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AAV Durability: The Road to a One-Shot Cure

Author: Joseph Moss, Market Analyst, Hanson Wade Intelligence

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Unlocking the Potential of AAV

Adeno-associated virus (AAV) vectors continue to dominate the gene replacement therapy landscape: 73% of clinical studies commenced within the last 2 years used AAV over other gene delivery systems. This is perhaps due to its broad applicability, ease-of-use, and relatively low immunogenicity. Recent approvals of Spark Therapeutics' Luxturna and Novartis' Zolgensma boosted confidence in the field. They provided proof of concept that AAV can selectively deliver different gene constructs to target cells, effectively replacing a defective gene to provide therapeutic effect. However, those remain the only two approvals, representing a clinical success rate of <1%.

Amongst other concerns, one of the principal reasons for the lack of approvals has been durability. Long-term clinical data for some therapies has revealed declining transgene expression over time. This perhaps owes to intrinsic features of AAV itself. The viral genome remains episomal in the nucleus, meaning the therapeutic gene is naturally lost following cell division. Consequently, expression wanes over time depending upon the rate at which the cell divides. This poses a unique issue for treating young people as the vector may become 'diluted' during development. This is further compounded by the immune response to an exogenous viral vector such as AAV – another means by which transgene expression is diminished. Unfortunately, AAV vectors cannot be re-administered using current technology due to the formation of neutralizing antibodies upon delivery. For AAV to be a true one-time therapy, these concerns regarding durability need to be addressed.

The scope of diseases being targeted by AAV gene therapy is continually increasing. The vast majority of such conditions require long-term gene expression to sustain therapeutic benefit. This report shares the available clinical data on the durability of therapies and summarises the key findings that we observed across studies.

Methodology

We observed significant variability in the availability and content of data reported across clinical trials. Therefore, we developed a consistent methodology to collect data that allowed for comparison of different trials. Data throughout the report is accurate as of February 2022.

Inclusion Criteria

We included all clinical trials of AAV-based gene therapies that have reported data on durability with a minimum of 6 months post-administration follow-up of participants. As a primary measure of durability, we collected data on therapeutic protein expression or activity. We believe that this provides the most representative measure of long-term durability. This is because other measures such as metabolic parameters or clinical outcomes may be sustained despite changes in transgene expression or therapeutic protein activity.

To provide a complete picture of the landscape, we included studies that report data on clinical outcomes at least 6 months after treatment when data on protein expression or activity were not available.

Although clinical data may not reflect subtle changes in protein expression, in most cases it indicates whether expression is present. Overall, a total of 121 different trials fit our criteria and are represented in the data throughout this report.

Data Collection and Summary

Different trials use different assays to measure protein expression or activity. Different assays often produce different quantities for the same relative level of expression or activity. Furthermore, many studies do not report quantitative data for expression or activity levels. Therefore, we have summarised any changes in expression or activity as sustained or not sustained, rather than reporting specific quantities. Hence, there may be instances where studies report sustained expression or activity which is below the therapeutic threshold.

We observed significant variability in the extent of durability data reported across the landscape. Some studies reported protein expression or activity levels over time for individual patients, whereas some studies only reported mean values. Therefore, we have summarised data at the level of detail in which it is presented in each study.

To supplement expression and activity data, we have summarised the clinical outcomes for each study. Due to the vast extent of clinical data for each study we based our assessment on a clinical outcome that is common across studies for a particular disease indication. Hence, there may be instances where there are improvements in other clinical outcomes which we have not reported.

Categorization of Data

To allow for comparison of different trials, we allocated each trial into one of four distinct categories based on the type of target tissue. The target tissue is based on the principal cell type that a therapeutic gene is to be delivered. All therapies could be grouped under 4 distinct groups: liver, central nervous system (CNS), muscle and ocular-targeted therapies.

Within each category, there was a range of different disease indications. To allow for more specific comparison of durability, we

sub-categorized trials by disease indication when there was ≥5 trials for a particular indication. We grouped all disease indications for which there was <5 trials together, under the category 'other'.

Assessing Durability

In our assessment of durability for each clinical study, we grouped trials into 3 categories which can be observed in the tables throughout this report. We used the following criteria for each study:

Green – durability of protein expression or activity was comprehensive: there were no reports of a lack of expression or activity in any patients throughout the duration of follow-up.

Amber – there were reports of a lack of expression or activity over time in at least 1 patient in the study. However, expression was not lost in all patients at the latest follow-up visit.

Red – Expression or activity was absent or lost in all patients followed up at the latest visit (there were no reports of sustained expression or activity).

Firstly, it is important to note that we have designated each trial a colour based on data that is publicly available. Some studies only report mean expression/activity levels for patients, so it is not possible to determine whether expression/activity declined in individual patients. Secondly, in some studies it was not possible to determine whether a lack of expression/activity was due to issues with efficacy rather than durability.

Our Sources

All clinical data was collected using the Beacon database (www.beacon-intelligence.com). Additional information was collected from clinicaltrials.gov, company press releases, original articles and review articles. All sources are cited throughout the report.

Liver-Directed Therapies

The liver is one of the most promising targets for gene replacement therapy via AAV, primarily due to its role in the production of various metabolic enzymes. It effectively acts as a factory, producing proteins which are then secreted into the circulation. This makes hepatocytes an ideal target for replacement of missing coagulation factors in hemophilia [1]. However, concerns have emerged with regards to the durability of AAV gene therapies delivered to the liver. Cell division, particularly during childhood development, may lead to reduced genome copy number. Moreover, direct administration to the liver is not possible. Therapies must be administered intravenously, requiring high doses which may induce a detrimental immune response against the vector. Therefore, there is a need to review available data on durability to provide a complete picture of the current landscape.

26 different AAV gene therapy trials targeted at the liver fit our criteria. To allow for representative comparison of different studies, we have grouped therapies according to disease indication. Disease indications that have less than 4 trials have been grouped under 'other'.

There were some key observations across studies:

- There was significant inter-patient variability in the durability of response to treatment, even within individual trials.
- Therapies for hemophilia B have the most convincing evidence of long-term durability of those targeting the liver.
- Declines in transgene expression occur either rapidly or gradually over the long-term, suggesting two distinct mechanisms.
- Dose is very much dependent upon the vector serotype used. The relationship between serotype and dose may have implications for durability.
- Specific vector serotypes produced more durable gene expression than others.

Hemophilia A

A total of 9 clinical studies of AAV gene therapies for hemophilia A reported data on durability, according to our criteria. All studies reported data on Factor VIII (FVIII) expression or activity levels over time. As shown in Table 1, 4/9 studies reported comprehensive durability of expression or activity at the latest follow-up visit. However, 5/9 studies reported declining expression/activity over time in ≥1 patient, 1 of which reported a loss of activity in all patients. This indicates that there is significant inter-patient variability in the durability of response to treatment, perhaps owing to differences in factors such as age or immunogenicity amongst patients.

We observed that expression/activity either declined rapidly or progressively decreased in a longer-term fashion. 2/5 studies reported short-term (<6 months) declines in expression. The respective studies suggested that this may be due to acute immune responses in susceptible patients. Longer term reduction (>6 months) was observed in the other 3/5 studies and is in line with the mechanism of action of AAV – vector genomes do not typically integrate into the host genome, so they become diluted over time as liver cells divide.

Increases in FVIII expression/activity were dose-dependent in some of the studies using multiple doses. In these studies (indicated in Table 1 as 'dose-dependent increase'), peak expression/activity was higher in some patients receiving higher doses. This might mean that therapeutic benefit persists for longer in these patients, as expression declines from a higher level and may take longer to drop below a therapeutic threshold. However, high doses may be more likely to induce an immune response which clears the AAV capsid from the body [2]. Many developers are attempting to find the optimal balance between high dose and reduced immunogenicity.

One of the ways in which the effects of

dose are being tackled is by using different AAV serotypes. As shown in Table 1, 6 different serotypes were used across 9 trials. Of note, BioMarin's Roctavian which uses AAV5 produced durable FVIII activity at up to 5 years post-treatment. Patients also experienced a sustained reduction in bleeding rate at 5 years, demonstrating that FVIII activity was sustained at therapeutic levels. However, activity slowly declined over time from 1 year post-dosing, perhaps due to liver cell proliferation. It is therefore possible that expression may eventually decline to a level which is not sufficient to maintain clinical benefit, meaning longer term follow-up is needed. Spark Therapeutics demonstrated that durability can be achieved using low vector doses in trials of SPK-8011 and SPK-8016. Though the serotype used in SPK-8016 is undisclosed, SPK-8011 uses low doses of AAV-LK03, a novel engineered capsid, and produced durable FVIII expression in 89% of patients at up to 4 years post-treatment. Engineered capsids and lower doses may reduce any potential immune responses [3], reducing the chance that the immune system will clear the vector from the body.

Hemophilia B

A total of 8 clinical studies of AAV gene therapies for hemophilia B reported data on durability, according to our criteria. All studies reported data on Factor IX (FIX) activity levels over time. As shown in Table 2, 4/8 studies reported comprehensive durability of FIX activity at the latest follow-up visit. However, 4/8 studies reported a lack of durable FIX activity in ≥1 patient. As in hemophilia A, this demonstrates that there is significant variability amongst patients in the durability of response to some therapies. Declining FIX activity either occurred rapidly or gradually over time, providing further evidence of two distinct mechanisms of decline.

6 different vector serotypes were used across 8 trials, demonstrating the heterogeneity of therapies for hemophilia. Interestingly, 3 different studies of UniQure's AMT-060 and AMT-061 showed durable FIX activity up to 5 years post-treatment using the AAV5 serotype. There were no reports of activity declining over time, in contrast to BioMarin's AAV5 gene therapy for hemophilia A. This suggests that FIX activity may be permanent and confirms the ability of AAV5 to produce durable outcomes. These studies used similar doses to the BioMarin studies, suggesting that there is an optimal dose range that leads to durable outcomes for each serotype. Furthermore, 50% of hemophilia B studies used bioengineered capsids which are said to have improved properties such as greater transgene expression [4]. The clinical data supports this as two of these vectors, AAVS3 and AAV-Spark100, produced durable FIX activity at therapeutic levels in most patients despite using relatively low doses. Within individual studies that used multiple doses, there was a trend towards higher peak FIX activity when higher doses were used. Depending upon immune responses, this might lead to longer-term therapeutic expression levels in some patients.

Overall, the clinical data from both hemophilia A and B studies is encouraging for developers of gene therapies for hematological disorders. Depending upon the treatment regimen and various patient-derived factors, durable transgene expression is certainly possible.

Other Liver-Directed Therapies

Other than hemophilia, there were 9 clinical studies of liver-targeted therapies with relevant data on durability, according to our criteria. A variety of disease indications were targeted, as shown in Table 3. 7 studies reported data on transgene expression or therapeutic protein activity, whereas 2 studies only reported data on clinical outcomes. 5/7 studies reported comprehensive durability of protein expression/activity at the latest follow-up visit. 2/7 studies reported a lack of durable expression in ≥1 patient. Though the longest duration of follow-up for these endpoints was only 2 years, these studies demonstrate that AAV can produce durable outcomes in the liver across a range of different diseases.

We observed no relationship between vector dose and durability of expression or activity across different studies, perhaps due to inherent differences between disease indications. However, we did observe dose-dependent increases in expression/activity within individual studies. For example, in a Phase 1/2 trial of HMI-102, only patients in the middle and high dose cohorts demonstrated durable PAH activity up to 1 year post-treatment, whereas those in the low dose cohort showed a lack of activity. This provides further evidence that a sufficient dose is required to produce a durable response.

Though all these therapies target the liver, 56% target lysosomal storage disorders (LSDs), perhaps due to largely unmet clinical need in the area [5]. Interestingly, all studies that reported comprehensive durability of protein expression/activity were LSDs. In particular, Fabry disease has emerged as a promising target for gene therapy, and this is reinforced by clinical data. Durable AGA expression/activity was demonstrated across 3/3 trials, using 3 distinct vectors. Protein activity was sustained at supranormal levels in trials of 4D-310 and ST-920, leading to sustained clinical outcomes. For example, AGA activity increased to 2506% of normal in one subject at 40 weeks post-treatment in the 4D-310 trial. In contrast, activity was sustained at a lower level in the Fabry trial of FLT190, perhaps explaining why there was a lack of long-term clinical benefit. Greater activity levels and hence clinical outcomes in the first two Fabry studies may be due to a combination of higher dose levels and the use of engineered vectors, which may produce more robust transgene expression.

Other than in the Phase 1/2 study of FLT190, clinical outcomes were mostly in line with sustained expression or activity. In the 2 trials that only reported data on clinical outcomes, clinical benefit was sustained at the latest follow-up visit in most patients. The correlation between transgene expression and clinical outcomes in the majority of studies suggests that transgene expression was also sustained in these patients.

Despite concerns over durability, clinical data demonstrates that AAV vectors can produce sustained expression of a range of different transgenes in the liver across different disease indications.

Table 1. Hemophilia A

Disease indication	Trial Identifier	Drug Name	Gene Delivered	Vector Serotype	Vector Dose (vg/kg body weight)	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Durability Endpoint	Durability Endpoint - Comments	Clinical Outcomes (Reduced Bleeding Rate)
Hemophilia A	NCT03001830	AAV2/8-HLP-FVIII-V3	FVIII	AAV2/8	$6x10^{11}$ / $2x10^{12}$	Intravenous	University College London	I	3	Increase in FVIII activity	Sustained at up to 47 weeks	Not reported
Hemophilia A	NCT03370172	BAX 888	FVIII	AAV8	$2x10^{12}$ / $6x10^{12}$	Intravenous	Shire	I/II	4	Increase in FVIII activity	Peaked at 4 - 9 weeks then declined; dose-dependent increase	Sustained in 1/4 patients at up to 10 months
Hemophilia A	NCT03588299	DTX 201	FVIII	AAVhu37	$0.5x10^{13}$ / $1x10^{13}$ / $2x10^{13}$	Intravenous	Bayer	I/II	9	Increase in FVIII expression	Sustained at up to 23 months	Sustained in most patients (two high dose cohorts) at up to 23 months
Hemophilia A	NCT03392974	Roctavian	FVIII	AAV5	$4x10^{13}$	Intravenous	BioMarin Pharmaceutical	III	1	Increase in FVIII activity	Sustained at up to 1 year	Sustained at up to 1 year
Hemophilia A	NCT02576795	Roctavian	FVIII	AAV5	$4×10^{13}$ / $6×10^{13}$	Intravenous	BioMarin Pharmaceutical	I/II	13	Increase in FVIII activity	Sustained at year 5 but declined from peak at 1 year; dose-dependent response	Sustained at up to 5 years
Hemophilia A	NCT03370913	Roctavian	FVIII	AAV5	$6×10^{13}$	Intravenous	BioMarin Pharmaceutical	III	134	Increase in FVIII activity	Sustained at year 3 but reduced from years 1 and 2	Sustained at up to 2 years
Hemophilia A	NCT03061201	SB-525	FVIII	AAV2/6	$9x10^{11}$ / $2x10^{12}$ / $1x10^{13}$ / $3x10^{13}$	Intravenous	Pfizer	II	11	Increase in FVIII activity	Sustained in high dose group at year 2 (results not reported for other doses) but slowly declining	Sustained only in the high dose cohort at up to 2 years
Hemophilia A	NCT03003533	SPK-8011	FVIII	AAV-LK03	$5×10^{11}$ / $1x10^{12}$ / $1.5x10^{12}$ / $2×10^{12}$	Intravenous	Spark Therapeutics	I/II	18	Increase in FVIII expression	Sustained in 16/18 subjects at up to 4 years; 2/18 subjects lost expression by week 26	Sustained in 16/18 subjects at up to 4 years
Hemophilia A	NCT03734588	SPK-8016	FVIII	Undisclosed	$5x10^{11}$	Intravenous	Spark Therapeutics	I/II	4	Increase in FVIII activity	Sustained at up to 66 weeks	Sustained at up to 18 months

FVIII, Factor FVIII

Table 2. Hemophilia B

Disease indication	Trial Identifier	Drug Name	Gene Delivered	Vector Serotype	Vector Dose (vg/kg body weight)	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Durability Endpoint	Durability Endpoint - Comments	Clinical Outcomes (Reduced Bleeding Rate)
Hemophilia B	NCT02396342	AMT-060	FIX	AAV5	$5×10^{12}$ / $2×10^{13}$	Intravenous	UniQure Biopharma	I/II	10	Increase in FIX activity	Sustained at up to 5 years	Sustained at up to 5 years
Hemophilia B	NCT03489291	AMT-061	FIX	AAV5	$2×10^{13}$	Intravenous	UniQure Biopharma	II	3	Increase in FIX activity	Sustained at up to 2 years	Sustained at up to 1 year
Hemophilia B	NCT03569891	AMT-061	FIX	AAV5	$2×10^{13}$	Intravenous	UniQure Biopharma	III	54	Increase in FIX activity	Sustained at up to 18 months	Sustained at up to 18 months
Hemophilia B	NCT01687608	BAX 335	FIX	AAV8	$2.0×10^{11}$ / $1.0×10^{12}$ / $3.0×10^{12}$	Intravenous	Shire	I/II	8	Increase in FIX activity	Sustained in 1 subject (middle dose) at 4 years; not sustained in others beyond 11 weeks; dose-dependent increase	Sustained in 1/4 subjects at up to 4 years
Hemophilia B	NCT02618915	DTX101	FIX	AAVrh.10	$1.6x10^{12}$ / $5.0x10^{12}$	Intravenous	Ultragenyx Pharmaceutical Inc	I/II	6	Increase in FIX activity	Increased from baseline at 40 weeks but variable throughout	Not sustained at 1 year
Hemophilia B	NCT03369444	FLT180a	FIX	AAVS3	$3.8x10^{11}$ / $6.4x10^{11}$ / $8.32x10^{11}$ / $1.28x10^{12}$	Intravenous	University College London	I/II	10	Increase in FIX activity	Sustained in 9/10 patients at up to 3.5 years but some declines were observed; dose-dependent increase'	Sustained in 9/10 subjects at up to 3.5 years
Hemophilia B	NCT00979238	scAAV2/8-LP1-hFIXco	FIX	AAV2/8	$2x10^{11}$ / $6x10^{11}$ / $2×10^{12}$	Intravenous	St. Jude Children's Research Hospital	I	10	Increase in FIX activity	Sustained at up to 4 years but declined in some patients; dose-dependent increase	Sustained in most subjects at up to 4 years
Hemophilia B	NCT02484092 NCT03307980	SPK-9001	FIX	AAV-Spark100	$5x10^{11}$	Intravenous	Pfizer	II	14	Increase in FIX activity	Sustained increase at year 5	Sustained at up to 5 years

FIX, Factor IX

Table 3. Other Liver-Directed Therapies

Disease indication	Trial Identifier	Drug Name	Gene Delivered	Vector Serotype	Vector Dose (vg/kg body weight)	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Durability Endpoint	Durability Endpoint - Comments	Clinical Outcomes
Acute intermittent porphyria	NCT02082860	rAAV2/5-PBGD	PBGD	AAV5	$5x10^{11}$ / $2x10^{12}$ / $6x10^{12}$ / $1.8x10^{13}$	Intravenous	Digna Biotech SL	I	8	Increase in PBGD expression	Sustained in 3/6 patients at 1 year	Sustained reduction in treatment requirement in some patients at 1 year
Fabry disease	NCT04519749	4D-310	GLA	4D-A101	$1x10^{13}$	Intravenous	4D Molecular Therapeutics	I/II	3	Increase in GLA activity	Sustained at up to 37 weeks	Sustained improvement in cardiac endpoints at up to 6 months
Fabry disease	NCT04046224	ST-920	GLA	AAV2/6	$0.5x10^{13}$ / $1x10^{13}$	Intravenous	Sangamo Therapeutics	I/II	4	Increase in GLA activity	Sustained at up to 1 year	Sustained improvement in symptoms in 3/4 patients at up to 1 year
Fabry disease	NCT04040049	FLT190	GLA	AAV8	$7.5x10^{11}$	Intravenous	Freeline Therapeutics	I/II	1	Increase in GLA expression	Sustained at up to 2 years	No sustained improvement at up to 2 years
Pompe disease	NCT03533673	ACTUS-101	GAA	AAV2/8	Undisclosed	Intravenous	Asklepios BioPharmaceutical, Inc.	I/II	3	Increase in GAA activity	Sustained at up to 6 months	Variable at 6 months
MPS type VI	NCT03173521	AAV2/8.TBG. hARSB	ARSB	AAV2/8	$6x10^{11}$ / $2x10^{12}$ / $6x10^{12}$	Intravenous	Fondazione Telethon	I/II	9	Increase in ARSB activity	Sustained at up to 1 year; dose dependent increase	Sustained reduction in treatment requirement at up to 1 year in high dose cohort (data not reported for other doses)
Phenylketonuria	NCT03952156	HMI-102	PAH	AAVHSC15	$2x10^{13}$ / $6x10^{13}$ / $1x10^{14}$	Intravenous	Homology Medicines, Inc	I/II	6	Increase in PAH activity	Sustained in 2/6 patients (mid and high dose) at up to 1 year	Not reported
Glycogen storage disease	NCT03517085	DTX401	G6Pase	AAV8	$2.0x10^{12}$ / $6.0x10^{12}$	Intravenous	Ultragenyx Pharmaceutical Inc	I/II	9	Not reported	Not reported	Sustained improvement in glucose control at up to 3 years
Ornithine transcarbamylase deficiency	NCT02991144	DTX301	OTC	AAV8	$3.4x10^{12}$ / $1x10^{13}$ / $1.7x10^{13}$	Intravenous	Ultragenyx Pharmaceutical Inc	I/II	9	Not reported	Not reported	Sustained improvement in ammonia control in 6/9 patients at up to 4 years

ARSB, arylsulfatase B; G6Pase, glucose-6-phosphatase; GAA, acid alpha-glucosidase; GLA, alpha-galactosidase A; MPS, mucopolysaccharidosis; OTC, ornithine transcarbamylase; PGBD, porphobilinogen deaminase; PAH, phenylalanine hydroxylase

Muscle-Directed Therapies

A range of different muscular diseases have been targeted with AAV, perhaps because several AAV serotypes can effectively transduce different types of muscle [6]. Treating many of these muscular diseases, particularly muscular dystrophies, requires lifelong production of a therapeutic protein. Healthy myocytes are capable of proliferation. However, the rate at which this occurs is markedly elevated in diseases such as Duchenne Muscular Dystrophy (DMD), which is characterized by progressive cycles of muscle degeneration and regeneration [6]. It is therefore expected that, at least in theory, transgene expression may decline over time. Examining the available clinical data will identify whether the current generation of therapies can provide long-term benefit.

19 different AAV gene therapy trials targeted at muscle fit our criteria. To allow for representative comparison of different studies, we have grouped therapies according to disease indication. Disease indications that have <5 trials have been grouped under 'other'.

There were some key observations across studies:

- Gene therapies for muscular dystrophies produce mostly durable outcomes, though the follow-up periods are limited.
- There were not many instances of short-term declines in expression, despite relatively high doses compared to therapies for other target tissues.
- AAV9 and AAVrh74 produce durable gene expression, though it is not known whether this persists longer term.
- There is a lack of substantial evidence for durable gene expression in muscle indications outside of muscular dystrophy.

Muscular Dystrophies

Since the defective proteins in various types of muscular dystrophy are often closely associated, we grouped studies targeting different muscular dystrophies together. A total of 11 studies reported data on durability, according to our criteria. 10/11 studies reported data on transgene expression over time, whilst 1 study only reported data on clinical outcomes. 9/10 studies reported comprehensive

durability of expression at the latest follow-up visit. Only 1 study reported a lack of expression in 1 patient. Overall, this suggests that gene expression in muscle persists long-term. However, the duration of follow-up was limited, with a median period of 5 months. Longer follow-up is required to determine whether transgene expression is permanent, especially since the mean age of study participants was ≤10 in 70% of trials with available data. However, it is important to note that muscular dystrophy quickly manifests in a severe phenotype - the median life expectancy of DMD patients is estimated to be just 22 years [7]. Therefore, gene therapy could still provide significant clinical value even if its effects are not lifelong.

We have included 5 studies that reported sustained transgene expression less than 6 months post-treatment. These studies still fit our criteria, as they reported clinical outcomes after 6 months. 3 of these studies reported sustained clinical benefit in all patients at the last follow-up visit. This suggests that transgene expression was also sustained, as we observed a correlation between sustained expression and clinical outcomes across muscular dystrophy trials.

The only report of a lack of transgene expression was in a Phase 1 study of SRP-9004 for limb girdle muscular dystrophy (LGMD). Expression could not be detected at 6 months in 1 of 3 patients. The study concluded that expression was probably lost shortly after gene transfer, as the patient was observed to have a rapid immune response to AAV. This demonstrates the importance of individual immune responses in achieving durable gene expression, supporting the need for careful selection of participants to receive a therapy.

We observed that high doses of vector were used in most studies, perhaps because many muscles around the body need to be transduced. The toleration of high doses in muscle may at least partly explain the durable treatment effect observed across most studies. Furthermore, either AAV9 or AAVrh74 were used in 91% of studies. Broadly positive results indicate that both these serotypes transduce muscle well and produce sustained expression of a range of different transgenes.

Though 90% of studies that are included in our analysis reported sustained transgene expression, it is important to note that 60% of studies had <1 year follow-up. Though the available data is still promising, a total of just 20 patients across 4 studies were shown to have sustained gene expression >1 year after treatment. Longer term monitoring of a great number of patients, particularly as they grow into adulthood, will determine the ability of AAV to provide a one-time treatment for this group of diseases.

Other Muscle-Directed Therapies

There were 5 disease indications that had <5 clinical studies containing data on durability, according to our criteria. Although these indications are distinct, the therapies studied primarily target muscle tissue. 7 of these studies reported data on the durability of transgene expression. As before, we have also included a single study that reported data on clinical outcomes over time, due to the observed correlation between transgene expression and clinical outcomes.

3/7 studies reported comprehensive durability of transgene expression at the latest follow-up visit. 4/7 studies reported a lack of durable expression in ≥1 patient, 1 of which reported a complete loss of expression in the sole patient followed up. However, there were limited reports of declining expression over time. It is therefore possible that a lack of durable expression in some patients is due to low initial expression levels rather than declines over time.

Though it is difficult to make definitive comparisons across different disease indications, it is notable that 2 of the 3 trials reporting comprehensive durability of expression used the highest doses. However, this may be because these studies used intravenous administration which might require higher doses to achieve therapeutic expression levels.

The third study that reported durable transgene expression demonstrated long-term (5 years) expression in muscle. Interestingly, this Phase 2 study of AGTC-0106 is the first gene therapy to report such results without using immunosuppression. Though there was evidence of a persistent immune response at up to 5 years, this did not appear to have a significant effect on expression levels. Expression levels were mostly sub-therapeutic, so it is not known whether similar durability would be observed with higher doses. Nonetheless, this suggests that immunosuppression might not always be essential to maintain expression long-term.

There were 5 studies of therapies that primarily target cardiomyocytes (heart failure and Danon disease studies). Due to their inability to replicate, cardiomyocytes may produce durable gene expression if transduced successfully [8]. Clinical outcomes were positive in high dose patients in a Phase 1/2 study of MYDICAR, prompting further investigation of the therapy in Phase 2 studies. However, these further studies failed to demonstrate similar clinical improvements. Notably, a large Phase 2 study showed that vector DNA was only present at very low levels, perhaps explaining the lack of therapeutic effect across 121 treated patients. It is therefore possible that the positive outcomes in the Phase 1/2 trial were due to chance, since only 9 patients showed clinical improvement. It was also suggested that the ratio of full (containing the transgene) to empty viral capsids used in the Phase 2 study may have affected gene transfer and therefore transgene expression [9]. Thus, it remains unclear whether long-term expression in cardiomyocytes can be consistently achieved.

Further evidence of durability is required to show whether AAV vectors can produce durable transgene expression in muscle indications outside of muscular dystrophies. This would unlock significant potential for the treatment of a vast array of rare diseases affecting muscle, in addition to common polygenic disorders such as heart failure.

Table 4. Muscular Dystrophies

Disease indication	Trial Identifier	Drug Name	Gene Delivered	Vector Serotype	Vector Dose	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Durability Endpoint	Durability Endpoint - Comments	Clinical Outcomes (Improved Motor Function)
BMD	NCT01519349	AAV1-Follistatin	Follistatin	AAV1	3×10^{11} / 6×10^{11} vg/kg/leg	Intramuscular	Nationwide Children's Hospital	I	6	Not reported	Not reported	Variable at up to 1 year
DMD	NCT04240314	AT702	U7snRNA	scAAV9	$3.0x10^{13}$ vg/kg	Intravenous	Nationwide Children's Hospital	I/II	2	Increase in dystrophin expression	Sustained at 3 months	Sustained in 1/2 patients at up to 1 year
DMD	NCT04626674	SRP-9001	Mini-dystrophin	AAVrh74	$1.33x10^{14}$ vg/kg	Intravenous	Sarepta & Roche	I	11	Increase in Mini-dystrophin expression	Sustained at 12 weeks	Sustained at up to 6 months
DMD	NCT03769116	SRP-9001	Mini-dystrophin	AAVrh74	$6.29x10^{13}$ / $8.94x10^{13}$ / $1.33x10^{14}$ vg/kg	Intravenous	Sarepta & Roche	II	20	Increase in Mini-dystrophin expression	Sustained at 12 weeks	Sustained at up to 48 weeks
DMD	NCT03362502	Fordadistrogene movaparvovec	Mini-dystrophin	AAV9	$1x10^{14}$ / $3x10^{14}$ vg/kg	Intravenous	Pfizer	I	19	Increase in Mini-dystrophin expression	Sustained at 1 year	Sustained at up to 1 year
DMD	NCT03375164	SRP-9001	Mini-dystrophin	AAVrh74	$2x10^{14}$ vg/kg	Intravenous	Sarepta	I/II	4	Increase in Mini-dystrophin expression	Sustained at 3 months	Sustained at up to 3 years
DMD	NCT03368742	SGT-001	Mini-dystrophin	AAV9	$5x10^{13}$ / $2x10^{14}$ vg/kg	Intravenous	Solid Biosciences	I/II	3	Increase in Mini-dystrophin expression	Sustained at up to 2 years	Sustained at up to 18 months
DMD	NCT03333590	rAAVrh74.MCK.GALGT2	B4GALNT2	AAVrh74	$5x10^{13}$ / $1x10^{14}$ vg/kg	Intravascular	Nationwide Children's Hospital	I/II	2	Increase in GALGT2 expression	Sustained at 4 months	Sustained at up to 2 years in high dose but not low dose subject
LGMD	NCT00494195	SRP-9004	α-SG	AAVrh74	3.25×10^{11} vg	Intramuscular	Nationwide Children's Hospital	I	3	Increase in α-SG expression	Sustained in 2/3 patients at 6 months	Not reported
LGMD	NCT01976091	SRP-9004	α-SG	AAVrh74	$1x10^{12}$ / $3x10^{12}$ vg/kg	Undisclosed	Nationwide Children's Hospital	I/II	6	Increase in α-SG expression	Sustained at 6 months	Not reported
LGMD	NCT03652259	SRP-9003	SGCB	AAVrh74	$1.85x10^{13}$ / $7.41x10^{13}$ vg/kg	Intravenous	Sarepta Therapeutics	I/II	6	Increase in SGCB expression	Sustained at up to 2 years	Sustained at up to 2 years

α-SG, alpha-sarcoglycan; BMD, Becker muscular dystrophy; B4GALNT2, Beta-1,4-N-Acetyl-Galactosaminyltransferase 2; DMD, Duchenne muscular dystrophy; SGCB, beta-sarcoglycan; U7snRNA, U7 small nuclear RNA.

Table 5. Other Muscle-Directed Therapies

Disease indication	Trial Identifier	Drug Name	Gene Delivered	Vector Serotype	Vector Dose	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Durability Endpoint	Durability Endpoint - Comments	Clinical Outcomes
X-linked myotubular myopathy	NCT03199469	AT132	MTM1	AAV8	1×10^{14} / 3×10^{14} vg/kg	Intravenous	Astellas Gene Therapies (Audentes Therapeutics)	I/II	23	Increase MTM1 expression	Sustained at up to 48 weeks	Sustained improvements in neuromuscular function at up to 48 weeks
Alpha-1 antitrypsin deficiency	NCT01054339	AGTC-0106	M-AAT	AAV1	$6x10^{11}$ / $1.9x10^{12}$ / $6x10^{12}$ vg/kg	Intramuscular	Applied Genetic Technologies Corporation	II	9	Increase in M-AAT expression	Sustained at up to 5 years; dose-dependent increase	Not reported
Lipoprotein lipase deficiency	NCT01109498	AMT-011	LPL	AAV1	3×10^{11} / $1x10^{12}$ vg/kg	Intramuscular	Amsterdam Molecular Therapeutics	II/III	14	Increase in LPL expression	Sustained in 4/7 subjects at 6 months	Sustained improvement in most patients at up to 2 years
Heart failure	NCT01966887	MYDICAR	SERCA2a	AAV1	1×10^{13} particles	Intracoronary	Assistance Publique - Hôpitaux de Paris & Celladon	II	5	Detection of vector DNA	Not detected at 1.5 years (n=1)	No sustained improvements in cardiac remodelling at 6 months
Heart failure	NCT00534703	MYDICAR	SERCA2a	AAV1	1×10^{13} particles	Intracoronary	Imperial College London	II	4	Detection of vector DNA	Detected at very low levels in 1/2 patients at 22 months; declined in one patient	No sustained improvements in cardiac function at up to 3 years
Heart failure	NCT00454818	MYDICAR	SERCA2a	AAV1	$1.4x10^{11}$ / $6x10^{11}$ / $3x10^{12}$ / $1x10^{13}$ particles	Intracoronary	Celladon Corporation	I/II	37	Not reported	Not reported	Sustained improvements in cardiac function at up to 12 months only in high dose patients (n=9)
Heart failure	NCT01643330	MYDICAR	SERCA2a	AAV1	1×10^{13} particles	Intracoronary	Celladon Corporation	II	121	Detection of vector DNA	Detected at very low levels at up to 29 months	No sustained improvements in disease progression at up to 29 months
Danon disease	NCT03882437	RP-A501	LAMP2B	AAV9	$6.7x10^{13}$ / $1.1x10^{14}$ vg/kg	Intravenous	Rocket Pharmaceuticals Inc	I	6	increase in LAMP2B expression	Sustained at up to 1 year	Stabilization or reducton in severity of heart failure at up to 2 years

M-AAT, M-specific alpha-1 antitrypsin; MTM1, myotubularin; LAMP2B, lysosome-associated membrane protein 2 isoform B; LPL, lipoprotein lipase; SERCA2a, sarco/endoplasmic reticulum Ca2+-ATPase

CNS-Directed Therapies

Of the two primary mechanisms by which it is thought transgene expression is lost – immune responses and cell division – only one of these is pertinent for CNS-directed therapies. The rate of neuronal cell division is extremely limited compared to other cell types [10]. Therefore, AAV vector genomes should persist long-term in the absence of any detrimental immune responses. We have reviewed the available clinical data to determine whether therapies targeting the CNS provide long-term benefits.

34 different AAV gene therapy trials targeted at the CNS fit our criteria. To allow for representative comparison of different studies, we have grouped therapies according to disease indication. Disease indications that have <5 trials have been grouped under 'other'.

There were some key observations across studies:

- Long-term durability across multiple disease indications is evident, though sample sizes are often small.
- 51% of trials did not report data on transgene expression or activity, making it difficult to identify long-term declines in expression
- Durable outcomes are demonstrated across multiple dose levels, showing that a wide range of doses can produce long-term expression. However, peak expression levels are often dose-dependent.
- There did not appear to be a clear relationship between age and durability of response.

Aromatic L-Amino Acid Decarboxylase (AADC) Deficiency

A total of 5 clinical studies for AADC deficiency reported data on durability, according to our criteria. All studies reported data on AADC expression or activity over time, all of which reported durable outcomes. Clinical benefit was also observed in all studies, showing that transgene expression was sustained at therapeutic levels.

Durable outcomes were achieved despite the use of relatively low doses compared to other CNS disease indications, such as Batten disease or SMA. This indicates efficient transduction of cells by AAV2 as well as robust gene expression. This is supported by data from other CNS trials, as AAV2 was often associated with the use of low doses but durable outcomes.

Interestingly, outcomes were durable despite the youth of trial participants. This may be due to the inherently low rate of neuronal cell division, so vector genomes persist. Therefore, in contrast to other disease areas, age may not be a significant factor when evaluating which patients may be unsuitable for CNS-directed gene therapies.

Batten Disease

A total of 5 clinical studies for Batten disease reported data on durability, according to our criteria. This included 3 different types of Batten disease, each requiring a different transgene. Just 1 study reported data on transgene expression over time, whereas others only reported data on clinical outcomes. The one study with expression data demonstrated durable CLN2 expression for up to 1 year post-treatment. 3 of the other 4 studies reported that clinical benefit was sustained at the last visit, indicating persistence of transgene expression.

A range of doses were used across trials. Interestingly, a Phase 1/2 trial of AAVrh.10CUCLN2 used the AAVrh.10 serotype, which has the lowest seropositivity of the 12 most common AAV serotypes in humans [10]. This may explain why durable CLN2 expression was observed despite the use of relatively low doses.

Though 80% of studies reported durable outcomes up to 2 years post-treatment, durability after 2 years was a concern in a long-term follow-up study of patients receiving AT-GTX-501. Stabilization of disease progression was not sustained, suggesting that transgene expression declined. This recently prompted Amicus Therapeutics to discontinue the programme [11]. Hence, the extent of long-term transgene expression in Batten disease is still relatively unknown. Quantitative measurements of expression over time in future studies will help to solve this problem.

Mucopolysaccharidosis (MPS)

There were 6 clinical studies of AAV gene therapies for MPS that reported data on durability, according to our criteria. This included 4 different types of MPS, each requiring a different transgene. All 6 studies reported data on protein expression or activity over time. 3/6 studies reported comprehensive durability of expression at the last follow-up. However, 3/6 studies reported a lack of durable outcomes in ≥1 patient. This suggests that there is inter-patient variability in response to treatment.

Differences in the reported units make it difficult to determine the effect of dose on durability. However, it does seem as if direct delivery to the CNS can produce durable gene expression at lower doses compared to systemic delivery. Despite a relatively low dose, UniQure reported stable expression up to 5.5 years after treatment with AMT110 delivered via intracerebral injection. Lower doses may reduce the overall immune response to the vector, reducing the likelihood that it will be cleared by the immune system [2]. Interestingly, this study used a long-term immunosuppressive regimen which lasted through the entire duration of follow-up. This may at least explain the long-term gene expression observed, highlighting the impact of differences in immunosuppressive regimens on durability.

All trials using multiple doses demonstrated dose-dependent increases in protein activity. In the long-term, this may have an impact on durability. If peak activity is higher, it may be sustained above a therapeutic threshold for a longer period. This dose-dependent effect was demonstrated in a Phase 1/2 trial of ABO-101 – NAGLU activity was sustained for longer in the high dose cohort. However, as we previously referred to, this response may differ between patients as higher doses can induce greater immune responses which also impact durability [2].

Durability of protein activity did not always translate to therapeutic effect. Clinical improvements were only sustained in 1 of 4 patients at 5.5 years post-treatment with AMT110, indicating that expression was sub-therapeutic. Interestingly, therapeutic effect was sustained in younger patients in studies of AMT110 and ABO-120. This opposes the notion that AAV gene therapies may be less durable in younger patients due to vector dilution. This may be due to inter-individual differences in immune responses, or because early gene replacement is essential before neurodegeneration is too advanced.

Parkinson's Disease

8 clinical studies of AAV gene therapies for Parkinson's disease reported data on durability, according to our criteria. This included trials for 3 different therapies, each delivering a unique transgene. Of these, 3 studies reported data on protein expression or activity over time, whereas others only reported data on clinical outcomes. All 3 of these studies reported durable outcomes, with a maximum follow-up of 4 years post-treatment. Though these results are promising, the other 5 studies reporting only clinical outcomes had mixed findings, indicating that gene expression did not persist in all patients.

One therapy, CERE-120, accounted for most studies reporting a lack of durable clinical outcomes. This may have been because NTN was not expressed at sufficient levels to provide therapeutic benefit. It is difficult to determine the reason for this, as different doses and routes of administration were used. However, we cannot rule out the possibility that restoring NTN expression in Parkinson's patients is insufficient to produce therapeutic effect. The other study reporting declining clinical benefit was a Phase I study of VY-AADC. Despite durable AADC expression at up to 4 years post-treatment, clinical improvements slowly declined from 1 year onwards. The observed disconnect between expression and clinical outcomes may have been due to the low doses used in the trial. Two subsequent trials of VY-AADC used higher doses and reported more durable clinical outcomes.

Durability of transgene expression and clinical outcomes in various Parkinson's disease studies provides promise to gene therapy developers aiming to target more common diseases. Although such diseases often don't have a monogenic cause, durable expression of a single transgene can produce long-term clinical benefits.

Spinal Muscular Atrophy (SMA)

No clinical studies for SMA reported data on the durability of transgene expression or protein activity over time. Therefore, we used clinical outcomes to measure durability. Although transgene expression or protein activity do not always translate to clinical outcomes, we observed a common relationship between both endpoints in our analysis.

A total of 7 studies reported data on the durability of clinical outcomes over time, all of which were studies of Novartis-AveXis's Zolgensma. All studies used improvements in motor function over time as an endpoint. For consistency, we used this as our measure of durability. It is worth noting that other endpoints were used that are not included in our analysis, such as survival without permanent mechanical ventilation.

Durable clinical benefit in at least some patients was observed in all trials. 5/7 studies reported a lack of clinical benefit in ≥1 patient, at various timepoints. However, there were limited reports of declining efficacy over time. Therefore, absence of gains in motor function in some patients may be due to a lack of efficacy rather than issues with durability. The durable therapeutic effect observed in most patients suggests that transgene activity is persistent up to 6 years after dosing.

Relatively high doses were used compared to other therapies targeted at the CNS. This was perhaps because all but one study used intravenous administration which might require higher doses to reach clinically relevant transgene expression levels. This might have contributed to high SMN1 expression levels and hence the durable clinical outcomes observed.

It has been suggested that dosing patients early on in disease progression has a positive impact on their response to treatment [12]. This opposes the idea that AAV therapies may be less beneficial in younger patients due to vector dilution during development. It will be interesting to see the extent of clinical benefit as patients grow into adulthood, particularly because there is no current data to determine whether transgene expression is declining in treated SMA patients.

Other CNS-Directed Therapies

There were 3 disease indications that had <5 clinical studies containing data on durability. Although these indications are distinct, the therapies studied all target the CNS. 1 of these studies reported data on the durability of gene expression. As before, we have also included studies that report data on clinical outcomes over time, due to the observed correlation between transgene expression and clinical outcomes.

UniQure's Phase 1/2 study for Huntington's disease is unique in that a positive outcome would equate to decreased HTT expression, rather than increased expression as in most studies. HTT expression was highly variable in the 2 patients followed up thus far. The small sample size makes it difficult to form any conclusions on the durability of the therapy. UniQure plan to release further data for more patients in the second quarter of 2022.

A Phase 2 trial of CERE-110 for Alzheimer's disease failed to demonstrate durable clinical benefit at up to 2 years post-treatment. This may have been due to a number of reasons such as the low dose used, unsuitable delivery method, or perhaps disease progression was too far advanced. In contrast, a Phase I trial of TSHA-120 for Giant Axonal Neuropathy demonstrated durable clinical outcomes at up to 3 years post-treatment. Notably, this study used high doses of AAV9, comparable to those in Zolgensma trials. Both these factors could have contributed to the durable outcomes observed.

Table 6. AADC Deficiency

Disease indication	Trial Identifier	Drug Name	Gene Delivered	Vector Serotype	Vector Dose (vector genomes)	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Durability Endpoint	Durability Endpoint - Comments	Clinical Outcomes (Improved Motor Function)
AADC deficiency	UMIN000017802	Ezaladcigene resoparvovec	DDC	AAV2	Undisclosed	Intracerebral	Jichi Medical University	I/II	6	Increase in AADC expression	Sustained at up to 2 years	Sustained at up to 2 years
AADC deficiency	jRCTs033180309	Ezaladcigene resoparvovec	DDC	AAV2	Undisclosed	Intracerebral	Jichi Medical University	I/II	8	Increase in AADC expression	Sustained at up to 5 years	Undisclosed
AADC deficiency	NCT02852213	Ezaladcigene resoparvovec	DDC	AAV2	1.3×10^{11} / 4.2×10^{11}	Intracerebral	Ohio State University	I	7	Increase in AADC activity	Sustained at up to 2 years	Sustained at up to 1.5 years
AADC deficiency	NCT01395641	Eladocagene exuparvovec	DDC	AAV2	1.8×10^{11}	Intraputaminal	National Taiwan University Hospital	I/II	10	Increase in AADC activity	Sustained at up to 5 years	Sustained at up to 5 years
AADC deficiency	NCT02926066	Eladocagene exuparvovec	DDC	AAV2	1.8×10^{11} / 2.4×10^{11}	Intraputaminal	National Taiwan University Hospital	II	8	Increase in AADC activity	Sustained at up to 5 years	Sustained at up to 5 years

AADC, aromatic l-amino acid decarboxylase; DDC, dopa decarboxylase

Tabe 7. Batten Disease

Disease indication	Trial Identifier	Drug Name	Gene Delivered	Vector Serotype	Vector Dose	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Durability Endpoint	Durability Endpoint - Comments	Clinical Outcomes (Reduced Rate of Neurological Decline)
Batten disease	NCT00151216	AAV2CUhCLN2	CLN2	AAV2	$2.5\text{x}10^{12}$ particle units	Intracranial	Weill Medical College of Cornell University	I	10	Not reported	Not reported	Sustained at up to 1.5 years
Batten disease	NCT01414985	AAVrh.10CUCLN2	CLN2	AAVrh.10	$2.85–9.0\times10^{11}$ vg	Intracerebral	Weill Medical College of Cornell University	I/II	8	Increase in TPP1 expression	Sustained at up to 1 year	Sustained at up to 1.5 years
Batten disease	NCT02725580	AT-GTX-501	CLN6	scAAV9	$1.5\text{x}10^{13}$ vg	Intrathecal	Amicus Therapeutics	I/II	13	Not reported	Not reported	Sustained at up to 2 years
Batten disease	NCT04273243	AT-GTX-501	CLN6	scAAV9	$1.5\text{x}10^{13}$ vg	Intrathecal	Amicus Therapeutics	LTFU	10	Not reported	Not reported	Initial stabilization at 2 years not sustained longer term
Batten disease	NCT03770572	AT-GTX-502	CLN3	scAAV9	6×10^{13} / 1.2×10^{14} vg	Intrathecal	Amicus Therapeutics	I/II	4	Not reported	Not reported	Sustained at up to 15 months

CLN, ceroid lipofuscinosis, neuronal; LTFU; long-term follow up: TPP1, tripeptidyl peptidase 1

Table 8. Mucopolysaccharidosis

Disease indication	Trial Identifier	Drug Name	Gene Delivered	Vector Serotype	Vector Dose	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Durability Endpoint	Durability Endpoint - Comments	Clinical Outcomes
MPS type I	NCT03580083	RGX-111	IDUA	AAV9	$1\text{x}10^{10}$ gc/g brain mass	Intracisternal	Regenxbio Inc.	I/II	5	Increase in IDUA activity	Sustained in 4/5 patients at up to 59 weeks	Sustained improvements in neurodevelopmental function at up to 20 months
MPS type II	NCT03566043	RGX-121	IDS	AAV9	$1.3\text{x}10^{10}$ / $6.5\text{x}10^{10}$ / $2.9\text{x}10^{11}$ gc/g brain mass	Intracisternal	Regenxbio Inc.	I/II	8	Increase in I2S activity	Sustained in majority of patients at up to 2 years; dose-dependent increase	Sustained improvements in neurodevelopmental function in some patients at up to 2 years
MPS type IIIA	NCT03612869	SAF-302	SGSH	AAVrh.10	$7.2\text{x}10^{12}$ vg	Intracerebral	Lysogene	II/III	16	Increase in SGSH activity	Sustained at 1 year	Not reported
MPS type IIIA	NCT02716246	ABO-102	SGSH	AAV9	$5\text{x}10^{12}$ / $1\text{x}10^{13}$ / $3\text{x}10^{13}$ vg/kg	Intravenous	Abeona Therapeutics	I/II	18	Increase in SGSH activity	Sustained at 2 years; dose-dependent increase	Sustained cognitive benefit only in younger patients (≤30 months) at up to 3 years
MPS type IIIB	NCT03315182	ABO-101	NAGLU	AAV9	$1\text{x}10^{13}$ / $5\text{x}10^{13}$ / $1\text{x}10^{14}$ vg/kg	Intravenous	Abeona Therapeutics	I/II	11	Increase in plasma NAGLU activity	Not sustained beyond 3 months (low dose) and 6 months (mid and high dose)	Not reported
MPS type IIIB	NCT03300453	AMT110	NAGLU	AAV2/5	$4\text{x}10^{12}$ vg	Intracerebral	UniQure Biopharma	I/II	4	Increase in NAGLU expression	Sustained at up to 5.5 years	Sustained improvements in neurocognitive development in 1/4 patients at up to 5.5 years

IDUA, alpha-L-iduronidase; IDS/I2S, iduronate-2-sulfatase; NAGLU, alpha-N-acetylglucosaminidase; SGSH, N-sulfoglucosamine sulfohydrolase

Table 9. Parkinson's Disease

Disease indication	Trial Identifier	Drug Name	Gene Delivered	Vector Serotype	Vector Dose (vector genomes)	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Durability Endpoint	Durability Endpoint - Comments	Clincal outcomes (Improved Motor Function)
Parkinson's disease	NCT00985517	CERE-120	NTN	AAV2	$9.4x10^{11}$ / $2.4x10^{12}$	Intranigral, Intraputaminal	Sangamo Therapeutics	I/II	30	Not reported	Not reported	No improvement vs controls at up to 2 years
Parkinson's disease	NCT00252850	CERE-120	NTN	AAV2	$1.3×10^{11}$ / $5.4x10^{11}$	Intrastriatal	Ceregene	I	12	Not reported	Not reported	Variable - sustained in some patients at up to 1 year
Parkinson's disease	NCT00400634	CERE-120	NTN	AAV2	$5.4x10^{11}$	Intracerebral	Ceregene	II	38	Not reported	Not reported	No improvement vs controls at up to 1 year
Parkinson's disease	NCT00195143	AAV-GAD	GAD	AAV2	Undisclosed	Undisclosed	NeuroLogix	I	12	Not reported	Not reported	Sustained at up to 1 year
Parkinson's disease	NCT00643890	AAV-GAD	GAD	AAV2	$7x10^{10}$	Subthalamic	NeuroLogix	II	22	Increase in GAD activity	Sustained at up to 1 year	Sustained at up to 1 year
Parkinson's disease	NCT01973543	VY-AADC	DDC	AAV2	$≤7.5x10^{11}$ / $≤1.5x10^{12}$ / $≤4.7x10^{12}$	Intrastriatal	Neurocrine Biosciences	I	15	Increase in AADC activity	Sustained at 6 months; dose-dependent increase	Sustained at up to 3 years
Parkinson's disease	NCT00229736	VY-AADC	DDC	AAV2	$9×10^{10}$ / $3×10^{11}$	Intrastriatal	Genzyme	I	10	Increase in AADC expression	Sustained at 4 years; dose-dependent increase	Improvements slowly declined from 1 to 4 years
Parkinson's disease	NCT03065192	VY-AADC	DDC	AAV2	$≤9.4×10^{12}$	Intrastriatal	Neurocrine Biosciences & Voyager Therapeutics	I	7	Not reported	Not reported	Sustained at up to 3 years

AADC, aromatic l-amino acid decarboxylase; DDC, dopa decarboxylase; GAD, glutamic acid decarboxylase; NTN, neurturin

Table 10. Spinal Muscular Atrophy

Disease indication	Trial Identifier	Drug Name	Gene Delivered	Vector Serotype	Vector Dose	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Durability Endpoint	Durability Endpoint - Comments	Clinical Outcomes (Improved Motor Function)
SMA	NCT03306277	Zolgensma	SMN1	AAV9	$1.1×10^{14}$ vg/kg	Intravenous	Novartis Gene Therapies (AveXis)	III	22	Not reported	Not reported	Sustained at up to 1 year in 13/22 patients
SMA	NCT03461289	Zolgensma	SMN1	AAV9	$1.1×10^{14}$ vg/kg	Intravenous	Novartis Gene Therapies (AveXis)	III	32	Not reported	Not reported	Sustained at up to 1 year in 14/32 patients
SMA	NCT03381729	Zolgensma	SMN1	AAV9	$6.0×10^{13}$ / $1.2×10^{14}$ / $2.4×10^{14}$ vg	Intrathecal	Novartis Gene Therapies (AveXis)	I	31	Not reported	Not reported	Sustained at up to 9 months in majority of patients
SMA	NCT03505099	Zolgensma	SMN1	AAV9	$1.1×10^{14}$ vg/kg	Intravenous	Novartis Gene Therapies (AveXis)	III	29	Not reported	Not reported	Sustained at up to 14 months in 19/29 patients
SMA	NCT04042025	Zolgensma	SMN1	AAV9	Undisclosed	Intravenous	Novartis Gene Therapies (AveXis)	4	6	Not reported	Not reported	Sustained at up to 17 months
SMA	NCT02122952	Zolgensma	SMN1	AAV9	$6.7×10^{13}$ / $1.1×10^{14}$ vg/kg	Intravenous	Novartis Gene Therapies (AveXis)	I	15	Not reported	Not reported	Sustained at up to 2 years in most patients (data only available for high dose cohort)
SMA	NCT03421977	Zolgensma	SMN1	AAV9	$6.7×10^{13}$ / $1.1×10^{14}$ vg/kg	Intravenous	Novartis Gene Therapies (AveXis)	U	13	Not reported	Not reported	Sustained at up to 6 years (data only available for high dose cohort)

SMN1, survival of motor neuron 1

Table 11. Other CNS-Directed Therapies

Disease indication	Trial Identifier	Drug Name	Gene Delivered	Vector Serotype	Vector Dose	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Durability Endpoint	Durability Endpoint - Comments	Clinical Outcomes
Alzheimer's disease	NCT00876863	CERE-110	NGF	AAV2	$2.0×10^{11}$ vg	Intracerebral	Sangamo Therapeutics	II	26	Not reported	Not reported	No sustained reduction in rate of cognitive decline at up to 2 years
Giant axonal neuropathy	NCT02362438	TSHA-120	GAN	scAAV9	$3.5x10^{13}$ / $1.2x10^{14}$ / $1.8x10^{14}$ / $3.5x10^{14}$ vg	Intrathecal	National Institute of Neurological Disorders and Stroke	I	14	Not reported	Not reported	Sustained improvements in motor function at up to 5 years
Huntington's disease	NCT04120493	AMT-130	HTT	AAV5	$6x10^{12}$ / $6x10^{13}$ vg	Intrastriatal	UniQure Biopharma	I/II	2	Decrease in HTT expression	Highly variable and inconclusive at 1 year	Not reported

GAN, gigaxonin; HTT, huntingtin NGF, nerve growth factor

Ocular-Directed Therapies

In the context of durability, the eye has some key distinctions from the previous target tissues. Firstly, it is a compartmentalized system, meaning that AAV vector administration is more targeted. Secondly, the eye has immune privileged status. This might mean that there is a reduced immune response to the vector, increasing its persistence in ocular tissues [13]. One may therefore expect that AAV gene therapies for ocular disorders exhibit greater durability relative to other indications. We have reviewed all available clinical data to determine whether this expectation is sufficiently evidenced.

42 different studies comprising a range of different disorders fit our criteria. However, outcome measures used in ocular studies varied significantly compared to other target tissue types. <10% of studies reported data on transgene expression over time. As before, we have also included all studies that only reported data on clinical outcomes at least 6 months post-treatment. This included various visual outcomes, which we have summarised as either stable or improved visual function. To account for the lack of data on transgene expression, we will be using this clinical data more extensively in our analysis of durability. This may mean that positive outcomes were reported for durability, but we cannot be sure whether gene expression was declining over time.

There were some key observations across studies:

- There was significant variation in the durability data for different disease indications.
- Ocular disorders tend to require lower doses to produce durable therapeutic effect.
- Route of administration may impact the therapeutic response and durability, though we found no consistent evidence of this across all trials.
- Early treatment intervention seemed to produce more positive outcomes.
- Durable gene expression and therapeutic effect is possible for common, acquired disorders.

Age-related Macular Degeneration (AMD)

6 clinical studies for AMD reported data on clinical outcomes at least 6 months after treatment. Furthermore, AMD was the only disease indication where some studies reported data on protein expression. This may be due to fundamental differences in the transgenes used for AMD. Instead of replacing a defective gene with a functional copy, the transgene encodes a protein that acts to inhibit the disease process. We have included data for clinical outcomes as well as protein expression in our analysis. For consistency with other ocular studies, we have only colour coded clinical outcomes based on our criteria.

3/6 studies reported comprehensive durability of therapeutic effect. The other 3/6 studies reported a lack of durable therapeutic effect in ≥1 patient. We cannot rule out that some of these cases were due to a lack of efficacy rather than durability issues, since there were limited reports of declining outcomes over time. However, a slight decline in VEGFR1 expression from 6 months to 1 year in a Phase 1 trial of AAV2-sFLT01 indicates that long-term durability is uncertain in some patients.

There did not appear to be any relationship between durability and dose, vector serotype or route of administration across trials. It might be expected that subretinal administration would produce the most durable outcomes, as this method delivers the transgene to the immune privileged compartment [14]. In theory, this should lead to a reduced immune response to the vector so that it continues to persist. However, we observed no correlation between subretinal delivery and greater durability in AMD.

As we previously discussed, dose is dependent on multiple factors such as vector serotype and route of administration. We have observed various cases within individual studies, such as hemophilia B trials, where higher doses lead to higher protein activity. This was also the case in a Phase 1/2 trial of RGX-314. However, despite lower transgene expression, therapeutic effect was more profound in mid-dose subjects rather than high dose subjects. Though this may be due to limited sample sizes, it suggests that higher doses might not always generate the best outcomes.

These results should provide promise to developers of ocular disease therapies as they demonstrate that durable treatment effect is possible in common acquired disorders that don't have a monogenic cause.

Choroideremia

7 clinical studies of AAV gene therapies for choroideremia reported data on clinical outcomes at least 6 months post-treatment, all of which were studies of the AAV2-based BIIB111. All studies used changes in visual function as an endpoint. Only 1 of these studies reported comprehensive durability of therapeutic effect, which was only at 6 months post-treatment. 6 studies reported a lack of durable therapeutic effect in ≥1 patient, 2 of which reported no improvement at the latest follow-up visit. However, we cannot be certain whether these results are due to a lack of initial efficacy or durability issues, due to the lack of transgene expression data over time. For example, Biogen's Phase 3 study of 169 choroideremia patients reported a lack of efficacy at 12 months, but we cannot confirm whether this was this was due to declining expression.

As shown in Table 13, a small number of patients demonstrated a durable therapeutic response. It appears unlikely that differences in therapeutic response amongst patients are due to dose, as multiple studies reporting different results used equivalent doses. Different responses may be due to differences between patients and their disease progression, indicating a need for careful selection of participants. It has also been reported that there are inconsistencies in subretinal delivery in choroideremia [15], which could also account for the variable results observed. Biogen are yet to report retrospective data from their Phase 3 trial. Data from such a large sample size may indicate where future trial or vector modifications can be made so that choroideremia gene therapy is efficacious and durable.

Leber's Congenital Amaurosis (LCA)

A total of 9 clinical studies for LCA reported data on clinical outcomes at least 6 months post-treatment. All studies reported changes in visual function as an endpoint. 3/9 studies reported comprehensive durability of therapeutic effect, up to a maximum of 5 years post-treatment. However, 6/9 studies reported a lack of durable therapeutic effect in ≥1 patient. There were various reports of long-term declines in therapeutic effect, perhaps because of slow declines in transgene expression over time.

As shown in Table 14, all 3 studies reporting comprehensive durability of therapeutic effect were of Spark's Luxturna. Durability appeared to be unrelated to vector dose, as other studies used higher doses of AAV2 and demonstrated outcomes that were less durable. However, durability could be at least partly related to the vector construct itself, which was optimized in several ways to enhance transgene expression and reduce immune responses [16]. This may mean that high levels of RPE65 are produced long-term, perhaps explaining the long-term therapeutic effect compared to other trials. Although it is difficult to determine the reasons for differences in durability between trials, it was suggested that differences in vector features as well as surgical procedures may be contributing factors [16].

It has been suggested that younger patients with LCA may benefit more from treatment, in contrast to other disease indications such as hemophilia. This may be because disease progression is less advanced in these patients [16], perhaps meaning that irreversible molecular changes have not yet occurred. As shown in Table 14, this is reinforced by the Phase I/II study of AGTC rAAV2-CB-hRPE65, where clinical benefit was only maintained in paediatric patients.

There appears to be a range of factors that influence the durability of therapies for LCA. However, the long-term therapeutic benefit observed in patients receiving Luxturna provides evidence that durability is achievable for developers of therapies targeting RPE65 mutations. It remains to be seen whether improvements in vision are permanent, especially since we cannot be sure whether expression is waning over time.

Leber's Hereditary Optic Neuropathy (LHON)

7 clinical studies for LHON reported data on clinical outcomes at least 6 months post-treatment. All studies reported changes in visual function as an endpoint. 5/7 studies reported comprehensive durability of therapeutic effect. 2/7 reported a lack of durable therapeutic effect in ≥1 patient, 1 of which reported no improvement in all patients followed up for 6 months post-treatment. Overall, these results demonstrate that current gene therapies for LHON mostly produce long-term clinical benefit up to 7.5 years post-treatment – the longest duration of follow-up we observed across all AAV gene therapy trials.

The studies reporting a lack of durable therapeutic effect in ≥1 patient did not report declining outcomes over time. Hence, these results may have been due to a lack of efficacy in the respective patients rather than issues with durability. Of note, the Phase 1 trial of University of Miami scAAV2-P1ND4v2 used a low dose of vector. This may account for the lack of clinical benefit observed at 6 months, although it is difficult to be sure of this due to the small sample size.

All five studies demonstrating comprehensive durability of therapeutic effect were of GenSight's GS010. This included four Phase 3 studies with large sample sizes relative to other ocular studies, allowing for more representative assessment of durability. Interestingly, results were mostly consistent across studies despite assessing patients with different times since disease onset. This is important because the therapy may be more broadly applicable to a greater proportion of LHON patients. However, clinical outcomes were still superior for those treated within one year of disease onset, highlighting the benefit of early diagnosis and intervention.

LHON is one of the most promising targets for ocular gene therapy, evidenced by durable outcomes using relatively low doses. GS010 is current under review by the European Medicines Agency, which may well lead to another breakthrough in gene therapy for a severe disorder with high unmet clinical need.

Retinitis Pigmentosa

We grouped various types of retinitis pigmentosa together, though they are a heterogenous group of disorders caused by a variety of defective genotypes. 7 clinical studies for retinitis pigmentosa reported data on clinical outcomes at least 6 months post-treatment. All studies reported changes in visual function as an endpoint. 4/7 studies reported comprehensive durability of therapeutic effect. The other 3/7 studies reported a lack of durable therapeutic effect in ≥1 patient. However, a lack of transgene expression data over time makes it difficult to determine whether these cases were due to a lack of initial efficacy or durability issues.

Noticeably, durability was observed despite differences in disease severity amongst different types of retinitis pigmentosa. XLRP is the most severe form of the disease, and durable outcomes in at least some patients were observed across all 4 XLRP studies. This suggests that AAV gene therapy for retinitis pigmentosa can be exploited against a range of phenotypes.

Various vector serotypes, both wildtype and engineered, were used across trials. Interestingly, 3 of the 4 studies reporting comprehensive durability of therapeutic effect used novel engineered capsids (AAV2tYF, rAAV2.7m8 and 4d-R100). These capsids may have enhanced properties such as enhanced cell transduction or transgene expression, which could have contributed to the durable outcomes observed.

Retinitis pigmentosa has the greatest number of ongoing clinical trials amongst all inherited retinal diseases. However, the longest duration of follow-up of outcomes across trials was only 2 years. Longer term

follow-up is necessary to determine whether improvements in visual function persist.

Other Ocular-Directed Therapies

4 diseases indications had <5 studies with data on durability, according to our criteria. This included 6 different trials, each reporting data on clinical outcomes at least 6 months after treatment. 1/6 studies reported comprehensive durability of therapeutic effect. The other 5/6 studies reported a lack of durable therapeutic effect in ≥1 patient, 1 of which reported no clinical benefit. In some of these patients, this may have been because transgene expression declined over time.

Though some clinical benefit was sustained at 3 years in a Phase 1/2 trial of rAAV.hCNGA3 for achromatopsia, most endpoints were not met. This suggests that transgene expression was present at a low level, perhaps not high enough to produce major functional improvements. That may also be the reason for the lack of clinical benefit observed in some patients across other trials.

Interestingly, two of AGTC's programmes for achromatopsia used the same dose and vector serotype to deliver a different transgene, despite differences in clinical outcomes. The trial of AGTC-401 produced more positive outcomes in some patients. The study suggested that this may be because the defective gene in these patients causes a complete lack of protein, whereas patients receiving AGTC-402 present with non-functional protein [17]. The presence of non-functional protein may interact with the vector, perhaps explaining inferior clinical outcomes. This suggests that there may be differences in outcomes depending upon the type of genetic mutation.

There were 2 trials for acquired diseases (diabetic macular edema [DME] and diabetic retinopathy) with relevant data. The Phase 2 trial of ADVM-022 for DME reported outcomes that were less durable compared to a trial of the same therapy in AMD, despite using the same doses. The therapy was subsequently discontinued for used in DME [18]. This demonstrates that there are marked differences in efficacy and durability based on disease etiology, even for the same therapy.

Overall, there is a lack of evidence of durable outcomes for therapies targeting the disease indications in Table 17. However, each of these disease indications currently have a limited number of trials reporting data on durability. More studies with long-term follow-up will determine the potential scope of ocular gene therapies.

Conclusion

Overall, we cannot be certain of the length of persistence of episomal transgenes due to variable results across clinical studies. However, persistence seems to depend on a wealth of different factors related to the therapy itself. Across multiple disease indications, the dose level, vector serotype and route of administration all seemed to have an impact on long-term protein expression, perhaps due to how they affect the immune response. Furthermore, these factors may interact, and as such they must be synchronized in order to produce a durable response.

We also observed that patient-derived factors can impact the durability of response. Though, many of these factors were perhaps less important than we envisaged, as no specific patient demographic seemed to show a less durable response overall. That being said, variables such as individual differences in immunogenicity and stage of disease progression have major impacts on both efficacy and durability.

Ultimately, all of the above variables are either within a developer's control or, in the instance of patient-derived variables, can be measured. Therefore, it is important that gene therapy developers review the current clinical data to discern the characteristics of trials that show durability of response.

Table 12. Age-related Macular Degeneration

Disease indication	Trial ID	Drug Name	Gene Delivered	Vector Serotype	Vector Dose	Route of Administration	Sponsor	Phase	No. of Participants	Mean Age / Age Range	Clinical Outcomes (Stable/Improved Visual Function)	Durability Endpoint
Neovascular AMD	NCT04514653	RGX-314	anti-VEGF fab	AAV8	$2.5x10^{11}$ / $5x10^{11}$ vg/eye	Suprachoroidal	Regenxbio Inc.	II	15	75	Stable in 14 of 15 patients followed up at 6 months	Not reported
Neovascular AMD	NCT03066258	RGX-314	anti-VEGF fab	AAV8	$3x10^{9}$ / $1x10^{10}$ / $6x10^{10}$ / $1.6x10^{11}$ / $2.5x10^{11}$ gc/eye	Subretinal	Regenxbio Inc.	I/II	42	80	Stable or improved at 3 years in subjects that responded to treatment; dose-dependent response	Sustained increase in anti-VEGF fab expression at 2 years; dose-dependent increase
Neovascular AMD	NCT01024998	AAV2-sFLT01	VEGFR1	AAV2	$2x10^{8}$ / $2x10^{9}$ / $6x10^{9}$ / $2x10^{10}$ vg	Intravitreal	Genzyme	I	19	77 (median)	Sustained improvement in only a few patients at 1 year	VEGFR1 expression slightly declined from 6 months to 1 year in those who showed expression
Neovascular AMD	NCT03748784	ADVM-022	Aflibercept	AAV.7m8	$2x10^{11}$ / $6x10^{11}$ vg/eye	Intravitreal	Adverum Biotechnologies	I	30	77	Stable at up to 2 years	Sustained increase in Aflibercept expression at up to 2 years
Neovascular AMD	NCT01494805	AVA-201	VEGFR1	AAV2	$1x10^{11}$ vg	Subretinal	Lions Eye Institute	I/II	24	80 (median)	Sustained improvements in a few patients at 3 years but not statistically significant	Not reported
Dry AMD	NCT03846193	GT005	CFI	AAV2	$2x10^{10}$ / $5x10^{10}$ / $2x10^{11}$ vg	Subretinal	Gyroscope Therapeutics Limited	I/II	13	Undisclosed	Stable at 48 weeks	Sustained increase in CFI expression in 11/13 patients up to 84 weeks

Anti-VEGF fab, anti-vascular endothelial growth factor fragment antigen-binding; CFI, complement factor I; VEGFR1, vascular endothelial growth factor receptor 1

Table 13. Choroideremia

Disease indication	Trial ID	Drug Name	Gene Delivered	Vector Serotype	Vector Dose (vector genomes)	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Mean Age / Age Range	Clinical Outcomes (Stable/Improved Visual Function)
Choroideremia	NCT03496012	BIIB111	CHM	AAV2	Undisclosed	Subretinal	Biogen Idec, Nightstar Therapeutics	III	169	Undisclosed	No sustained improvement at 1 year
Choroideremia	NCT01461213	BIIB111	CHM	AAV2	$1x10^{11}$	Subretinal	University of Oxford	I/II	6	50	Stable at 6 months
Choroideremia	NCT02077361	BIIB111	CHM	AAV2	$1x10^{11}$	Subretinal	University of Alberta	I/II	6	Undisclosed	No sustained improvement at 1 year
Choroideremia	NCT02553135	BIIB111	CHM	AAV2	$1x10^{11}$	Subretinal	University of Miami	II	6	Undisclosed	Sustained improvement in only some patients at 6 months
Choroideremia	NCT03507686	BIIB111	CHM	AAV2	$1x10^{11}$	Subretinal	Biogen Idec, Nightstar Therapeutics	II	6	32-72	Sustained improvement in some patients at 2 years
Choroideremia	NCT02671539	BIIB111	CHM	AAV2	$1x10^{11}$	Subretinal	STZ eyetrial	II	6	55	Stable or improved in most patients at 2 years but no significant difference from control eyes
Choroideremia	NCT03584165	BIIB111	CHM	AAV2	Undisclosed	Subretinal	Biogen Idec, Nightstar Therapeutics	U	6	Undisclosed	Variable - improvements in only some measures in some patients at 5 years

Table 14. Leber's Congenital Amaurosis

Disease indication	Trial ID	Drug Name	Gene Delivered	Vector Serotype	Vector Dose	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Mean Age / Age Range	Clinical Outcomes (Stable/Improved Visual Function)
LCA	NCT03872479	EDIT-101	CEP290	AAV5	$6x10^{11}$ / $1.1x10^{12}$ vg/ml	Subretinal	Editas Medicine	I/II	6	41	Sustained improvement in some patients in high dose group but not others at 6 months
LCA	NCT00749957	AGTC rAAV2-CB-hRPE65	RPE65	AAV2	$1.8×10^{11}$ / $6×10^{11}$ vg/eye	Subretinal	Applied Genetic Technologies Corporation	I/II	12	25	Sustained improvement at up to 5 years only in 4 paediatric subjects; improvement in others declined from 2 years
LCA	NCT03920007	SAR439483	GUCY2D	AAV5	$3.3x10^{10}$ vg/ml	Subretinal	Atsena Therapeutics	I/II	3	34	Improvements in some measures at 9 months
LCA	NCT00516477	Luxturna	RPE65	AAV2	$1.5×10^{10}$ vg	Subretinal	Spark Therapeutics	I	12	20	Sustained improvement at 3 years
LCA	NCT01208389	Luxturna	RPE65	AAV2	$1.5x10^{11}$ vg	Subretinal	Spark Therapeutics	I/II	11	14	Sustained improvement at 4 years
LCA	NCT01496040	rAAV2/4.hRPE65	RPE65	AAV2/4	$1.22x10^{10}$ - $4.8x10^{10}$ vg	Subretinal	Nantes University Hospital	I/II	9	24	Variable - improvements in only some measures in some patients at 2 years
LCA	NCT00643747	tgAAG76	RPE65	AAV2	$1x10^{11}$ / $1x10^{12}$ vg	Subretinal	University College London	I/II	12	14	Variable - some improvements in some patients at 1 year which declined at 3 years
LCA	NCT00481546	AAV-2/2.RPE65	RPE65	AAV2	$5.96×10^{10}$ vg	Subretinal	University of Pennsylvania	I	3	20	Sustained improvements at 3 years deteriorated at up to 6 years
LCA	NCT00999609	Luxturna	RPE65	AAV2	$1.5x10^{11}$ vg/eye	Subretinal	Spark Therapeutics	III	25	15	Sustained improvements in those who responded to treatment at 5 years

CEP290, centrosomal Protein - 290 kDa; GUCY2D, guanylate cyclase 2D; RPE65, retinoid isomerohydrolase

Table 15. Leber's Hereditary Optic Neuropathy

Disease indication	Trial ID	Drug Name	Gene Delivered	Vector Serotype	Vector Dose	Route of Administration	Sponsor	Phase	No. of Patients Followed Up	Mean Age / Age Range	Clinical Outcomes (Improved Visual Function)
LHON	NCT03293524	GS010	ND4	AAV2	Undisclosed	Intravitreal	GenSight Biologics	III	98	Undisclosed	Sustained at 2 years in those subjects that responded to treatment
LHON	NCT02161380	University of Miami scAAV2-P1ND4v2	ND4	AAV2	5×10^{9} vg	Intravitreal	University of Miami	I	2	50	No improvement in patients followed up for 6 months
LHON	NCT03406104	GS010	ND4	AAV2	$9x10^{10}$ vg	Intravitreal	GenSight Biologics	III	61	35	Improvements at 2 years sustained at 4 years
LHON	NCT02064569	GS010	ND4	AAV2	$9x10^{9}$ / $3x10^{10}$ / $9x10^{10}$ / $1.8x10^{11}$ vg/eye	Intravitreal	GenSight Biologics	I/II	15	48	Sustained at 5 years
LHON	NCT02652767	GS010	ND4	AAV2	$9x10^{10}$ vg/eye	Intravitreal	GenSight Biologics	III	39	37	Sustained at 96 weeks in those subjects that responded to treatment
LHON	NCT01267422	NR082	ND4	AAV2	$1x10^{10}$ vg	Intravitreal	Huazhong University of Science and Technology	U	8	19	Sustained at up to 7.5 years in 6/8 patients
LHON	NCT02652780	GS010	ND4	AAV2	$9x10^{10}$ vg/eye	Intravitreal	GenSight Biologics	III	37	34	Sustained at 96 weeks in those subjects that responded to treatment

ND4, NADH dehydrogenase subunit 4

Table 16. Retinitis Pigmentosa (RP)

Disease indication	Trial ID	Drug Name	Gene Delivered	Vector Serotype	Vector Dose	Route of Administration	Sponsor	Phase	No. of Participants	Mean Age / Age Range	Clinical Outcomes (Improved Visual Function)
Advanced RP	NCT04919473	MCO-010	MCO1	AAV2	1.75×10^{11} / 3.5×10^{11} vg/eye	Intravitreal	Nanoscope Therapeutics	I/II	11	Undisclosed	Sustained at up to 1 year; dose-dependent response
RP	NCT01482195	rAAV2-VMD2-hMERTK	MERTK	AAV2	Undisclosed	Subretinal	King Khaled Eye Specialist Hospital	I	6	33	Sustained in some patients at 2 years
XLRP	NCT03316560	AGTC-501	RPGR	AAV2tYF (AAV2)	Undisclosed	Subretinal	Applied Genetic Technologies Corporation	I/II	29	Undisclosed	Sustained at 1 year in those who responded to treatment
RP	NCT03326336	GS030	ChrimsonRtd Tomato	AAV.7m8	$5x10^{10}$ / $1.5x10^{11}$ / $5x10^{11}$ vg	Intravitreal	GenSight Biologics	I/II	2	Undisclosed	Sustained at 1 year in those followed up
XLRP	NCT03252847	AAV-RPGR	RPGR-ORF15	AAV2/5	Undisclosed	Subretinal	MeiraGTx	I/II	7	24	Sustained at 1 year in 6/7 patients
XLRP	NCT04517149	4D-125	RPGR	4D-R100	$3x10^{11}$ / $1x10^{12}$ vg/eye	Intravitreal	4D Molecular Therapeutics	I/II	8	41	Sustained at up to 1 year in those followed up
XLRP	NCT03116113	BIIB112	RPGR	AAV8	5×10^{9} / 1×10^{10} / 5×10^{10} / 1×10^{11} / 2.5×10^{11} / 5×10^{11} gp	Subretinal	Biogen Idec, Nightstar Therapeutics	I/II	18	Undisclosed	Sustained in only some patients from 6 months to 1 year

MCO1, multicharacteristics opsin; MERTK, tyrosine-protein kinase mer; RPGR, retinitis pigmentosa GTPase regulator; XLRP, X-linked retinitis pigmentosa

Table 17. Other Ocular-Directed Therapies

Disease indication	Trial ID	Drug Name	Gene Delivered	Vector Serotype	Vector Dose	Route of Administration	Sponsor	Phase	No. of Participants	Mean Age / Age Range	Clinical Outcomes (Stable/Improved Visual Function)
Achromatopsia	NCT02610582	rAAV.hCNGA3	CNGA3	AAV8	1×10^{10} vg / 5×10^{10} 1×10^{11} vg	Subretinal	STZ eyetrial	I/II	9	40	Only some improvements which were sustained at year 3
Achromatopsia	NCT02935517	AGTC-402	CNGA3	AAV2	$4x10^{10}$ - $3.2x10^{12}$ vg/ml	Subretinal	Applied Genetic Technologies Corporation	I/II	20	51	No consistent evidence of improvements at 1 year (only some sporadic improvements)
Achromatopsia	NCT02599922	AGTC-401	CNGB3	AAV2	$4x10^{10}$ - $3.2x10^{12}$ vg/ml	Subretinal	Applied Genetic Technologies Corporation	I/II	25	40	Sustained improvement in some patients (high dose and paedeatric patients) at 1 year
Diabetic macular edema	NCT04418427	ADVM-022	Aflibercept	rAAV2.7m8 (AAV2)	$2x10^{11}$ / $6x10^{11}$ vg/eye	Intravitreal	Adverum Biotechnologies	II	34	60	Variable - improvements in only some measures in some patients at 36 weeks
Diabetic retinopathy	NCT04567550	RGX-314	anti-VEGF	AAV8	$2.5x10^{11}$ gc/eye	Suprachoroidal	Regenxbio Inc.	II	15	51	Stable in some patients at 6 months
X-linked retinoschisis	NCT02416622	BIIB-087	RS1	AAV2tYF (AAV2)	$1x10^{11}$ / $3x10^{11}$ / $6x10^{11}$ vg/eye	Intravitreal	Applied Genetic Technologies Corporation	I/II	27	Undisclosed	No improvements at 6 or 12 months

Anti-VEGF, anti-vascular endothelial growth factor; CNGA3, cyclic nucleotide gated channel subunit alpha 3; CNGB3, cyclic nucleotide gated channel subunit beta 3; RS1, retinoschisin

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Contact Us

Joe Moss
Market Analyst
Hanson Wade Intelligence
Email: joseph.moss@hansonwade.com

James Eslea-MacDonald
Group Director
Hanson Wade Intelligence
Email: james.eslea-macdonald@hansonwade.com

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