EXPANDING PATIENT ACCESS TO GENE THERAPIES THROUGH AAV CAPSID ENGINEERING

Abstract: Adeno-associated viral (AAV) vectors present a host of advantages for use as delivery vehicles for genetic material. They also have limitations that impact their safety, efficacy, and applicability. Capsid engineering has the potential to increase the specificity and transduction efficiency while reducing unwanted immune responses and facilitating the manufacturability of AAV capsids. Such improvements have the potential to broaden the range of diseases that can be treated with gene therapies and greatly expand access to patients.

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AAV Vectors Crucial to Gene Therapy

Most approved gene therapies on the market today leverage viral vectors for delivery of the new genetic material. Many are based on adeno-associated viruses (AAVs). Glybera (UniQure, EMA 2012), approved for the treatment of lipoprotein lipase deficiency, is based on an AAV1 vector. Luxturna (Spark Therapeutics, FDA 2017, EMA 2018) and Upstaza (PTC Therapeutics, EMA 2022), marketed for the treatment of RPE65 retinal dystrophy and AADC deficiency, respectively, are based on AAV2 vectors. Roctavian (Biomarin, EMA 2022) for the treatment of hemophilia A is based on an AAV5 vector, while Zolgensma (Novartis, FDA 2019, EMA 2020) for the treatment of spinal muscular atrophy leverages an AAV9 vector. Notably, all of these approved products use wild-type viral vector capsids.

Meanwhile, there are hundreds of gene therapies in clinical trials, many of which are based on AAV vectors spanning numerous serotypes, including wt 1, 2, 5, 8, and 9 capsids. However, a growing fraction of newer candidates entering the clinic leverage AAV vectors engineered for improved performance properties. Examples include the 2tYF, 7m8, LK03, and 4D-R100 capsids, which are used in gene therapies in development by AGTC, Adverum, Spark and LogicBio, and 4DMT, respectively.

AAV vectors predominate as delivery vehicles for gene therapy for several reasons. First, they are not pathogenic; many people have already been exposed to AAVs, and these viruses are not associated with human diseases. Second, recombinant AAV vectors typically do not integrate into the genomes of the cells they enter, but can exist as stable episomes for the lifetimes of the transduced cells. Consequently, one treatment can last for the patient's lifetime, depending on the tissue that is being targeted.

Limitations of Natural AAV Capsids

While readily accessible, wt or natural AAV vectors have limitations with respect to their performance as vehicles for the delivery of genetic material. Notably, natural capsids have poor specificity and tend to infect a wide range of tissues. At the same time, there are certain tissues that AAV vectors do not reach well, including the heart, brain, kidney, and lung.

In addition, the capsid surface interacts not only with cell-surface receptors on target cells that allow infection but also with antibodies generated during previous exposures, which are designed to prevent infections. The problem is only an issue for some people — specifically those who have high levels of preexisting neutralizing antibodies (NABs) that render the therapy ineffective. Screening of patients for the presence of such antibodies in their blood is therefore conducted before administration.

Unfortunately, many people who would otherwise benefit from the gene therapy are turned down because they happen to have the wrong antibodies. For example, in some parts of the world, more than 50% of the population exhibits NABs against AAV2. Globally, fewer people have NABs against AAV7 or AAV8 than against AAV2, but the numbers are still high for any natural human AAV.

Furthermore, after an AAV has entered a cell, the capsid surface composition influences the extent to which it is degraded before it can lead to transduction. Indeed, only a small fraction of the AAV particles that enter cells reach the nuclei, establish episomes, and proceed to long-term transgene expression, leading to poor transduction efficiency.

Safety and Efficacy Issues Remain

There are also safety issues associated with the use of wt AAV capsids in gene therapy applications. The primary concern is the potential for patients to experience an excessive, dose-dependent immune response. High doses of AAV can trigger a cellular immune response, resulting in the elimination of transduced cells and loss of transgene expression.

Maximizing safety requires achieving the desired therapeutic effect at lower doses. That can be made possible by improving the transduction efficiency in general or improving tissue specificity so that less AAV is wasted on infecting off-target tissues. Achieving these improvements requires a

move away from wt vectors to capsids that have been engineered to possess different surface properties. Engineered capsids are more likely to evade preexisting immunity and avoid causing aggressive immune responses because of their modified surfaces. Capsid engineering can therefore help increase patient access to gene therapies.

AAV Structure Presents Opportunities for Engineered Improvements

The AAV capsid is an assembly of 60 monomers comprising the proteins VP1, VP2, and VP3, which are all expressed from the same cap gene. Both VP1 and VP2 contain the entire sequence of VP3, with the elements that deviate from VP3 typically contained inside the capsid. The capsid surface, therefore, can be considered as an assembly of 60 identical subunits. Indeed, all AAVs have a very similar structure consisting largely of highly conserved regions but also including nine variable regions.

These variable regions are responsible for the differences in immune properties and tropism and are the main targets for capsid engineering. There is a limit to the possible variations that can be incorporated into AAV capsids because of the need for the 60 subunits to fit together and thus for the variations to be compatible with capsid assembly and genome packaging. Even with this requirement, an astronomically huge range of potential diversity remains to be explored (including capsids that would be very unlikely to occur in nature), such as shuffling of capsid regions between very distantly related AAVs, large peptide insertions, rare amino acid combinations, and so on.

Engineering is required because there is no selective pressure within nature to create AAV vectors with properties ideal for use as delivery vehicles for human gene therapies. In particular, there is no evolutionary driver for the development of tissue specificity. In order to obtain new capsids better suited to their use in gene therapy, rational design, directed evolution, or a combination of both must be employed.

Rational Design and Directed Evolution Drive Capsid Engineering

New capsid variants are engineered by rational design, directed evolution, or a combination of the two. Directed evolution using large libraries of mutants remains the most powerful approach. Many studies have been conducted to identify different epitopes and thus which positions interact with antibodies. For some AAV vectors, the positions involved in receptor recognition are known, and hence which sites can be targeted for modification.

With this information in hand, it is possible to use a few different engineering approaches. In one case, a large range of mutations along the surface can be investigated using high-throughput technologies. In other cases, it is possible to insert a peptide or perform DNA shuffling, in which interesting parts of different variants and/or cell types are identified and mixed in different combinations. Depending on how the behavior of the engineered capsid evolves, the strategy can be modified and elaborated further.

With the digital transformation underway in the biopharma industry, it can be anticipated that advanced digital technologies will ultimately be applied to capsid engineering. Artificial intelligence and machine learning, for instance, may enable the development of in silico modeling and discovery, thereby streamlining capsid engineering.

Considering Specificity, Immune Evasion Transduction Efficiency, and Manufacturability

Capsid optimization must take into consideration different factors that have different levels of importance depending on the specific gene therapy under development. The ultimate goal is to develop gene therapies that benefit as many people as possible. There are four primary criteria that must be addressed to achieve this goal: specificity, immune evasion, transduction efficiency, and manufacturability.

It is desirable to have a capsid that can selectively deliver its cargo to the target cell type. Doing so allows for the administration of lower doses, which contributes to greater safety and efficacy and reduced cost. Boosting the transduction efficiency can also provide the same benefits.

Evasion of preexisting immunity is necessary in order not to exclude patients from gene therapies. There are 13 different human AAV serotypes, and people are more or less likely to have antibodies to specific serotypes depending on their location (e.g., Asia vs. Europe vs. North America), as well as other factors, such as age or disease status. Developing AAV capsids that will not get degraded by preexisting antibodies is a challenge. One possible strategy would be to use non-human AAVs, because people do not have antibodies against them. However, non-human AAVs generally fail to infect human cells, so capsid engineering is typically performed on human AAVs.

Manufacturability is particularly important for gene therapies administered to large patient populations in large doses. Robust, large-scale, and cost-effective production of the viral vector is needed. Production yield is known to vary with serotype. For example, the yield of AAV2 is generally much lower than that observed for AAV5 or AAV9. As a result, AAV2 will not be the preferred parent serotype for capsid engineering if production yield is a crucial criterion.

Appropriate capsid engineering can address all of these factors. Problematic epitopes can be removed to avoid antibody recognition. Tissue specificity can be improved by surface modifications. Certain positions can be changed to improve transduction efficiency by reducing degradation of capsids before they can reach the nucleus. Capsids can also be selected to improve their compatibility with purification processes.

It is important to remember that specificity, immune evasion, transduction efficiency, and manufacturability are not all always important for all gene therapies. Local administration may eliminate the need for high specificity. Delivery into an immune-privileged site, such as the eyes or the central nervous system (CNS), may mean that preexisting antibodies are less of a concern. For gene therapies that require small doses and target small patient populations, manufacturability is less of an issue because it is generally easier to produce viral vectors on a small scale.

Finally, capsid engineering may enable optimal modes of delivery. Most gene therapies targeting the eye, for instance, are administered via subretinal injection, which is a surgical procedure that carries significant risk. Capsid engineering has the potential to enable delivery via intravitreal injection, which can be performed by a nurse in a doctor's office, making it safer and less costly.

Many Companies are Pioneering Novel AAV Capsids

Most of the pioneering work on AAV capsid engineering was initially pursued in an academic setting. The leading researchers involved in this work later created or joined startup companies. A few examples include the following:

- 4DMT (David Schaffer, Ph.D., co-founder)
- Voyager Therapeutics (Mathieu Nonnenmacher, Ph.D., Vice President, Capsid Discovery)
- Askbio (Jude Samulski, Ph.D., President)
- Stridebio (Aravind Asokan, Ph.D., Chief Science Officer)
- Aavigen (Dirk Grimm, Ph.D., Chief Science Officer)
- LogicBio (Mark Kay, M.D., Ph.D., founder)
- Affinia (Luk Vandenberghe, Ph.D., co-founder)
- Dyno (Eric Kelsic, Ph.D., Chief Executive Officer)

Most of these companies, in addition to being actively involved in capsid discovery, have their own therapeutic pipelines, often in partnership with other companies. Stridebio also provides AAV manufacturing services. Exegenesis Bio, Capsida, and Byongen are three newer companies that appear to be very committed to contributing to the capsid engineering field despite not having any high-profile experts on their teams and may end up as significant players as well.

Already Observing Impacts on Patient Safety and Therapeutic Effectiveness

The ultimate impact of AAV capsid engineering will be to increase patient access to gene therapies (through the development of capsids that evade preexisting NABs), enable safer gene therapies that are effective at lower doses (due to capsids with increased specificity and transduction efficiency), and allow the development of gene therapies that target a wider range of diseases, such as those of the lung, heart, and kidneys (due to expansion of the tissues that can be targeted by AAV capsids).

Some success has already been realized. Improvement in transduction efficiency was pioneered by my former colleagues in the Srivastava lab at the University of Florida approximately 15 years ago. They systematically explored the effects of surface amino acid substitutions (mostly Y to F) to enable evasion of phosphorylation, which leads to ubiquitination and eventually proteasome-mediated degradation of the capsid.

Patient safety and therapeutic effectiveness have also been improved. Engineered capsids for retina targeting such as 7m8 or 2tYF allow delivery by intravitreal injection instead of subretinal injection. For intravenous delivery, engineered capsids, such as LK03 or AAVS3, allow lower doses to be used, contributing to lower toxicity and higher likelihood of long-term transgene expression. Similarly, capsid engineering has enabled gene therapies for the treatment of CNS diseases to be delivered via systemic or lumbar intrathecal injection rather than intracranially.

Better AAV Capsids will Help Gene Therapy Become More Mainstream

At Porton Advanced Solutions, we anticipate that AAV capsid engineering will indeed enable gene therapy to become more mainstream. More patients will be eligible for treatment, costs will be reduced, and more diseases will be addressed. For the short to intermediate term, AAV will remain the vector of choice. Over the longer term, however, other alternatives will emerge that overcome some limitations of AAV vectors that cannot be addressed through capsid engineering. In particular, AAV capsids cannot package genomes larger than 5 kilobases or provide long-term transgene expression in tissues that undergo rapid cell division.

AAV Capsid Engineering at Porton Advanced Solutions

Porton Advanced recognizes the crucial roles that successful AAV capsid engineering solutions will play in enabling the success of next-generation gene therapies for much greater numbers of patients. We have a strong expertise in designing and building complex libraries with large numbers of mutations spread over the capsid surface. Such libraries are very effective at generating variants with strong immune evasion and altered tropism. We also have very strong bioinformatics and data analysis skills that allow Porton Advanced to identify promising candidates early in the evolution process and improve library design.

At this stage, we have constructed a large collection of highly complex capsid libraries and started several selection projects. Examples include a retinal pigmented epithelium (RPE)-targeting capsid for retinal diseases that is 10 times more efficient than capsids with the 7m8 mutation and a liver-targeting capsid that is more efficient than LK03 and much better at evading preexisting antibodies. In the future, Porton Advanced has plans to target the heart, muscles, kidneys, and CNS.

In addition to building our own portfolio of specialized novel capsids that clients can license directly, Porton Advanced is also positioned to apply our directed-evolution technology and expertise for the development of specific capsids that meet client requirements or improve their existing engineered capsids.

Identifying attractive mutations is only one part of the process, however; testing is another. To enable acceleration of capsid development by minimizing the use of animals, Porton is also currently developing 3D culture expertise. With 3D cultures that closely mimic real organs or tissues, the data generated is much more translatable than that obtained with 2D assays and vastly better than data obtained in animal models.

Underlying these activities is a belief that it is important to explore many different approaches, including some more far-fetched concepts. The latter, if successful, may lead to novel, disruptive, and unexpected technologies that may have a significant impact on patient lives. At Porton Advanced Solutions, we actively pursue a wide range of ideas and purposely go where others in the industry do not. For instance, we are working on developing new capsids that can package oversized recombinant AAV genomes. Packaging capacity is a significant limitation of AAV vectors that, if resolved, could significantly expand the potential applications for AAV-based gene therapy.

For these many reasons, Porton Advanced Solutions has the potential to become a world leader in capsid engineering. Five years from now, we anticipate having several capsid variants in clinical trials, including some with the highest transduction efficiencies and the best immune-evasion capabilities available to gene therapy developers.

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