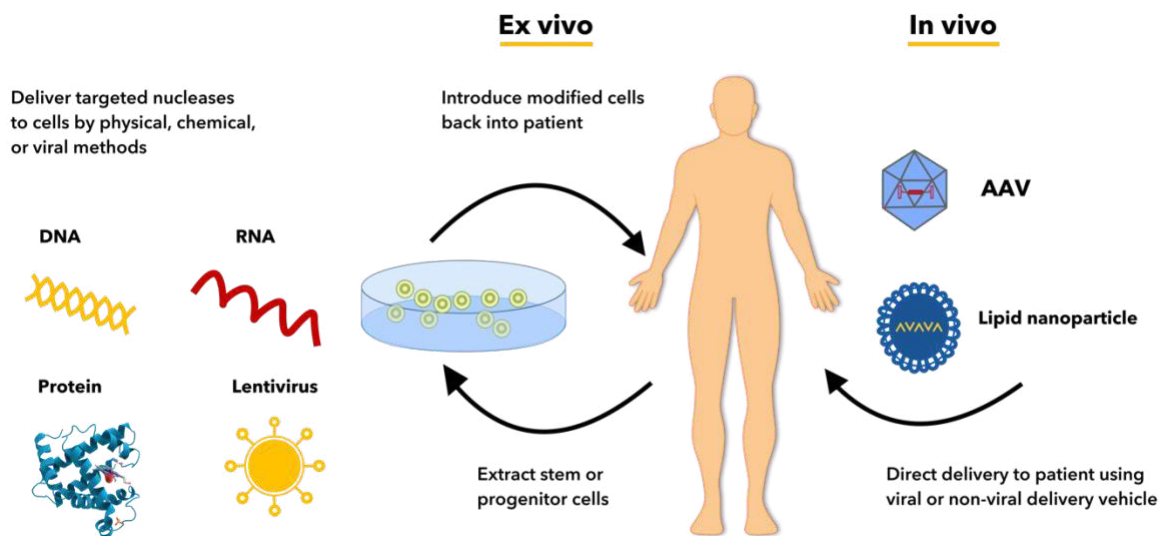


Figure 4: Ex vivo and in vivo gene therapy techniques

2.2 - Important milestones

As more and more underlying genetic causes for diseases are being identified, a lot of traction has been seen in gene therapy in the last decade. Even though antisense oligonucleotides have been approved since 1998, the first viral vector-based therapy was approved in 2003 in China⁸. Since 2017, when FDA approved the first gene therapy for an inherited disease, there has been a paradigm shift in approvals of such therapies with over a dozen therapies being approved till date. Developments in other technologies like CAR-T cell therapies have also been favoured with regulatory approvals. A few important milestones in this journey have been highlighted below:

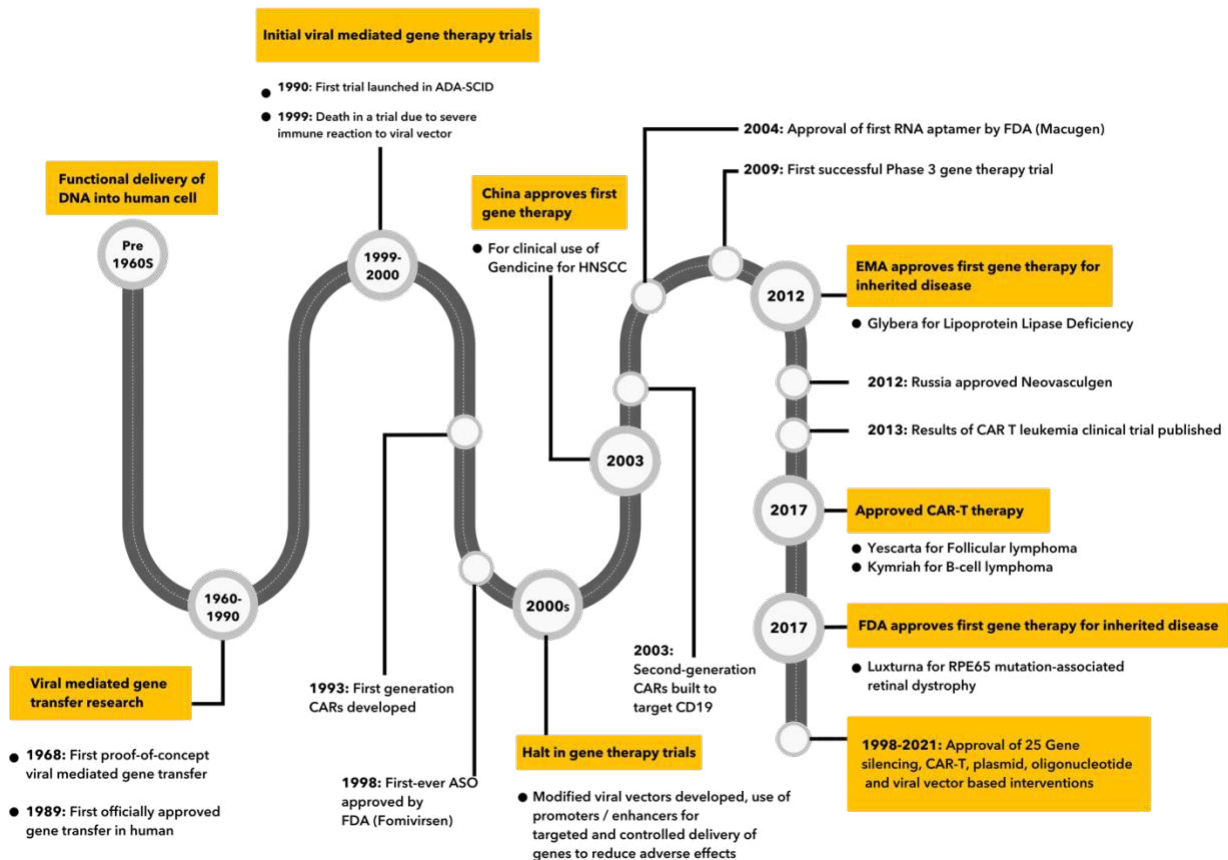
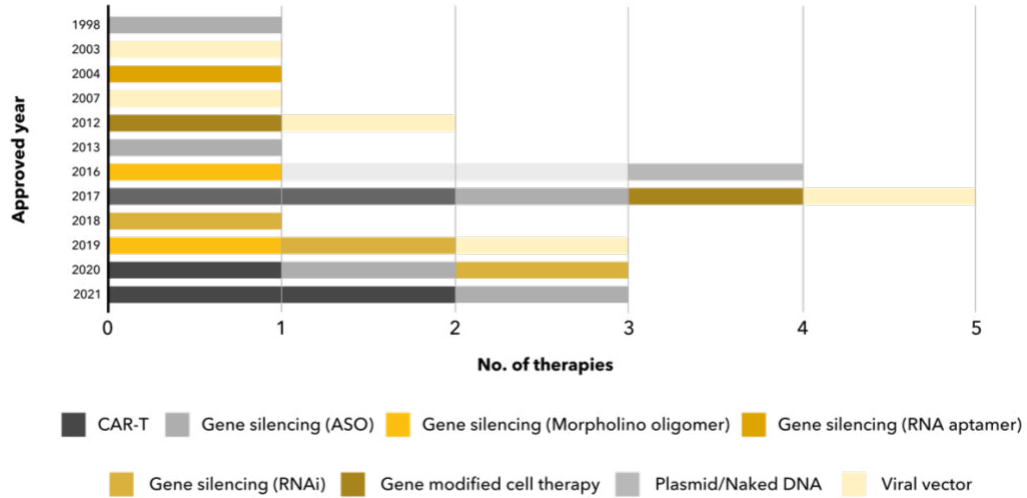


Figure 5: Developmental milestones of Gene and CAR-T cell therapies

2.3 - Gene therapy approvals

Gene silencing based therapies, which use antisense oligonucleotides or other RNA interference mechanisms that hinder production of specific proteins, have been the earliest approved gene therapies and also the most numerous. On the other hand, viral vectors used for inserting genetic materials have also seen major improvements in their design and have been approved for few genetically inherited diseases. The field of cancer immunotherapy saw a major boost when CAR-T

cell therapies were approved for difficult to treat advanced B-cell lymphomas. With the advent of ‘second-generation’ therapies in early stage clinical studies (including allogenic and off-the shelf therapies), several exciting therapies are in the pipeline.



Approved gene therapies by categories

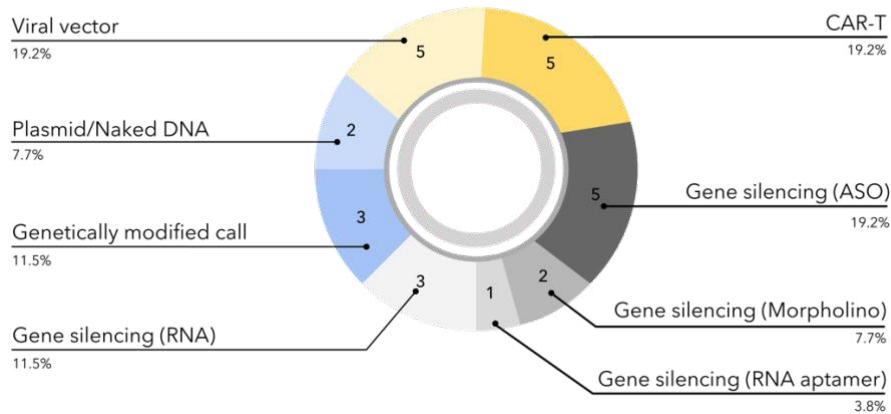


Figure 6: Approved gene therapies by categories over the years

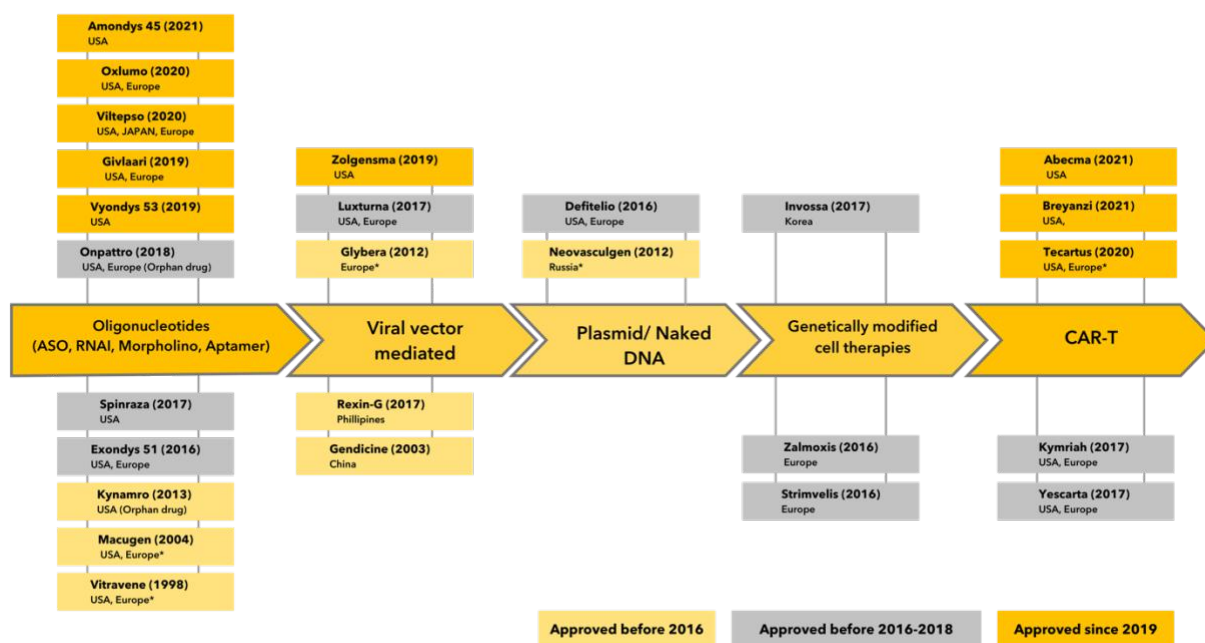


Figure 7: Gene therapies approved globally

2.4 - Gene therapy challenges

Clinical trial activities and publication on early PoC on genetic drugs witnessed high momentum in the mid 90's amid high expectations. But the researchers realised that focus is needed on basic science of gene transfer and technologies to better address the issues of safety along with efficacy. Though there are the following challenges^{2,9,10}, pharma industry has kept the research momentum ongoing:

- Vector-induced immune response (immunogenicity): In case of gene therapy being directly administered via virus vectors, any pre-existing immune responses to the wild-type virus from which the vector is engineered, or the transgene product itself, can impact the efficacy if not managed in advance.
- Reaching scale is difficult: As there are no stable cell lines with viral vectors, homogeneous production for the entire batch needs to be analyzed for characteristics and quality.
- Highly complex nature of both process and product: Irrespective of the gene delivery vehicle (meant to carry either the transgene, RNA or gene editing elements), the process and generated product are both highly complex.
- Development speed creates impediment: Robust, real time, easy-to-use and reproducible assays is crucial for further advancement of gene therapies. Development of certain autologous therapies (e.g. CAR-T cell therapy) have complex manufacturing and

transportation processes thereby making adherence to CGMP (current good manufacturing practice) standards difficult

- No clear established regulatory guidance: Regulatory authorities come up with guidelines as and when required for the evolving gene therapy field.
- Upfront cost along with long term durability and adverse events raise concern: Gene therapies address the root causes of disease with a single curative dose and can replace expensive maintenance treatment. This may lead to cost savings in the long run but the upfront costs are very high. Among payers and regulators, uncertainty surrounding long-term durability and adverse events leads to some concern. For example, Yescarta, a gene therapy indicated for adults with relapsed or refractory large B-cell non-Hodgkin's lymphoma and marketed by Kite/Gilead, is priced at \$373,000.

3. Landscape of gene therapy

To introduce genetic material into a patient's body, they need to be packaged within a vector. These vectors can act as delivery vehicles for the gene, which once entered into the cell, can then produce the relevant protein. To test the efficacy of the therapy in human patients, different phases of clinical trials are conducted. Commonly used vectors and the clinical landscape of various types of gene therapies are elaborated below.

3.1 - Gene therapy delivery systems using viral and non-viral vectors

The viral gene delivery systems are based on DNA, RNA and oncolytic viral vectors while non-viral gene delivery systems use other biological, physical and chemical methods.

DNA-based viral vectors typically have a larger transfer capacity, and some of the vectors have the ability to integrate their genetic information into the target cell genomes. DNA vectors include technologies based on poxviruses, adenoviruses, adeno-associated viruses, and herpes viruses¹¹. Though transient, RNA-based viral vectors have the ability to transcribe directly for infectious RNA transcripts. Oncolytic viruses are an emerging treatment option for cancer e.g. oncolytic adenovirus co-expressing IL-12 and IL-18 improves tumor-specific immunity.

Non-viral vectors have the advantage of less likely immune response than viral vectors. They use a system of biocompatible materials such as lipids, naked DNA, chromosomes, plasmid, cationic polymers, and conjugate complexes¹². In addition to better safety of administration, they may also allow almost unlimited transgene size and the possibility of repeated administration.

In some cases, bacteria may also be used as a vector - bacteria-mediated RNA interference (engineered bacteria to produce and deliver siRNA), bacterial protein delivery (genetically modified bacteria used to deliver bacterially expressed therapeutic proteins) and also DNA vaccination (bactofection of plasmids encoding a tumor-expressed antigens to induce immune response)¹³.

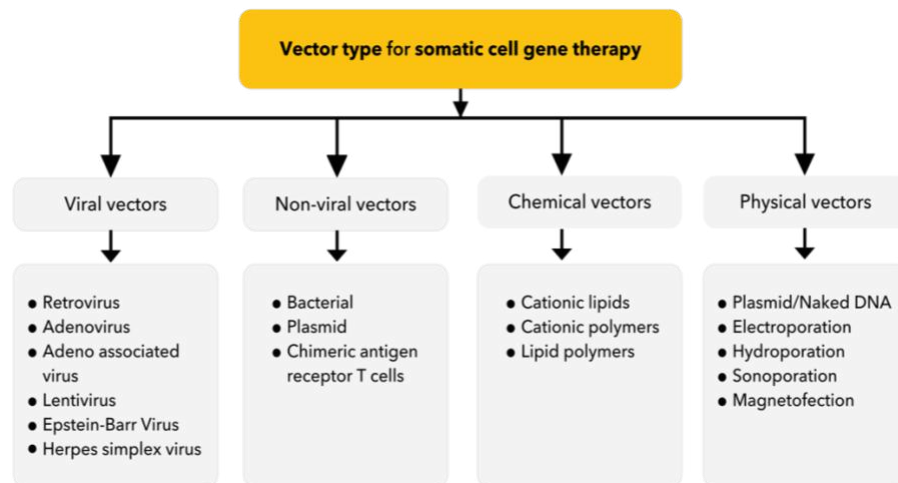


Figure 8: Gene therapy delivery types

As new vector types are being developed, focus is on reducing toxicity and immunogenicity related events thereby making gene therapies potentially safe and effective, e.g. rAAV2/HBoV1 chimeras that specifically transduce polarized human airway epithelia¹⁴, Anc80L65 using synthetic AAV capsids¹⁵.

3.2 - Clinical trials over the years

In this chapter, we explore 2330 trials of gene and adoptive cell therapies including CAR-Ts (from clinicaltrials.gov registry, considering interventional trials with a gene therapy as an experimental drug and excluding Phase 4 trials).

We analyze trends by trial start year, phase and primary completion year. We also look into trial proportion by status and indication for various gene therapy types.

3.2.1 - Year and phase-wise Gene & adoptive cell therapies trials distribution

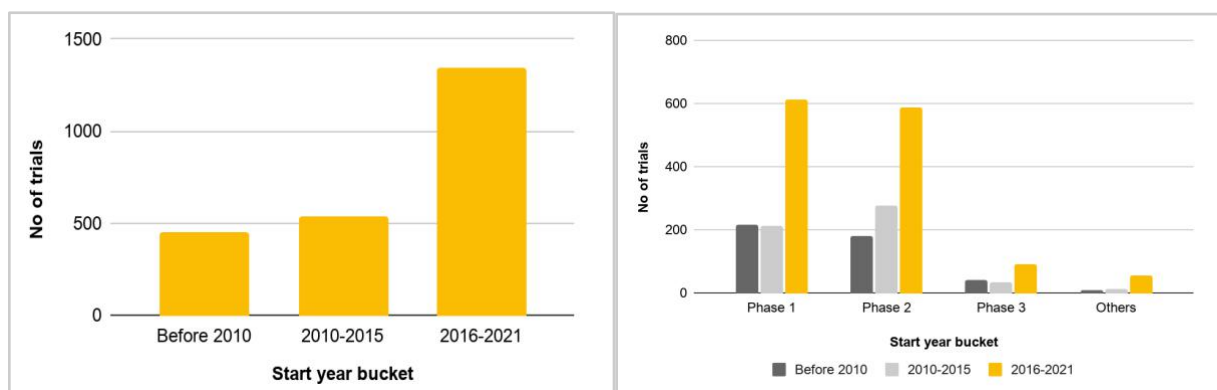


Figure 9: Trial count by phase and year bucket

58% trials for gene and adoptive cell therapies including CAR-T cells have initiated after 2016. From 2010, a higher number of phase 2 (864) trials have been started, followed by phase 1 (824) trials.

3.2.2 - Analysis by status of different type of gene therapy trials

Analysing the distribution of gene therapy trials using different categories like CAR-T cells, viral/bacterial vector, gene silencing and plasmid/naked DNA, by their status in the clinical trial, we observe that 29% of the total trials (n= 2330) have completed their studies while 34% trials are still recruiting the patients. Trials that are terminated either due to recruitment, business related reasons, or due to safety and efficacy issues contribute to 19% of the total trials for gene and CAR-T cell therapies.

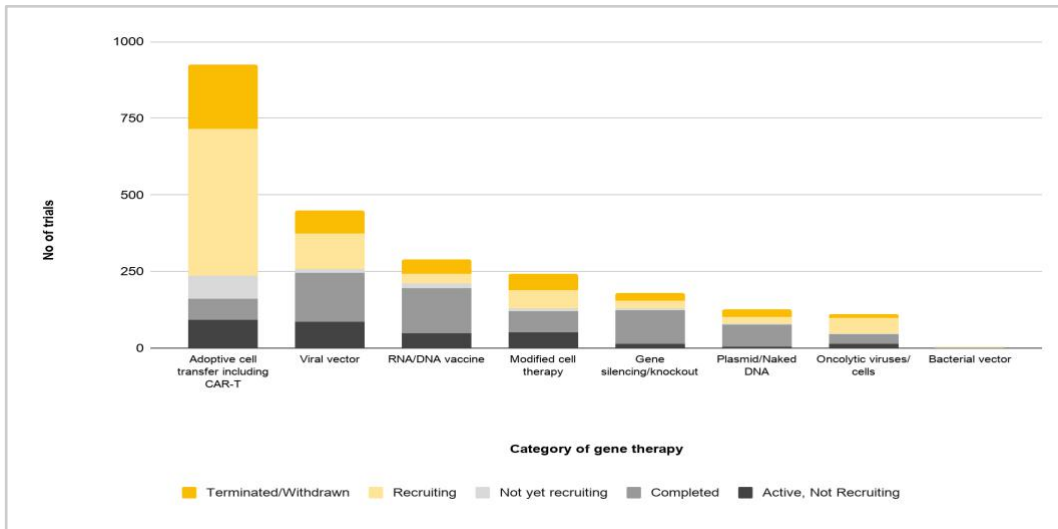


Figure 9: Trial proportion by status across various gene therapy types

3.2.3 - Analysis by condition for various therapy type

Analyzing gene therapy trials by conditions for which they are conducted - we observe that CAR-T cell therapy is mainly studied in oncological indications with leukemia/lymphoma being a major area of focus (Figure 10).

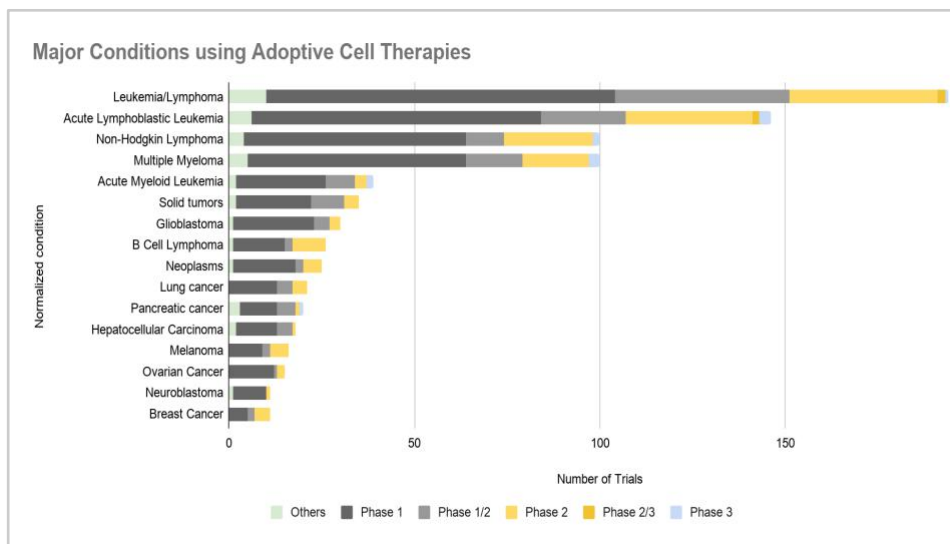


Figure 10: Trial proportion by phase for various conditions tried in adoptive cell therapy

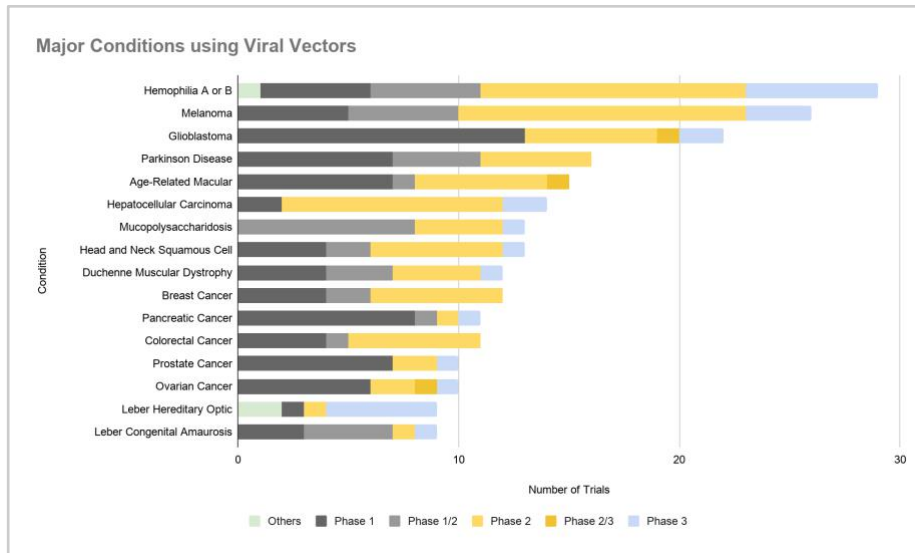


Figure 11: Trial proportion by phase for various conditions tried using viral vectors

The spectrum of gene therapies using viral vectors includes both delivery vehicles developed for transient short-term and permanent long-term expression. These vectors are represented by both RNA and DNA viruses with either single-stranded (ss) or double-stranded (ds) genomes. Large number of viral vectors are being evaluated for a number of disease indications like Hemophilia A or B, Melanoma, Glioblastoma, Parkinson’s disease and Age-related macular degeneration (Figure 11).

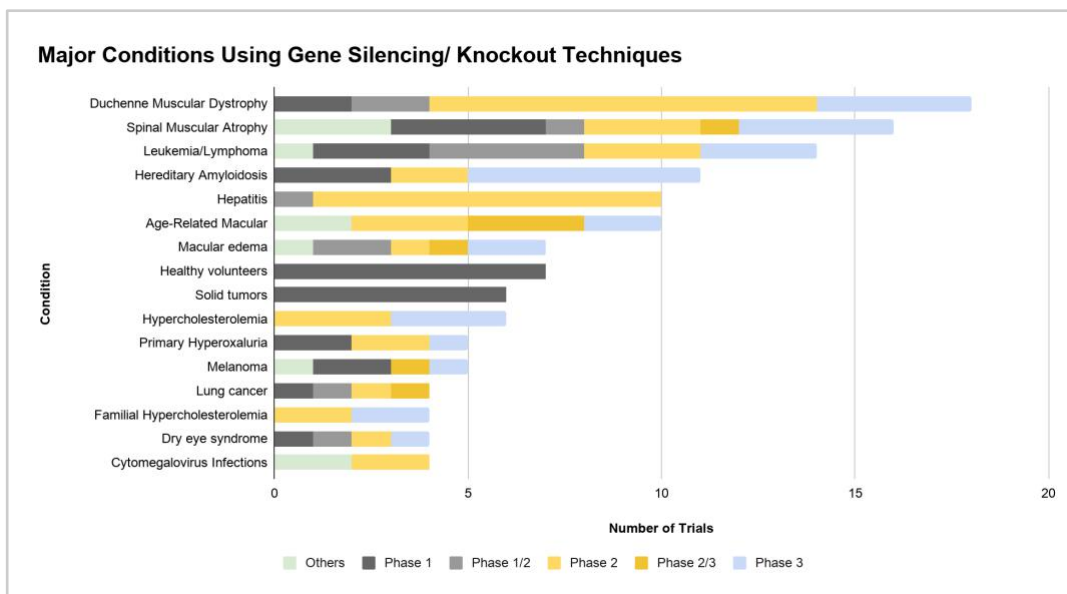


Figure 12: Trial proportion by phase for various conditions tried using gene silencing/knockout

Gene therapy using gene silencing or knockout technique is able to silence the expression of the abnormal gene in diseased conditions. These therapies are widely studied in Duchenne Muscular

Dystrophy, Spinal Muscular Atrophy, Leukemia/Lymphoma, Hereditary Amyloidosis, Hepatitis, Age-related Macular Degeneration (Figure 12).

3.2.4 - Analysis by completion year for all gene therapy trials

Analyzing trials by primary completion year and phase to gauge upcoming approvals - we observe that 262 gene and CAR-T cell therapies trials are expected to be completed in 2021, with 295 trials in 2022.

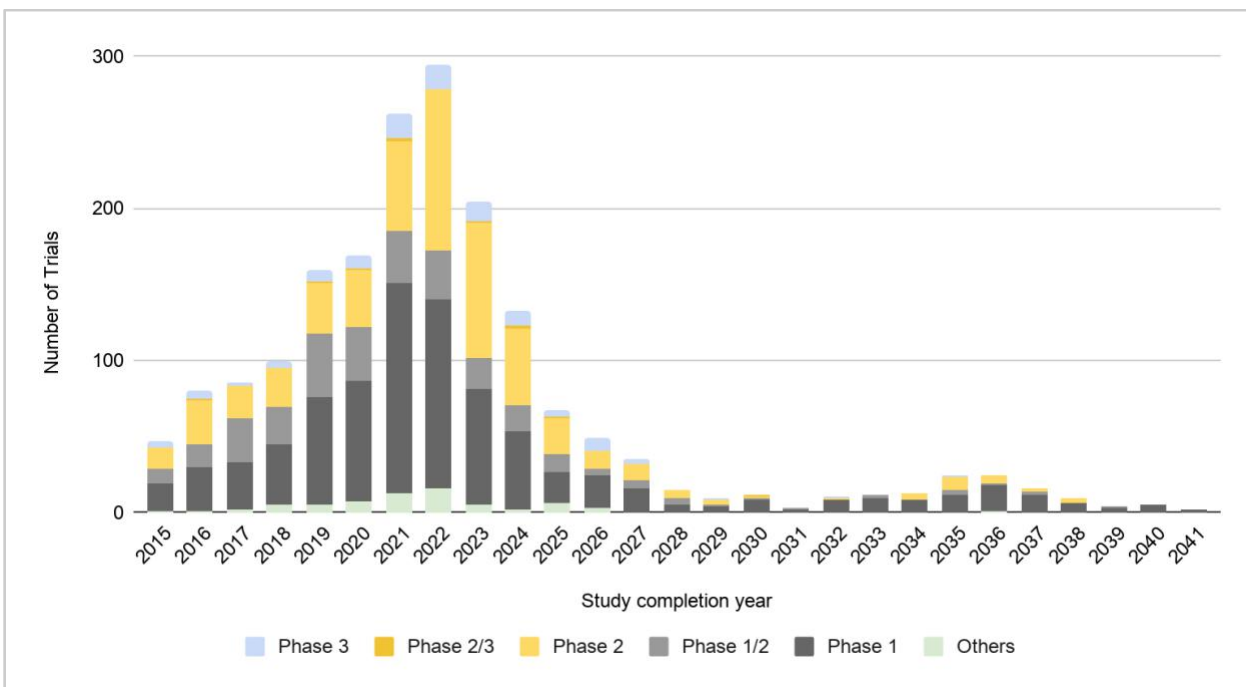


Figure 13: Trial proportion by phase and study completion year

Key points from this section:

1. Although viral vectors have been prominent delivery types for gene therapy, non-viral vectors (bacterial, plasmid and chemical) are gaining traction primarily due to less immunogenicity and higher genome capacity.
2. Gene therapy has been most active in recent times with more trials starting in the last 5 years than in previous all years combined with ~500 trials due for completion in the next 2 years.
3. While CAR-T cell therapy is mainly studied in leukemia/lymphoma, viral vectors have been more prominent in Haemophilia and Duchenne Muscular Dystrophy has been the focus area for gene silencing.

4. A Deeper Look Into AAV

Among the different categories of gene therapies, viral vectors are already widely studied in clinical trials¹⁶. This section provides an overview of adeno-associated virus associated trial landscape as one of the most prominent examples for gene therapy viral vectors. We analyze the clinical trial landscape for various aspects like therapeutic areas, routes of administration, sponsors, and adverse events.

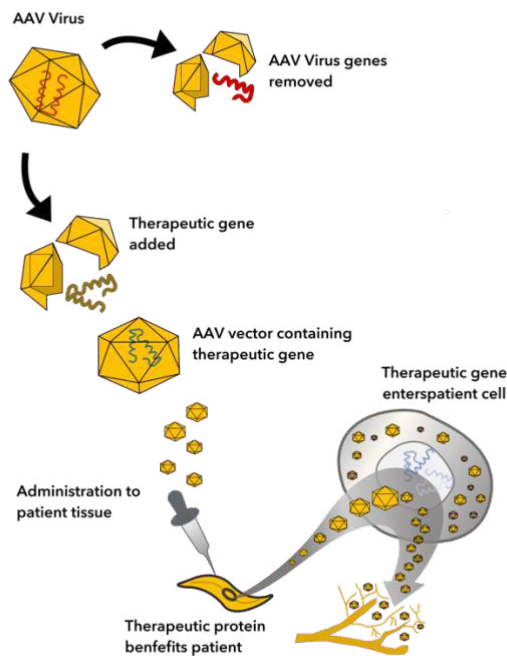


Figure 14: AAV vector mediated gene transfer in patients

4.1 - AAV - widely chosen viral vector for gene therapy

Adeno-associated virus (AAV) is a small (25 nm), nonenveloped virus that packages a linear single-stranded DNA genome. To use as a vector, AAV is transformed from a naturally occurring virus into a delivery tool: most of its viral components are replaced by genes of interest. It has the ability to generate recombinant AAV particles lacking any viral genes and containing DNA sequences of interest for various therapeutic applications.

AAV has shown specific functionality in gene therapy applications as:

- It has a unique life cycle and ability to infect different cell and tissue types (both non-dividing and dividing cells) with persistent expression⁷
- Wild-type AAV apparently lacks pathogenicity. Also, it doesn't replicate in human without a helper virus thereby making it safer to use¹⁷
- The virus does not elicit a cell-mediated immune response and inflammation, hence decreasing risks of adverse events¹⁸
- AAV vectors very rarely integrate into the host genomes, making them comparatively safer to use

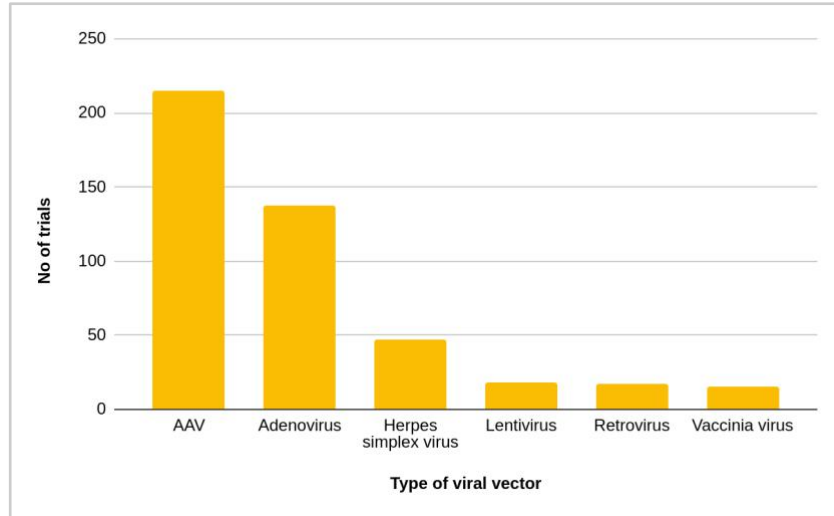


Figure 15: Trial distribution by viral vector type

4.1.1 - Approved AAV related gene therapy Interventions

The table below shows all three approved AAV related gene therapy interventions, their year of approval, conditions for which they have been approved, their capsid types and transgene products.

Drug	Brand Name	Condition approved for	Capsid type	Transgene Product	Year approved in
Alipogene Tiparvovec	Gly-bera	Familial Lipoprotein Lipase Deficiency	AAV1	LPLS447X	2012
Voretigene Neparvovec	Luxturna	Leber Congenital Amaurosis	AAV2	RPE65	2017
Onasemnogene Apeparvovec	Zolgensma	Spinal Muscular Atrophy	AAV9	SMN1	2019

Table 1: Details of all approved AAV-based therapies

Though AAV vectors are one of the most frequently used viral vectors in clinical trials with gene therapy (Figure 15), AAV vector production has limitations that cause issues with patient safety and efficacy, and the commercial success of the therapy. The challenges that AAV therapy is facing are¹⁹:

- Production of low vector concentrations
- Delivering the desired genetic sequence into the AAV capsids
- Neutralization of oncogenic DNA fragments in vectors
- Minimize the risk of immunogenicity
- Harvesting AAV from cells and removing impurities

4.2 - Analysis of AAV trials

In this section, we provide a detailed analysis of AAV-related clinical trials using viral parameters such as Capsid type, Promotor used, Gene delivery target, Route of administration, Purpose of gene therapy etc. AAV trials are further analysed for safety and efficacy objectives.

4.2.1 - AAV trials by phase and TA

To assess the interest of the industry in AAV related trials, we segmented the trends of AAV trials across different phases and therapeutic areas over the years.

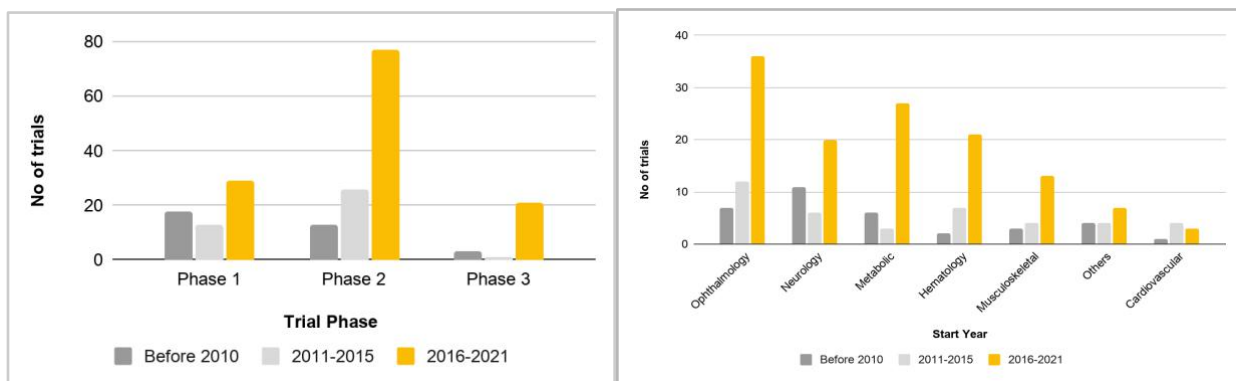


Figure 16a
Figure 16b
Figure 16: AAV trial distribution by year for various phases and TA

Of total 219 trials analysed, 203 are interventional and Phase 1, Phase 2 and Phase 3 trials. Increasing interest in AAV related gene therapy trials is observed in recent years as seen from peak in Phase 2 and Phase 3 trials (Figure 16a).

Most AAV trials are being conducted in ophthalmology followed by neurology and metabolic therapeutic areas (Figure 16b).

4.2.2 - Top sponsors by phase and year

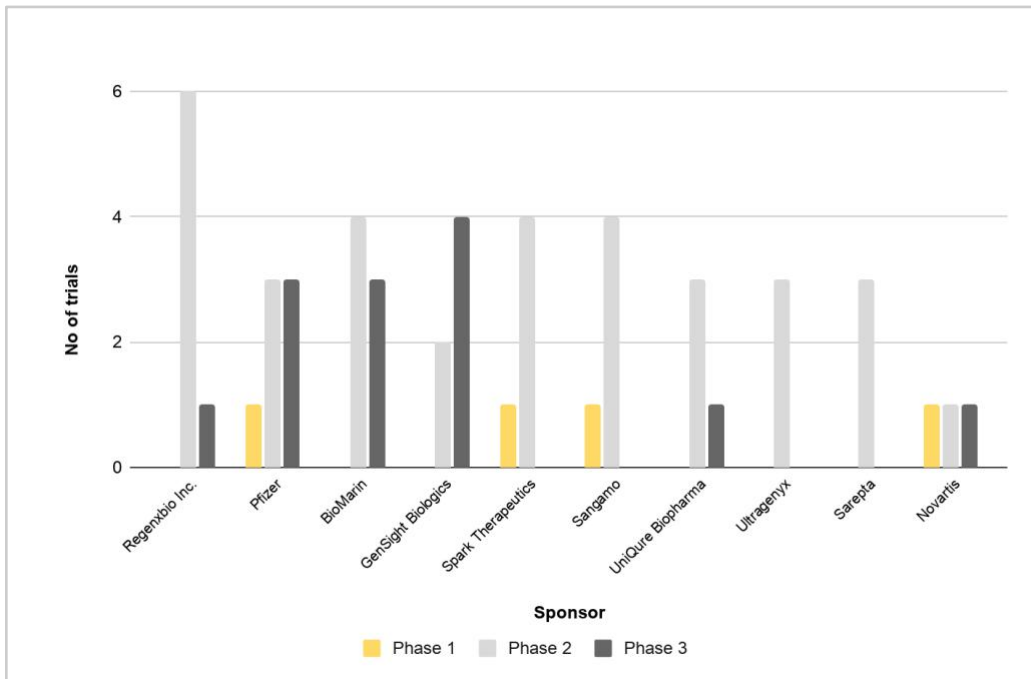


Figure 17: AAV trial distribution by phase for top sponsors (Note: The above table contains both historical and ongoing trials)

The sponsor studies for AAV trials showed RegenxBio Inc followed by Pfizer, BioMarin Pharmaceutical, GenSight Biologics and Spark Therapeutics are having the highest interest in AAV trials in recent years. GenSight Biologics, Pfizer, BioMarin, Novartis, RegenxBio Inc, UniQure Biopharma have the most advanced pipeline, with several trials ongoing in Phase 3 while RegenxBio Inc, BioMarin Pharmaceutical, Spark Therapeutics, Sangamo Therapeutics and Pfizer covered most of the AAV trials are in Phase 2 (Figure 17).

4.2.3 - Top diseases by phase and start year

Further, we observed the top diseases for which AAV trials are being conducted and the tables below show the count of those trials based on the start year and phase.

Top five indications that are highly studied for AAV vectors are Hemophilia B, Parkinson's Disease, Duchenne Muscular Dystrophy, Hemophilia A and Retinitis Pigmentosa (Figure 18).

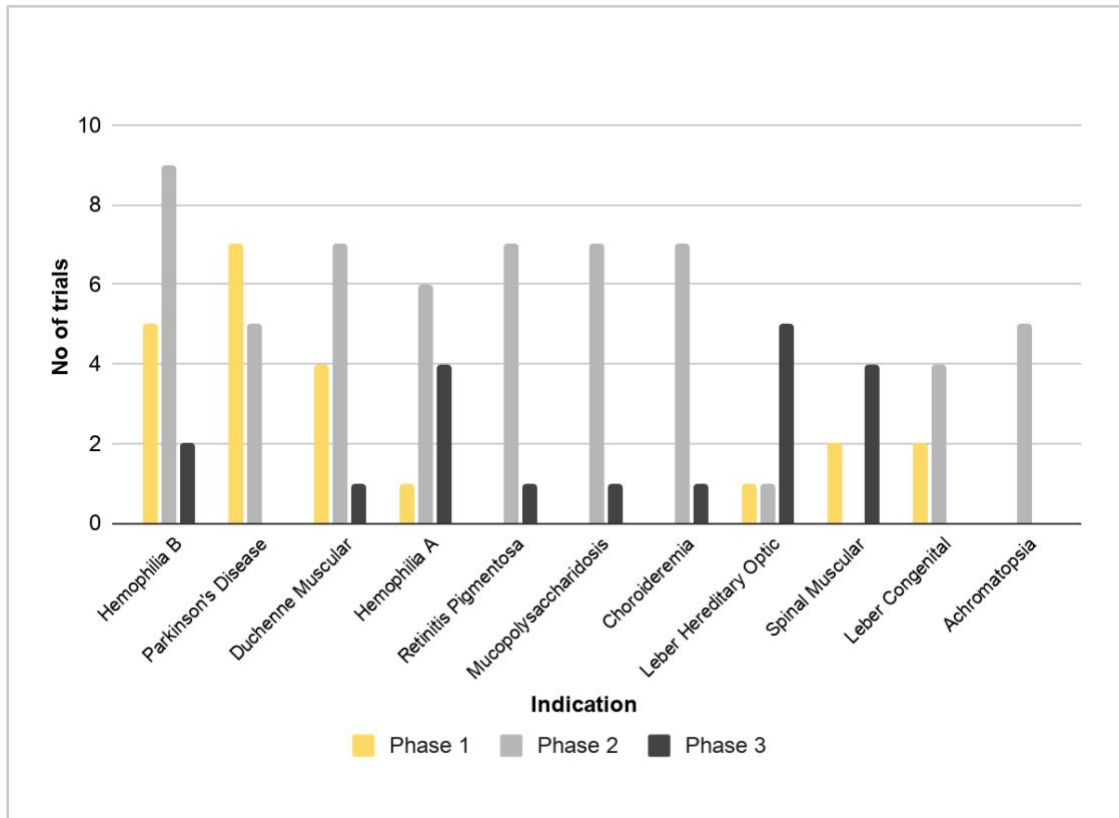


Figure 18: AAV trial distribution by indication and phase

Trials have reached a higher phase (Phase 3) for Hemophilia A/B, Leber Hereditary Optic Neuropathy and Spinal Muscular Atrophy while Hemophilia B, Duchenne Muscular Dystrophy, Retinitis Pigmentosa, Mucopolysaccharidosis, Choroideremia have reached Phase 2.

4.2.4 - Route of administration used in AAV trials

In this section, we compare the most common route of administration for gene therapy intervention (AAV trials) across different therapeutic areas.

Route of administration	Ophthalmology	Neurology	Metabolic	Hematology	Musculoskeletal	Others	Cardio-vascular	Total
Intravenous	-	8	22	29	9	4	1	73
Subretinal	37	-	-	-	-	-	-	37
Injection Into Brain	-	27	-	-	-	-	-	27
Intramuscular	-	1	7	1	10	3	-	22
Intravitreal	17	-	-	-	-	-	-	17
Others (Intranasal, Intra Salivary, Etc)	2	2	-	-	-	4	-	8
Intrathecal	-	7	-	-	-	-	-	7
Intracoronary	-	-	-	-	-	-	7	7
Intra-articular	-	-	-	-	1	4	-	5
Total	56	37	37	30	20	15	8	203

Table 2: AAV trials' route of administration for various therapeutic areas

1. Intravenous gene therapies targeting the liver are addressed in both hematological and metabolic indications
2. Subretinal or Injection into brain routes of administration in Ophthalmology and Neurology contribute to 31% of all gene therapy trials

4.3 - Capsid types and promoter types

4.3.1 - Commonly used capsid type

AAVs have been discovered with multiple serotypes and variable tropism. Besides genetic differences in virus capsids, tissue tropism may be influenced by cell surface receptors, cellular

uptake, intracellular processing, nuclear delivery of the vector genome, uncoating, and second-strand DNA conversion²⁰. The table below depicts the most frequently used capsid types in AAV trials across different therapeutic areas.

Capsid Type	Ophthalmology	Neurology	Metabolic	Hematology	Musculoskeletal	Others	Cardiovascular
AAV2	37	19	1	-	-	9	-
AAV8	10	-	7	3	1	2	-
AAV9	-	9	8	-	4	-	-
AAV1	-	1	5	1	3	2	6
AAV5	1	-	2	11	-	-	-
AAVrh74	-	-	-	-	10	-	-
AAVrh10	-	5	4	1	-	-	-
AAV2/5	2	-	2	1	2	2	-
AAV2/8	-	-	2	4	-	-	1
SPK100	-	-	-	4	-	-	-
AAV2/6	-	-	2	2	-	-	-
Others (AAV2/4, LK03 etc.)	4	2	2	3	-	-	-

Table 3: AAV trials distribution by capsid type across therapeutic areas

Commonly used Capsid types by TA are:

1. Ophthalmology: AAV2, AAV8
2. Neurology: AAV2, AAV9, AAVrh10
3. Metabolic: AAV9, AAV8, AAV1
4. Hematology: AAV5
5. Musculoskeletal: AAVrh74, AAV9
6. Cardiovascular: AAV1

4.3.2 - Commonly used promoter types

Promoters are essential to control the expression of a therapeutic gene being delivered by gene therapy vectors. These promoters could have ubiquitous (e.g. CMV, CAG promoter) or tissue-specific expressions (e.g. GAD-65, hRPE65, hAAT or liver specific promoters). A tissue-specific promoter can restrict unwanted transgene expression as well as facilitate persistent expression

whereas ubiquitous expression promoters are used when targeting a specific cell type is not required, i.e. transgene expression is required in the broadest possible spectrum of cells.

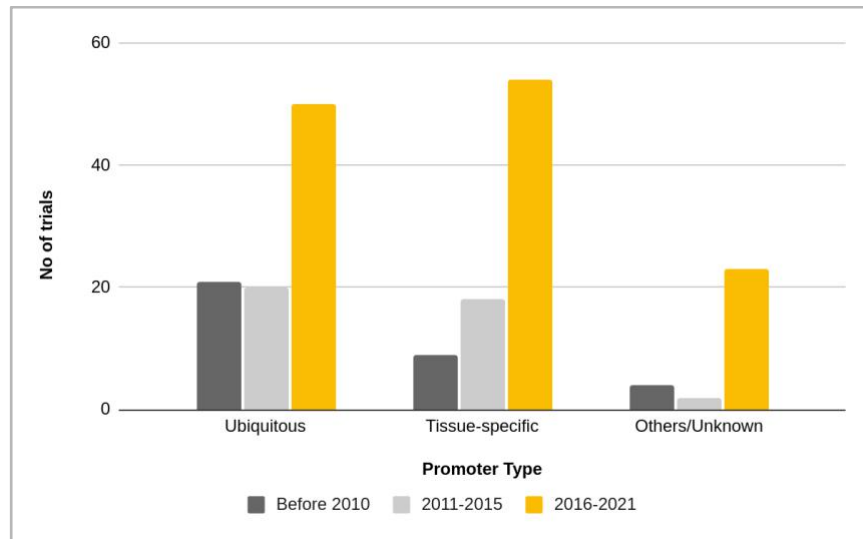


Figure 19: AAV trial distribution by promoter type for different years of start

In AAV trials conducted, most widely used promoters are Ubiquitous followed by Tissue specific. Also, higher increase observed in trials with Tissue specific promoters compared to Ubiquitous promoters for before and after 2016.

4.3.3 - Strategy used in AAV trials

In this subsection we have examined different strategies used in AAV trials across various therapeutic areas. Definitions for inclusion as a strategy are:

- Addition - Consists of the delivery of a new protein-coding gene to treat a disease
- Replacement - Directed at monogenic recessive diseases that are caused by non-functional genes, consisting in the delivery of a non-mutated form of the affected gene to treat the disease
- Inhibition - Introduction of a gene whose product either inhibits the expression of another gene or interferes with the activity of the product of another gene
- Silencing - Regulation of gene expression in a cell to prevent the expression of a certain gene during either transcription or translation (usually DNA/RNA interference mediated)
- Skipping - exon skipping (form of RNA splicing) is used to cause cells to “skip” over faulty or misaligned sections of genetic code, leading to a truncated but still functional protein despite the genetic mutation
- Editing - Technique that allow genetic material to be added, removed, or altered at particular locations in the genome of the hos

Therapeutic Area	Addition	Replacement	Inhibition	Silencing	Skipping	Editing	Total
Ophthalmology	42	11	3	-	-	-	56
Neurology	23	14	-	-	-	-	37
Metabolic	12	23	-	1	-	1	37
Hematology	9	21	-	-	-	-	30
Musculoskeletal	13	6	-	-	1	-	20
Others	12	2	-	1	-	-	15
Cardiovascular	1	7	-	-	-	-	8
Total	112	84	3	2	1	1	203

Table 4: AAV trials distribution by strategy for various therapeutic areas

1. Gene Addition most widely used in: Ophthalmology (75%), Musculoskeletal (65%), Neurology (62%)
2. Gene Replacement most widely used in: Metabolic (62%), Hematology (70%), Cardiovascular (88%)

4.4 - Efficacy profile in AAV trials

We looked at the 80 AAV trials which have efficacy related endpoints and where data was available (across trial registry, publications etc.) on those efficacy endpoints being met or not. We haven't made any observations on Phase 3 trials due to lack of data. Below are our observations:

1. By Therapeutic Area

- Phase 1: Trials in Ophthalmology shows the highest efficacy followed by Neurology and Hematology
- Phase 2: Trials in Hematology shows the highest efficacy followed by Ophthalmology and Neurology

2. By Route of Administration

- Phase 1 and 2: Subretinal shows the highest efficacy followed by Intravenous and Injection into brain

3. In intravenous route of administration, in Phase 2 trials AAV5 capsid type has better efficacy compared to AAV8

4. By primary Gene Delivery Target

- Phase 1: Delivery into eye shows the highest efficacy followed by delivery into CNS and Liver
- Phase 2: Delivery into muscle shows the highest efficacy followed by delivery into Eye and CNS

5. Capsid type AAV2 has highest efficacy in Phase 1 and Phase 2 followed by AAV1
6. Gene Addition has better efficacy in Phase 2 and Phase 3 compared to Gene Replacement. Gene Replacement has better efficacy in Phase 1 than Gene Addition.

4.5 - Analysis of adverse events

We analyzed adverse event data on 21 AAV related clinical trials reported in the clinical trials registry and associated publications. Majority of trials belonged to Ophthalmology, Neurology & Hematology with Hemophilia B and Parkinson's being the top conditions.

Although improving, gene therapy still has high rates of adverse events when compared to other therapy types. Below are our observations:

1. 24% subjects (82 out of 341) had serious adverse events in the 21 trials analyzed
2. Most frequent serious adverse events are
 - a. Cardiac related such as Cardiac Failure, Atrial Fibrillation, etc
 - b. Respiratory tract and Pneumonia related
 - c. Various viral infections - Rhinovirus, Parainfluenzae, Enterovirus, etc
3. Intramuscular, Intravitreal were safer routes of administration compared to Intracoronary, Intravenous and injection into the brain in terms of serious adverse events as well as mortality.
4. Among major capsid types, AAV2 (tried in Ophthalmology & Neurology) was safer than AAV1 (tried in Musculoskeletal, Cardiovascular & Metabolic) both in terms of serious adverse event and mortality, while AAV9 (only tried in Spinal Muscular Atrophy) had the most serious adverse event.

4.6 Trials with AAV therapies nearing completion

Amongst the ongoing AAV trials, few are slated for completion in the near future. Of these, Valoctocogene roxaparvovec, for the treatment of Hemophilia A, has been submitted for FDA approval by BioMarin. Fidanacogene elaparvovec (by Pfizer) received FDA breakthrough therapy designation and EMA orphan drug designation in 2016. Another Pfizer drug to have received orphan drug designation from FDA and EMA in 2017 is Fordadistrogene movaparvovec. Below are the development details of these interventions.

Intervention	Development phase	Disease	Sponsor	Primary completion date
Giroctocogene fitelparvovec	Phase 3	Hemophilia A	Pfizer	2022-08-12
AAV5-RPGR	Phase 3	X-Linked Retinitis Pigmentosa	Janssen, MeiraGTx	2022-07-05
Lenadogene nolparvovec	Phase 3	Leber Hereditary Optic Neuropathy	GenSight Biologics	2022-07-05
Valoctocogene roxaparvovec	Preregistration	Hemophilia A	BioMarin	2021-12-09
Etranacogene dezaparvovec	Phase 3	Hemophilia B	UniQure Biopharma	2020-09-25
Olenasuflogene relduparvovec	Phase 2/3	Mucopolysaccharidosis Type IIIA	LYSOGENE	2022-03-25
Fidanacogene elaparvovec	Phase 3	Hemophilia B	Pfizer	2022-05-25
RGX-314	Phase 2/3	Wet Age-related Macular Degeneration	Regenxbio Inc.	2023-03-13
Fordadistrogene movaparvovec	Phase 3	Duchenne Muscular Dystrophy	Pfizer	2022-09-29

Table 5: AAV trials with primary completion date in 2021-2022

5. Key takeaways and next in series

Gene therapy evolved through many milestones with a history of several decades filled with ups and downs in expectations and efforts to bring an optimal trade-off between the efficacy and safety. Given that gene therapies address the root causes of disease, often with a single curative dose that can replace expensive maintenance treatment, there are many challenges to bring gene therapeutics to the market in terms of higher cost, vector-induced immune response, evolving regulatory scenario, challenges related to manufacturing, transportation and scale. Despite these challenges, researchers and the biotech industry continued to invest in research activities. Moreover, encouraging data has also been obtained from some long-term gene therapies that further validate the use of this therapy as a potential permanent cure:

- Follow-up data for Glybera with eight patients for three years and fourteen patients for one year in two different trials showed a statistically significant, tenfold decrease in the incidence of acute pancreatitis²¹.
- A phase 3 trial of Luxturna showed improvements in ambulatory navigation, light sensitivity and visual field to be maintained at 4 years for the patients with biallelic RPE65 mutation-associated retinal dystrophy²².

- Data for Zolgensma from the START long-term follow-up study demonstrated the durability of a single, one-time dose in patients now up to five years post-dosing and some patients more than five years of age²³.

Though many diseases are in focus for the researchers, chronic conditions viz oncology, neurology, rare and genetic disease are most frequent where gene therapy has seen its uses. Higher research efforts led to an increase in approvals that in turn resulted in an increase in commercial interest in gene therapy. Though there are over 20 therapies that received market authorization in this field, it is possible that the 2020 breakthrough in terms of mRNA based vaccine for COVID19 within a short time brought renewed exuberance. Industry witnessed higher M&A deals and steep valuations, wherein established players started building up capacities in gene therapy through inorganic routes. M&A activity has been aggressive in the gene therapy sector, which recorded ~100 deals since 2016. Rising investors' interest led to steep valuations. In the last 2 years, the gene therapy stocks have beaten the benchmark biotechnology index indicating a rising interest. Following the secondary markets, primary markets too saw increased activity. Funds raised via IPO route gained significant momentum.

Our research on the pipeline of the gene therapy segment led us to following takeaways:

- A flurry of total trials (n=2330) for gene and CAR-T cell therapies showed 58% trials have been initiated after 2016 and 29% trials have completed their studies while 34% trials are still recruiting the patients.
- CAR-T cell therapy is mainly studied in oncological indications with leukemia/lymphoma being a major area of focus while viral vectors are being evaluated for Hemophilia A or B, Melanoma, Glioblastoma, Parkinson's disease and age-related macular degeneration.
- Of the ongoing gene and CAR-T cell therapy trials, 262 trials are expected to be completed in 2021, with 295 trials in 2022. Looking at the number of phase III trials getting completed over the next five years, we can expect new approvals for both gene and CAR-T cell therapies.

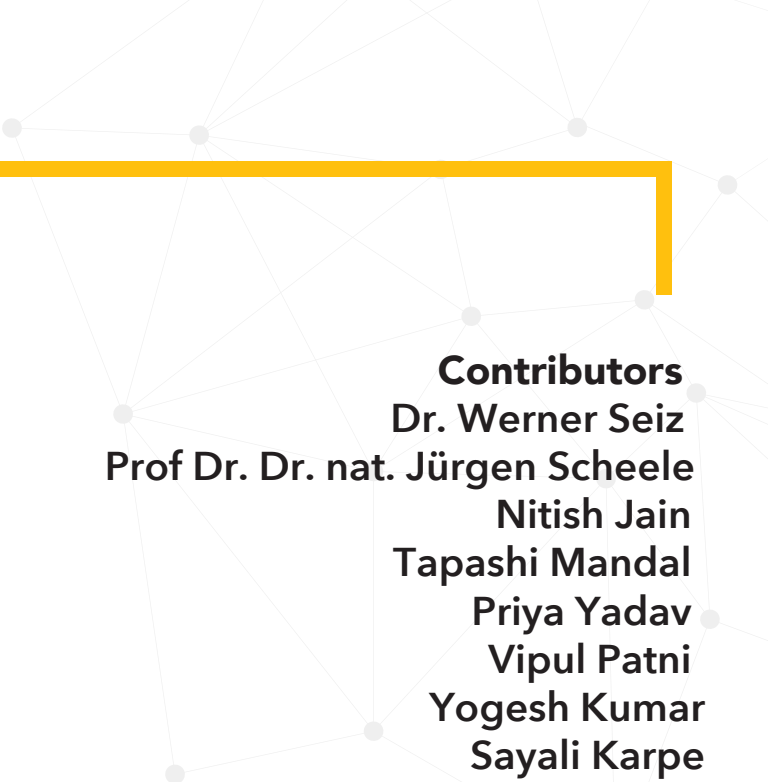
To conclude we believe that the gene therapy based R&D efforts are gaining momentum with optimum support from the industry and investors. Research activities are also throwing up advances and we strongly believe there will be a big breakthrough from the segment. Ultra chronic conditions viz oncology, neurology, immunology and rare diseases will continue to be prominent as far as solutions expected from gene therapy are concerned.

In the upcoming whitepaper in this series of our research on gene therapies, we will focus more on individual facets of adoptive cell therapies with special emphasis on CAR-T cell therapy.

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