FROM THE ANALYST'S COUCH

The clinical landscape for AAV gene therapies

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Gene therapy using adeno-associated virus (AAV) as a vector has emerged as a novel therapeutic modality that has the potential to lead to substantial disease modification in many monogenic disorders, or perhaps even cures. Given the interest in the approach, which has been boosted by the recent approval of two AAV-based gene therapies by the US FDA, we have conducted a systematic review of the landscape of clinical trials of AAV-based gene therapies (see Supplementary Fig. 1 and Supplementary Table 1 for details). Here, we highlight the key trends and discuss the implications.

Analysis of AAV gene therapy trials

The analysis identified 149 unique clinical trials, 94 of which had been completed and 51 for which the efficacy end point was reached (Supplementary Fig. 2). The number of trials initiated annually increased from 5 in 2010 to 26 in 2017 (FIG. 1a). Most of the studies that reached primary completion were phase I/II studies with both safety and efficacy as their end points, with more than 80% of all studies backed by industrial sponsors (FIG. 1b). The average duration of studies seems to have decreased over time, possibly reflecting the establishment of the trial designs as well as the increased comfort of the regulators with the modality (Supplementary Fig. 3).

Capsids and cassettes. Analysis of the evolution of the use of AAV capsids (based on 144 out of 149 studies that disclosed the capsid) and promoters (based on 104 out of 149 studies that disclosed the promoter) showed that the originally engineered AAV2 serotype remains the most used throughout the study period (FIG. 1c) and has the most safety and efficacy evidence, with more than 40 completed trials. Since 2015, the number of trials of agents using AAV8 and AAV9 capsids for delivery to the central nervous system (CNS) has grown, reflecting the increased use of gene therapy for CNS diseases. Novel capsids such as AAV-LK03, SPK-100 and AAV-HSC15 have gained traction, but evidence of their safety and efficacy is limited, with a total of seven trials between them.

Similarly, the most used promoters, both historically and in recent years, are ubiquitous promoters with a demonstrated track record of efficiency, such as CBA, CAG and CMV (FIG. 1d). Between 2015 and 2019, 45% of trials that used constructs with disclosed promoters used one of these three. In recent years, three trends away from the use of ubiquitous promoters emerged. First, more than 25 trials reported using tissue-specific strong promoters such as albumin and synapsin to achieve expression restricted to a particular tissue. Second, improvements in cassette design and the ability to manufacture larger doses of AAV, with up to 2×10^{14} vg per kg tested in patients with no serious side effects, enabled the use of promoters native to the therapeutic gene. However, with recent research suggesting that high vector copy numbers of AAV9 can cause severe toxicity in animal models (*Hum. Gene Ther.* **29**, 285–298; 2018), it is unclear whether the use of native promoters relying on high vector copy numbers is sustainable. Biogen recently announced the termination of development of BIIB089, an investigational SMA1 gene therapy, citing similar toxicity concerns. Third, synthetic promoters designed to target genetically or functionally defined subsets of cells have shown success in animal studies, but no clinical trials using such promoters reached primary completion by the cut-off date.

Safety. We analysed all studies with available safety data ($n = 101$) and classified safety events as either administration-related, defined as arising within the 28-day period following administration of the therapy, or as treatment-emergent, attributed to the action of the transgene itself, the presence of the capsid, or the response of the patient's immune system to the transgene or the capsid (FIG. 2). For the 3,328 patients treated in trials in the analysis period, there were nine grade 4/5 serious adverse events (SAEs) deemed treatment-emergent (TESAEs) and no patient deaths attributed directly to the transgenes or capsids. No studies were

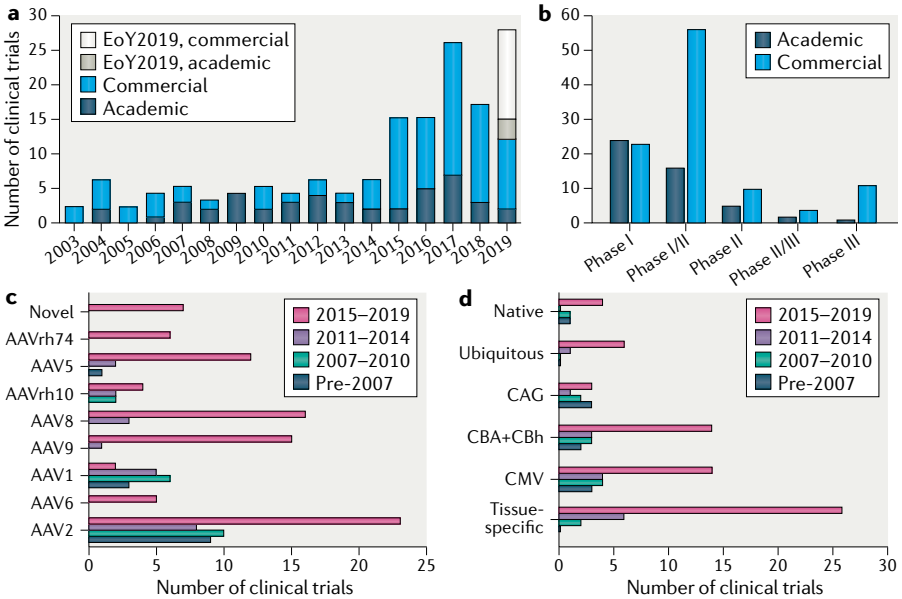

Fig. 1 | **Trends for AAV gene therapies in clinical trials. a** | Number of studies started per year. **b** | Number of clinical studies by phase. **c** | Adeno-associated virus (AAV) capsid type by period. **d** | Promoter use by period. See Supplementary Information for details on data and analysis. EoY, end of year.

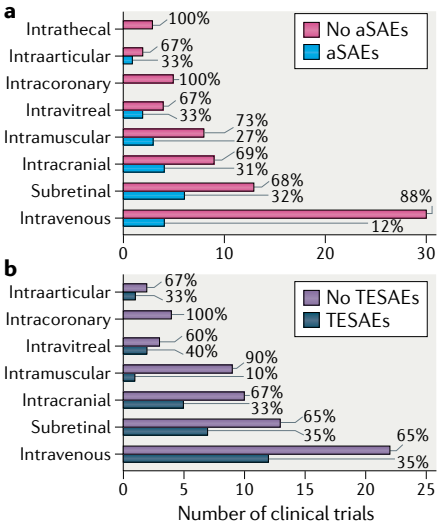

Fig. 2 | **Safety trends for AAV gene therapies by route of administration. a** | Administration-related serious adverse events (aSAEs; $n = 96$). **b** | Treatment-emergent serious adverse events (TESAEs; $n = 99$). See Supplementary Information for details on data and analysis.

terminated on safety grounds as of the cut-off date, and all completed studies reached their safety end points ($n = 51$). However, reports after the cut-off date of this study indicate that 3 of 17 patients who received the higher dose of an experimental gene therapy, AT132, for X-linked myotubular myopathy, died of progressive hepatobiliary disease and subsequent sepsis and gastrointestinal bleeding. An investigation into the potential role of the immune response to the capsid used is ongoing.

An average of 21% of all trials had grade 1–5 administration-related SAEs ($n = 96$ reporting administration safety). Intravenous and intrathecal administration were safer than most other procedures (FIG. 2a). Intracranial administration was on par with others in terms of SAE rate, but the SAEs experienced tended to be higher grade and more clinically significant. Nausea, injection site reactions and headaches were the most prevalent administration-related adverse events.

An average of 35% of all trials analysed had SAEs that were deemed TESAEs and not directly relatable to the administration of the therapy ($n = 99$ trials reporting treatment-emergent adverse events). Notably, intramuscular gene therapies resulted in the lowest TESAE counts (FIG. 2b). The data indicated no significant differences in administration-related SAEs by capsid type for the most commonly used capsids, AAV1 and AAV2, and that AAV1 might be marginally safer than AAV2 with regard to TESAEs (Supplementary Fig. 4).

Efficacy. Most of the 94 completed trials that were available for analysis focused on four organs: the eye, liver, muscles and the CNS (Supplementary Fig. 5). Intravenous gene therapies targeting the liver were shown to address both metabolic and haematological conditions. Parkinson disease as well as haemophilia A and B are the three indications with the most clinical trials over time.

Overall data on success rates are inevitably limited by the small numbers of agents. Based on the 11 agents that reached the stage of new drug application (NDA), the probability of progressing from an investigational new drug (IND) application to an NDA was 36%, and the median duration from an IND to an NDA was 86 months. The probabilities of success for gene therapies were higher than the historical averages across all five therapeutic areas with completed trials (TABLE 1). Haematological gene therapies had the highest overall probability of success (56% from IND to NDA). However, the number of completed trials is very low, and the investigational product closest to market, valoctocogene roxaparvovec, was recently refused registration by the US FDA, which requested longer-term efficacy data.

Outlook

Available clinical data, covering more than 3,000 patients treated over more than 20 years, indicate that AAV gene therapy is a safe, well-tolerated and efficacious modality. However, several major challenges remain unresolved. First, the durability of response to gene therapy is uncertain owing to a lack of lifelong follow-up. The issue of steroid use and the role of the immune response in durability of response remains unresolved (Supplementary Fig. 6). We view pushback by the regulators, resulting in longer pivotal studies needed to collect more robust follow-up data, as highly likely in conditions without rapid physiological decline, such as haemophilia and genetic diseases of the retina. Life-saving products in diseases such as spinal muscular atrophy will probably still benefit from shorter trials and rapid approval, provided they show convincing evidence of sustained improvement.

Second, the improvement in manufacturing led to an increase in the average doses of AAV used in clinical trials since 2015. Although this allows for the potential use of higher vector doses in diseases requiring high proportions of transduced cells in the body, most of the vector still ends up in the liver and can cause potential toxicity there and elsewhere. We expect better cassette engineering to be important in decreasing the viral load necessary to achieve comparable therapeutic effects.

Finally, our analysis shows that the proportion of trials for agents outside of the eye, liver, muscle and the CNS is low ($n = 11/94$). Major organs, such as the heart, the kidney and the lung, remain almost inaccessible to AAV gene therapies. An ongoing switch to engineered capsids and synthetic promoters, allowing the therapy to avoid the liver, escape immune oversight and make the transgene functional only in a narrowly defined subset of cells, is necessary to further advance the clinical significance of AAV gene therapy.

Table 1 | **Transition probabilities for gene therapies by phase**

Therapeutic area	Phase I	Phase I/II or phase II	Phase II/III or phase III	IND to NDA	IND to NDA for all drugs[a]
Ophthalmology	83% (5/6)	62% (8/13)	60% (3/5)	31%	24%
Neurology	73% (8/11)	56% (5/9)	67% (2/3)	30%	19%
Metabolic	NA	43% (3/7)	100% (2/2)	43%[b]	16%
Haematology	75% (3/4)	75% (6/8)	100% (1/1)	56%	47%
Musculoskeletal	38% (3/8)	60% (3/5)	NA	23%[b]	29%

[a]Historical success rates for any drug in this therapeutic area (source: Global Data). [b]No therapy completed the investigational new drug (IND) to new drug application (NDA) path successfully as of the cut-off date. See Supplementary Information for details. NA, not available.

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Competing interests
D.A.K. is a non-executive director of Redpin Therapeutics, which develops AAV gene therapies. O.P.S. is a non-executive director of SparingVision SA, which develops AAV gene therapies. J.C.M.v.d.L. is a consultant to 4BIO Capital. The other authors declare no competing interests.

Supplementary information
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