Adeno-Associated Virus Gene Therapy Landscape

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Adeno-associated viruses (AAV) are virus particles composed of single-stranded DNA surrounded by a protein shell. Despite their simple structure, recombinant AAVs (rAAV) can perform the important role of delivering nucleic acids into cells during gene therapy. Due to the high demand for potentially curative treatments in areas of huge unmet need and the growing availability of AAV vectors, a race to launch successful gene therapy products is underway. Companies such as Novartis and Spark Therapeutics already have approved gene therapy products and the field is growing, with at least 50 companies/institutions actively developing AAV gene therapy products (Figure 1 and Table 1).

Figure 1: Companies developing ≥5 AAV based gene therapies

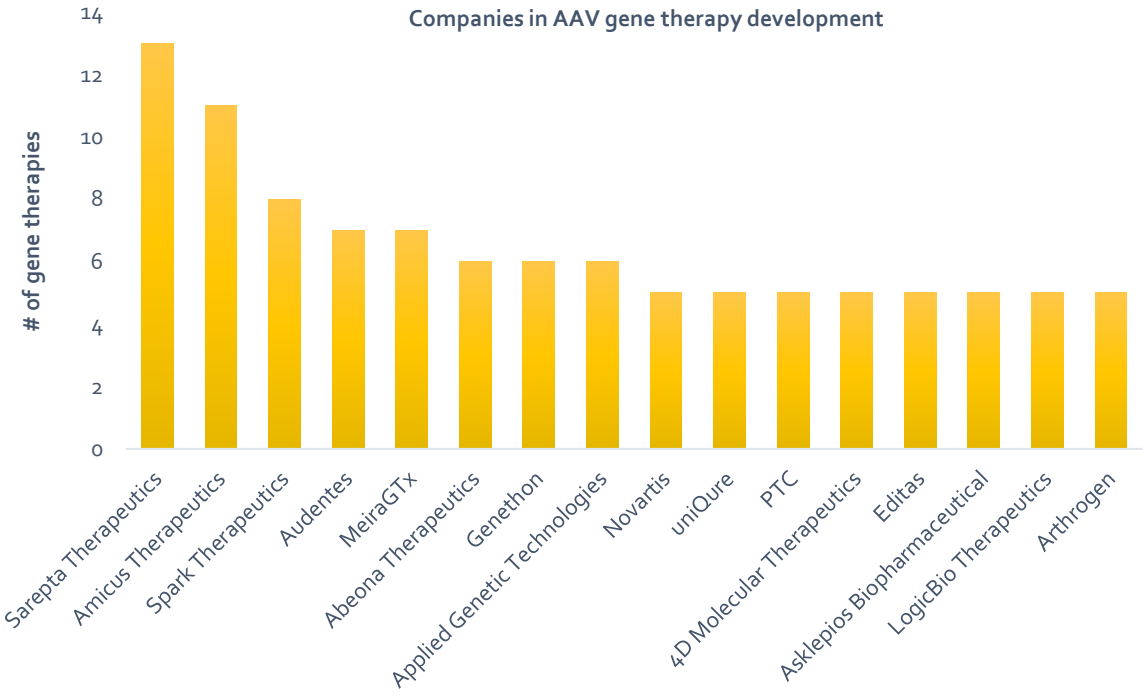

Table 1: Companies developing <5 AAV based gene therapies

Company	# gene therapies in each company
Pfizer	4
Vivet	
Ultragenyx	
Sangamo	
Freeline	
REGENXBIO	
Adverum Biotechnologies	
Voyager Therapeutics	
University of Massachusetts Medical School	
University of Pennsylvania	
University of Florida	3
Benitec Biopharma	
Passage Bio	
University College London	
Biogen	
Esteve	
BioMarin	2
Takeda	
Axovant Sciences	
Astellas Pharma	
Royal Free and University College Medical School	
University of Melbourne	
Fondazione Telethon	1
NIH	
Huazhong University of Science and Technology	
Gene Therapy Research Institution	
McGill University	
University of California San Francisco	
Janssen Pharmaceuticals	
Akouos	
University of Texas System	
Solid Bio	
Rocket Pharmaceuticals	
Prevail Therapeutics	

Advantages of using AAVs for gene therapy include their ability to infect both non-dividing and dividing cells, lack of pathogenicity and relatively poor immunogenicity. In addition, AAVs act episomally by behaving as separate extrachromosomal elements in the nucleus of target cells – this decreases their propensity to cause cancer. However, AAVs are not without their disadvantages. One limitation is their nucleic acid capacity, with AAVs having a genome size 5-10x smaller than adenoviruses or lentiviruses. Another non-trivial challenge facing effective and safe AAV gene delivery is the host immune response.

The immune response

The humoral immune response is particularly problematic for AAV gene therapy, with 96% of humans having antibodies against AAV, of which 32% neutralize AAVs completely (Chirmule et al., 1999). The presence of neutralizing antibodies is one of the major factors that led to early clinical failures in this approach (Greenberg et al., 2015). The issue is further complicated by site-specific immune responses to different AAV serotypes; hence when delivering an AAV based gene therapy, an understanding of the best route of administration for a specific AAV is required (Brockstedt et al., 1999).

There are four main strategies that can be adopted to address the presence of anti-AAV neutralizing antibodies. The first strategy involves using less-seroprevalent capsids or switching serotype. Boutin and colleagues demonstrated that the prevalence of anti-AAV1 and -AAV2 total IgG is higher (67% and 72% respectively) than that of anti-AAV5 (40%), anti-AAV6 (46%), anti-AAV8 (38%) and anti-AAV9 (47%) (Boutin et al., 2010). Serotype switching has been shown to be successful but is only a short-term solution that circumvents the challenge posed by anti-AAV neutralizing antibodies.

A second option would be to perform plasmapheresis. Plasmapheresis is the process by which neutralizing antibodies can be filtered from patient blood. This method has proven successful in reducing the titers of neutralizing factors for AAV types 1,2,6, and 8 in a sample of ten human patients (Monteilhet et al., 2011). In addition to addressing issues with primary infection with AAVs, this method can also be applied to patients requiring re-administration of vector. Limitations include the fact that the method requires multiple cycles of plasmapheresis and has been shown to be less efficient in patients with high titers of neutralizing antibodies.

A third suggestion has been to use immunosuppressants. This method appears to be the preferred short-term solution, primarily due to the ease of access to low cost immunosuppressive drugs. The potential of immunosuppressants in addressing the problem was shown by Velazquez and colleagues (Velazquez et al., 2017). The research group found that combination administration of prednisolone and rapamycin reduced serum AAV9 neutralizing antibodies by 70-80% at 4 weeks and 85%-93% at 8 weeks of treatment. One major issue with using immunosuppressants is that it makes the user vulnerable to infections. These drugs may also have unwanted consequences on tissue biodistribution and transduction efficiency of the gene therapy.

A fourth option is to increase the capsid dose or to use capsid decoys. Mingozzi et al. demonstrated that introducing empty capsids along with the gene therapy vector would titrate out any neutralizing antibodies to AAV - these empty capsids served as a decoy to allow successful transgene delivery (Mingozzi et al, 2013). However, this method has yet to be tested in humans and is likely to be ineffective with high antibody titers. Furthermore, as found by Johnson and Samulski, empty capsids would also enter target cells, leading to an increased presentation of capsid epitopes on MHC class I molecules (Johnson and Samulski, 2008). This would in theory increase the harmful T cell responses against the AAV gene therapy capsid.

Clearly the immune system response against the AAV capsid still poses the biggest challenge for successful AAV gene therapy. Despite this, companies continue to develop gene therapies using a wide variety of serotypes for many indications.

Gene therapy landscape by AAV serotype

At present, 12 human serotypes and more than 100 non-human primate serotypes have been identified. A landscaping exercise of companies developing gene therapy products (see Figure 2) revealed that while the specific serotypes utilized in many programs remain undisclosed, largely due to their early stage in development, AAV2, AAV8 and AAV9 are the most popular serotypes being utilized amongst those programs for which AAV serotype is publicly known. There are of course many factors that contribute to serotype selection, one of which is tissue tropism.

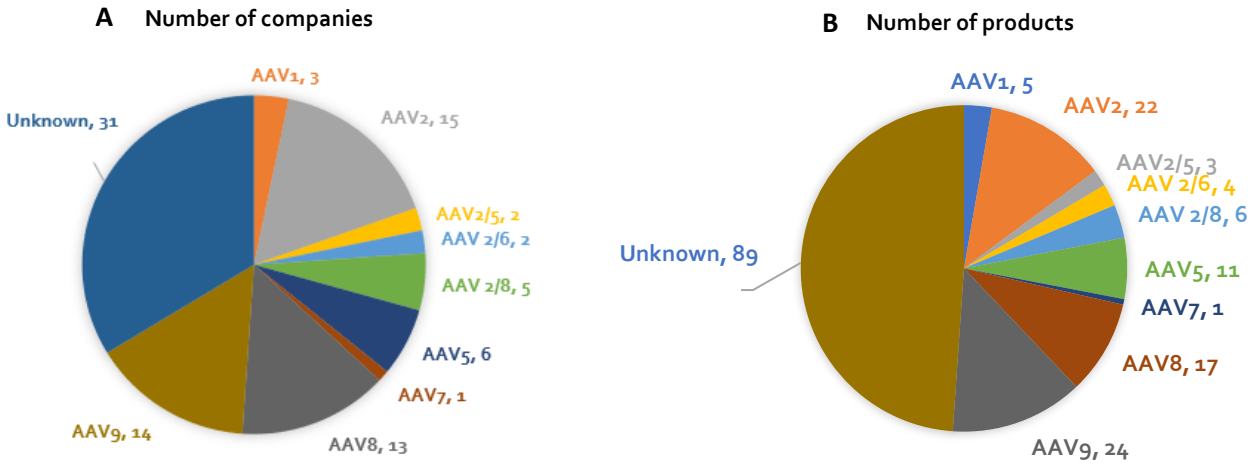

Figure 2: (A) Number of companies developing gene therapy products, by AAV serotype (serotype, # of companies); (B) Number of gene therapy products in development, by AAV serotype (serotype, # of products)

AAV serotypes differ in their tropism i.e. their ability to infect certain cell types, principally due to their different cell surface receptors. Our current understanding of inherent AAV tropism is outlined in Table 2. Following the discovery that cell surface proteins influence AAV tropism, researchers have been able to genetically modify surface proteins to target certain AAVs to specific cell types. For example, investigators (Ried et al., 2002) have incorporated ankyrin repeat proteins (DARPins, sections of protein A) and cytokines into an AAV capsid for specific cell targeting. This method has been subsequently applied to create AAVs targeting tumors and CD_4+ T cells, with implications for cancer treatment (Münch et al., 2013). As we continue develop our understanding of AAV biology, we will undoubtedly find more opportunities to modify the properties of AAVs to better perform their function as gene therapeutics.

Table 2: Tissue tropisms by AAV serotype (AddGene)

Tissue	Optimal serotype
CNS	AAV1, AAV2, AAV4, AAV5, AAV8, AAV9
Heart	AAV1, AAV8, AAV9
Kidney	AAV2
Liver	AAV7, AAV8, AAV9
Lung	AAV4, AAV5, AAV6, AAV9
Pancreas	AAV8
Photoreceptor Cells	AAV2, AAV5, AAV8
RPE (Retinal Pigment Epithelium)	AAV1, AAV2, AAV4, AAV5, AAV8
Skeletal Muscle	AAV1, AAV6, AAV7, AAV8, AAV9

AAV2 was one of the first AAVs to be identified and is one of the serotypes for which we have the most detailed understanding. It is therefore no surprise that a large proportion of late clinical stage gene therapy products utilize AAV2, as can be seen in Figure 3. Many gene therapies also utilize AAV8 or AAV9 due to their well-established tropism for cells in the liver, the heart and skeletal muscle following intravascular delivery. For example, Solid Biosciences is using AAV9's specificity for skeletal muscle in their treatment of Duchenne Muscular Dystrophy (Phase 2, SGT-001). AAV9's efficiency in traversing the blood-brain barrier has also brought new opportunities for the treatment of CNS disorders, including those involving non-neuronal cells. An example of such application would be Amicus Therapeutics' AAV products to target neurons in the treatment of Batten Disease.

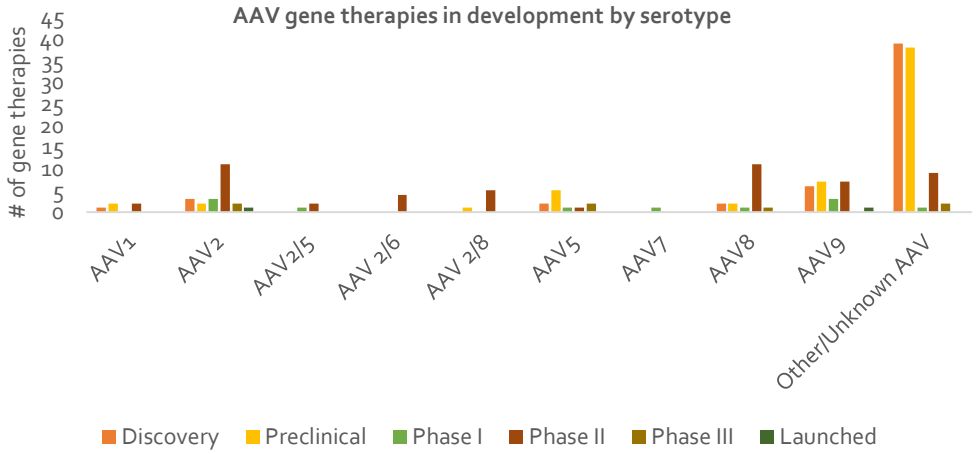

Figure 3: Number of AAV gene therapy products under different stages of development, by serotype

Gene therapies utilizing AAV5 are also being developed centred around its tropism. For example, Editas Medicine is utilizing AAV5's specificity for retinal photoreceptors in their treatment of Leber's Congenital Amaurosis (Phase 2, EDIT-101).

Disease areas being targeted by gene therapy

As seen in Figure 4, at present, most companies are focusing their efforts on developing gene therapies to treat metabolic, ophthalmic and neurological disorders, for which many indications result from loss-of-function genetic alterations. The eye's immune privileged status, accessibility and compartmentalization significantly reduce systemic spread of locally delivered gene therapy product and therefore reduce the risk of antibody neutralization of the vector – one of the key issues in gene therapy today. Many metabolic and bleeding disorders are a result of dysfunctional enzymes expressed predominantly or exclusively in the liver. Because of the liver's unique dual blood supply, it receives nearly 25% of the cardiac output of blood. This means that administration of an AAV systemically leads to effective delivery of the vector to hepatocytes. The use of AAV in muscular disorders is promising due to its high transgene expression when injected intramuscularly and whole-body muscle transduction when delivered intravenously.

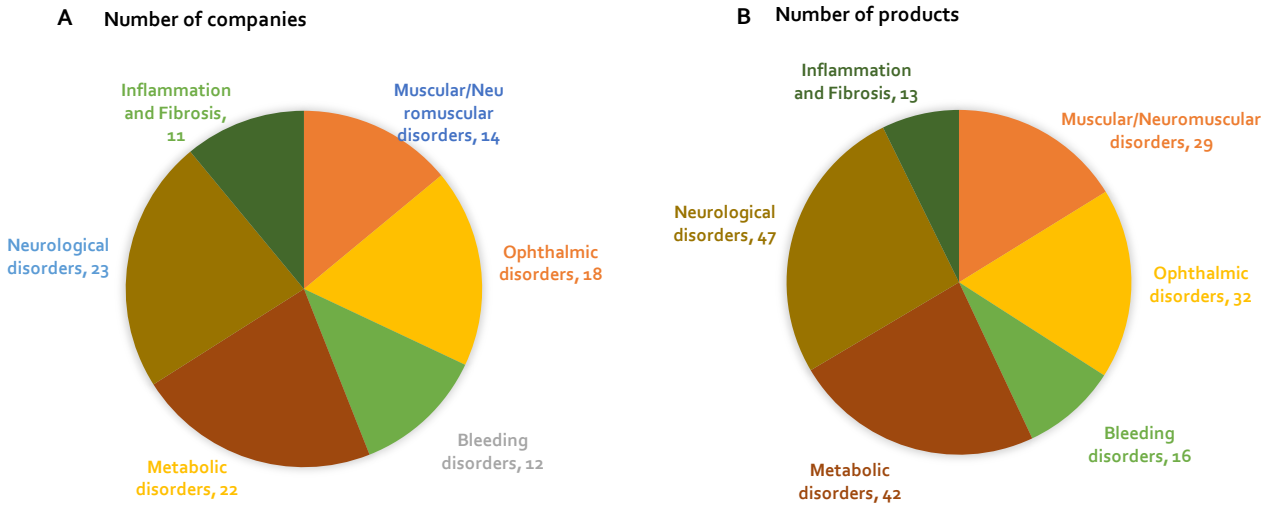

Figure 4: (A) Number of companies developing an AAV product, by disease area; (B) Number of AAV gene therapy products being developed, by disease area

A review of current gene therapies in development by disease area and stage of development shows that while activity is relatively evenly spread, there are notably more gene therapies in preclinical stage for neurological disorders than other disease areas. On the other hand, bleeding disorders account for the highest number of gene therapies in Phase 3 (see Figure 5). Gene therapies for Hemophilia make up 13 out of the 16 products addressing bleeding disorders; given the big unmet need for treatments in this indication, it is no surprise that so many gene therapy products are progressing rapidly through development. Interestingly, gene therapies actively in development for inflammation/fibrosis have only progressed to Phase 1. Four out of the 13 products addressing inflammation/fibrosis disorders are for treating cystic fibrosis. A major challenge for treatment of this indication has been to fit the CFTR gene, alongside a suitably strong promoter, into a limited capacity AAV. Furthermore, gene therapy-mediated expression of the CFTR gene is quickly lost due to high turnover rates of airway surface cells.

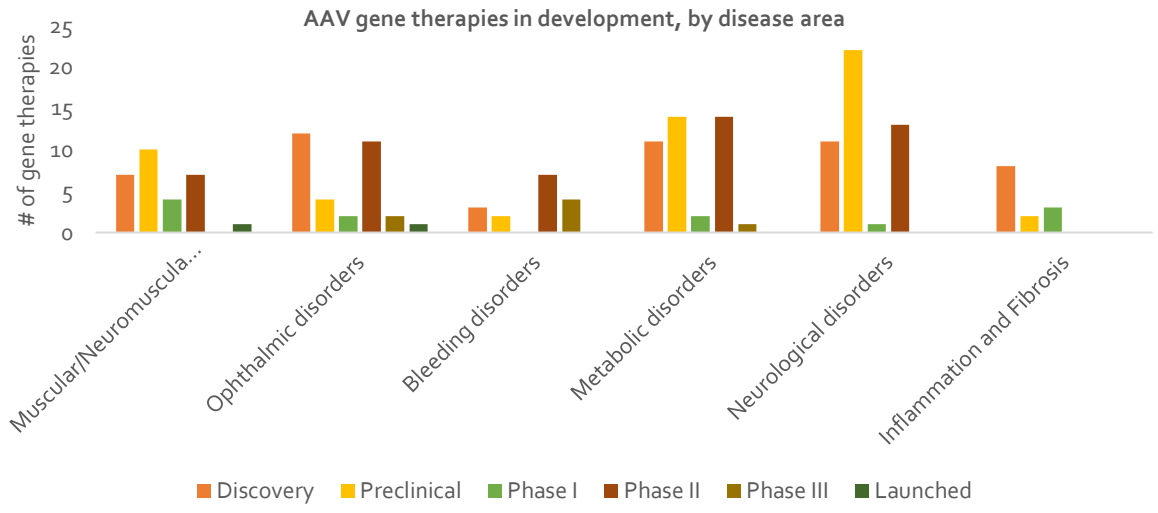

Figure 5: Number of AAV gene therapy products under different stages of development, by disease area

The use of AAV as vectors in gene therapy has many advantages. They provide stable transgene in a variety of tissue types as well as being minimally pathogenic. As we enhance our understanding of AAV capsids and their role in transduction, we will continue to improve their versatility. However, a major challenge that has yet to be addressed is the issue presented by the humoral immune response. There is no doubt though that with the huge potential of AAV technology, alongside the recent expansion in our understanding of gene therapy, an interesting era in gene therapy is underway -- one in which AAV is playing a leading role.

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