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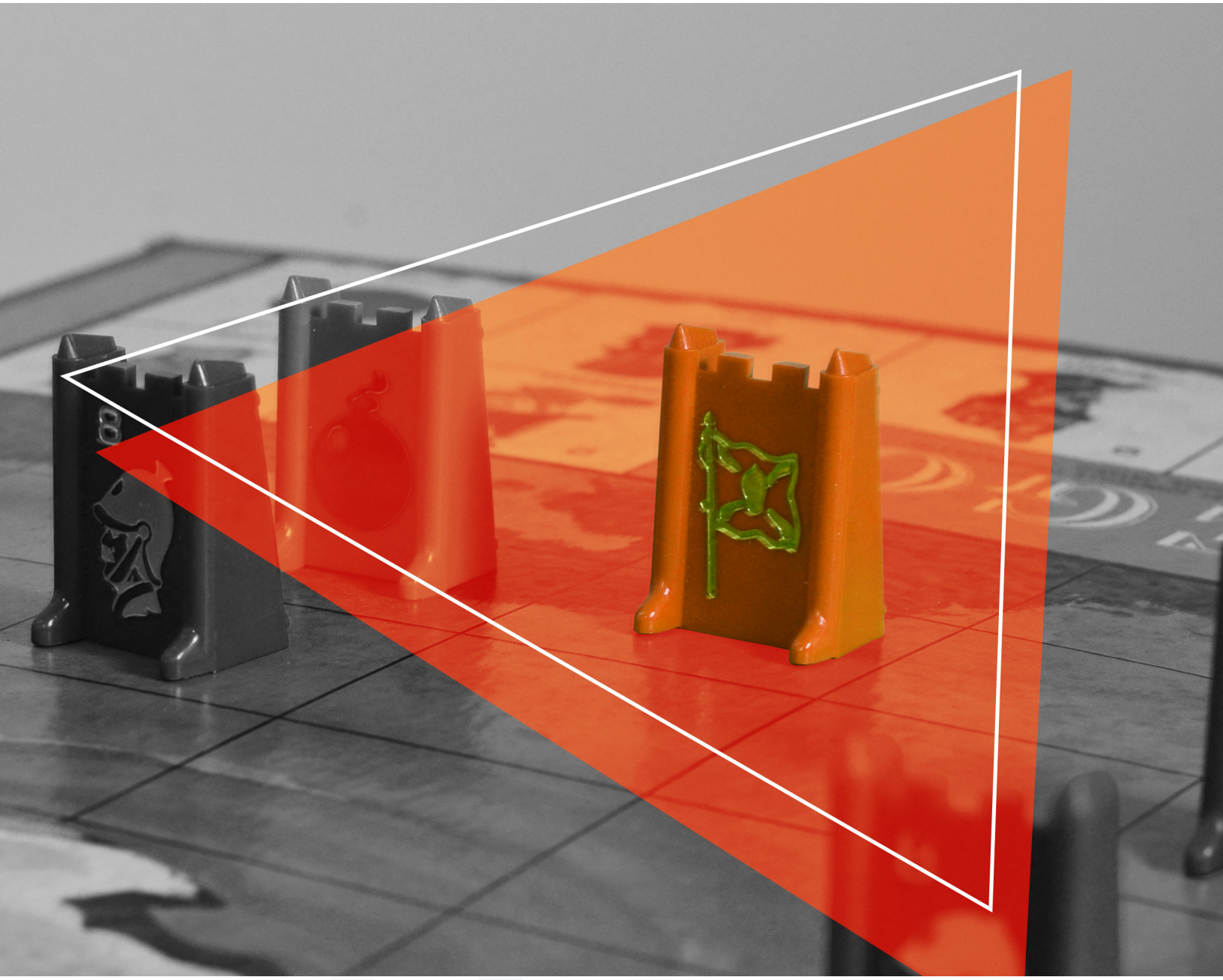


BETTING ON HIDE AND SEEK WITH AAV

In the COVID world, the field of classic virology – the study of virus structure, function, and interactions with the host environment – has been reinvigorated with the rapid development of several types of vaccines that trigger the immune system. This same type of research is being conducted in the field of gene therapy, however the goal is to achieve the completely opposite outcome: develop viral vectors that evade the immune system and "infect" target cells with greater efficiency. Development in the gene therapy field has exploded in recent years, largely due to a renewed interest in the investment community and Large Pharma, who are betting on over 30 years of basic research becoming a clinical and commercial reality. While the United States has only seen two gene therapy product approvals, Zolgensma (Novartis) and Luxturna (Roche), two products are currently under review and over 20 are in phase 3. Of these late-stage gene therapies, 6 are being developed or marketed by Large Pharma, the remaining programs are being developed by small and midsize biotechs with an average market cap of $2.7B.

At the forefront of this renewed interest is a virus called adeno-associated virus (AAV). Originally discovered in the 1960s, AAV has been affectionately referred to as "almost a virus" due to its limited pathogenicity compared to other viruses. AAV-based therapies comprise a significant amount of clinical stage gene therapies; out of the gene therapies in phase 3 clinical trials, 11 use AAV vectors. However, recent clinical holds due to adverse events and three recent patient deaths have highlighted the limits to AAV caused by a heightened immune response at higher doses. They have underscored the consistent and crucial challenge of the field that has been the focus of innovation for decades: how to achieve sufficient and long-term gene expression without encountering dose-limiting toxicities.

To understand potential ways in which AAV gene therapy can be improved, it is important to first understand the steps between injecting an AAV vector and achieving gene expression. The pathway of AAV transduction and therapeutic gene expression includes four key steps:

01

Upon administration, AAV must arrive at, bind to, and enter a target cell.

02

Once inside, the vector must traffic through the cell and enter the cell's nucleus.

03

The vector must then "uncoat" to reveal the therapeutic transgene, which becomes expressed.

04

Expression must persist in transduced cells.

Along each step in this process, there are cellular or immune system obstacles that have been designed by nature to impede this process. Innovation in the field thus can be categorized according to where advancements are tackling each step in the pathway.

01

Targeting Cells While Remaining Undetected by Neutralizing Antibodies

Most of the work dedicated to improving AAV vectors has been focused on developing capsid variants that 1) better target the desired tissue or cells, and/or 2) avoid detection by neutralizing antibodies (NAbs). A number of naturally occurring AAV serotypes that can deliver gene therapies to a specific tissue have been identified, and the below table lists several common AAV serotypes, their tropism, and the number of Phase III/Approved gene therapies using them:

Table 1: Common Human AAV Serotypes, Marketed or in Late-Stage Development

Serotype	Tissue Tropism	Phase 3 / Approved Gene Therapies	Programs
AAV1	Muscle, Liver, CNS	1	*None Currently**
AAV2	CNS, Eye, Liver, Kidney, Muscle	2	BIIB111 (Biogen, Choroideremia) Lumevoq (GenSight, LHON)
AAV5	CNS, Liver, Muscle, Eye, Lung	2	Roctavian (BioMarin, Hemophilia A) Etranacogene Dezaparvovec (UniQure, Hemophilia B)
AAV6	Lung, Liver, Muscle, Heart	1	SB-525 (Sangamo, Hemophilia A)
AAV8	Liver, Muscle, Heart, Eye, Pancreas	1	BIIB 112 (Biogen, XLRP)
AAV9	CNS, Heart, Liver, Muscle, Lung	2	Zolgensma (Novartis, SMA) PF-06939926 (Pfizer, DMD)

LHON: Leber Hereditary Optic Neuropathy; **XLRP:** X-linked Retinitis Pigmentosa; **SMA:** Spinal Muscular Atrophy; **DMD:** Duchenne Muscular Dystrophy

**Glybera, the first AAV gene therapy ever approved (Europe) was based on an AAV1 vector, but was suspended due to low demand*

However, before AAV can even reach its target cells, it is susceptible to detection by NAbs, a natural immune defense to pathogens that have previously been encountered. It has been estimated that 40-60% of the population harbors neutralizing antibodies (NAbs) to AAV. Developers have attempted to circumvent this issue by discovering or engineering capsids that "look" different and thus go unrecognized. The same techniques used to discover these stealthier capsids are also used to develop new variants with improved target tissue tropism. These approaches can be classified into four categories, which are highlighted below:

Table 2: Strategies to Develop Capsids with Enhanced Tropism or Immune Evasion Properties

	Discovery of Naturally-Occurring Variants	Rational Design	Directed Evolution	In Silico-Guided Design
Description	Discovery of AAVs with less exposure to humans (e.g. rare human variants or animal varieties)	Intentional targeted capsid modification based on preexisting knowledge of capsid structure and function	Library-based (e.g. capsid shuffling, peptide display) forced selection of variants with desired traits	Computational modeling to inform variant development
Examples	▶ LYS-SAF302: AAVrh.10(Sarepta, Phase 3) ▶ SRP-9001: AAVrh.74(Sarepta, Phase 2)	*No clinical examples yet*	▶ SPK-8011: Spark200(Spark, Phase 3) ▶ ADVM-022: AAV2.7m8 (Adverum, Phase 2)	*No clinical examples yet*

Several companies have emerged in recent years with a core focus on developing next generation AAV capsids. Stride Bio leverages its scientific expertise in capsid structure to guide the design of novel vectors with enhanced tissue targeting properties and/or reduced recognition by neutralizing antibodies. 4D Molecular Therapeutics draws from its proprietary capsid library and a directed evolution approach to engineer vectors tailored for specific diseases. In February, longtime European gene therapy leader Genethon partnered with artificial intelligence group WhiteLab Genomics to design vectors with enhanced tissue tropism and/or reduced immunogenic properties.

02

Improving Intracellular Trafficking

In general, AAV vectors that enter cells must complete a series of trafficking steps to enter the nucleus where the transgene is delivered. Intracellular trafficking, particularly nuclear entry, represents a significant barrier to transduction. Current approaches for enhancing AAV's ability to traffic to the cell nucleus while avoiding cellular barriers include capsid modification and small molecule augmentation. For its XLRP program, Applied Genetic Technologies Corporation is testing an AAV2 variant that has been modified to remove its surface-exposed tyrosine residues, which allow this variant to avoid ubiquitylation and proteasome degradation while at the same time improving nuclear entry. In addition to capsid modification, it is possible that future gene therapies include co-treatment with small molecules or biologics that enhance vector uptake in cells, improve trafficking, or block host factors from inhibiting transduction. While there are no known clinical trials to date employing this strategy, it could become an area of interest, especially as many large pharma companies have become involved in gene therapy development and can provide access to their proprietary small molecule libraries.

03

Optimizing Gene Expression

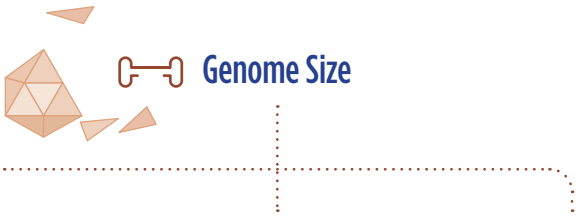
Once AAV enters the nucleus and releases the packaged genome, it must be expressed to yield the therapeutic product. Naturally occurring AAV contains a single-stranded DNA that must be converted to a double-stranded form prior to being expressed. The discovery of self-complementary (sc) AAV circumvents this rate-limiting step, resulting in faster and higher gene expression. Zolgensma is the first approved product that uses scAAV technology. Examples of additional scAAV programs include Abeona's phase 1/2 programs in mucopolysaccharidosis IIIA and IIIB, Amicus' phase 1/2 programs in CLN3 and CLN6 Batten disease, and Sarepta's phase 1/2 program in limb girdle muscular dystrophy 2D.

The drawback to self-complementary AAV, and single-stranded AAV for that matter, is the size limitation of the genome. AAV is limited to a packaging capacity of ~5kb, meaning that the entire packaged genome (i.e. transgene, promotor, and other elements) delivered via single-stranded AAV cannot exceed this size, and genomes delivered via

self-complementary AAV can only be half this size. For naturally occurring genes that are larger than ~5kb, two strategies have gained traction in recent years. The development of miniaturized genes, smaller transgenes that still result in expression of functional protein, is well established within the Duchenne muscular dystrophy pipeline, with Pfizer, Sarepta, and other competitors developing vectors containing some version of a smaller dystrophin gene. An alternative strategy has emerged, which involves delivering half-transgenes via separate vectors and relying on recombination within the target cell. This "dual vector" approach was in preclinical development by Nightstar Therapeutics to deliver the ABCA4 gene for Stargardt disease before the company was acquired by Biogen in 2019.

Another strategy to increase targeted gene expression is through the design of enhanced promotors. Many first-generation constructs include ubiquitous promotors, which enable robust gene expression but are not specific to any cell or tissue type. Without a tissue-tropic vector, gene expression will occur in any cell that has been transduced by AAV, which can result in off-target effects or activation of the immune system. Ubiquitous promoters also tend to be larger, taking up valuable real estate within the transgene. Promoter engineering thus aims to develop stronger, tissue-specific, and/or smaller promoters. For example, Solid Biosciences' SGT-001 and Sarapta's SRP 9001 use muscle-specific promoters for their Duchenne muscular dystrophy programs. Encoded Therapeutics aims to develop gene therapies with better cell selectivity and improved expression through promoter and other regulatory element engineering. In 2019, AskBio acquired Synpromics, a synthetic promoter development company, with the goal of enhancing tissue targeting and increasing gene expression.

Figure 1: Strategies to Modify Transgene for Enhanced Expression or Large Genes



Strategy	<~2.5kb Self-Complimentary	~2.5-5kb Conventional	>~5kb Miniaturized
Examples	▶ Zolgensma (Novartis) ▶ Etranacogene Dezaparvovec (UniQure, Phase 3) ▶ Lumevoq (GenSight, Phase 3)	▶ Luxterna (Spark) ▶ Roctavian (BioMarin, Phase 3) ▶ BIIB112 (Biogen, Phase 2/3)	▶ PF-06939926 (Pfizer, Phase 3) ▶ SRP-9001 (Sarepta, Phase 2)

04

Achieving Long-Term Persistence

For many of the gene therapy trials to date, the short-term effects seem promising. However, in several cases, long-term expression has been disappointing. Apart from transgene dilution in replicating cells, the biggest challenge to expression durability is the immune response, which can be triggered by the AAV vector, transgene, or transgene protein product. Major adverse responses, including CTL-mediated cytotoxicity and inflammation, appear to be dose-dependent, so vector- or transgene-directed strategies that increase AAV potency may address this challenge. Additional approaches that aim to alter the vector or the transgene to limit interactions with the immune system are underway. For example, Ally Therapeutics is a recently formed biotech company with a platform based on blocking TLR9 activation by incorporating short TLR-9 inhibitory sequences into the transgene.

Table 3: Key Deals and Recent Financing of Select AAV Gene Therapy Companies

	Company	Recent Deals and Financing
Companies with Marketed Products	Spark Therapeutics	**$161M** (2015)
	AveXis Therapautics	**$4.3B** (Roche, 2019)
Companies with Clinical-Stage Programs	AskBio	**$2B** upfront + **$2B** in milestones (Bayer, 2020)
	Nightstar Therapeutics	**$800M** (Biogen, 2019)
Capsid-Engineering Companies	4D Molecular Therapeutics	**$222M** (2020)
	Affinia Therapeutics	**$110M** (Series B, 2021)
	Dyno Therapeutics	**$100M** (Series A, 2021)
	StrideBio	**$81.5M** (Series B, 2020)
Promoter-Engineering Companies	Synpromics	Undisclosed terms (AskBio, 2019)
	Encoded Therapeutics	**$135M** (Series D, 2020)
Immune System-Targeting Companies	Ally Therapeutics	Undisclosed seed funding

IPO
Acquisition
Venture Capital

LOOKING AHEAD

Acknowledging the current challenges, the future is bright for AAV and gene therapy at large. In lock step with the innovation in gene therapy, huge strides have been made in the field of human genomics, enabling more patients with rare genetic disorders to be identified and diagnosed much earlier than ever before. With the ubiquity of social media and digital enablement, advocacy groups have become a stronger force in gene therapy development and awareness. The promise of gene therapy is further evidenced by greater collaboration offered by regulatory agencies and widespread acceptance of currently approved treatments by payer organizations. In the development pipeline, there is a growing shift in innovation from rare, life-threatening disease dominating the landscape to more mainstream conditions. Several viral vector-based COVID vaccines are in development (including some based on AAV), and Johnson & Johnson's recently-approved vaccine will become the first mass-administered viral vector in the world. While there are still challenges to overcome before gene therapy becomes a commonly-accepted therapeutic modality, a dedicated body of innovators, backed by Large Pharma and the investment community, is committed to unlocking the promise of this powerful technology.

Figure 2: Innovation Along the Pathway of AAV Transduction