ADVERTISEMENT FEATURE

THE FUTURE OF GENE THERAPY DEPENDS ON EFFECTIVE, SELECTIVE DELIVERY

Improved AAV tropism is essential for gene therapy to reach its full potential. NEW CAPSID ENGINEERING TECHNIQUES are taking on this challenge.

Clinical translation of gene therapies has a history of moving two steps forward, one step back. A key bottleneck in the field has been the limited availability of safe, selective and effective methods for delivering therapeutic genes. Notable progress has been made over the past decade, and landmark gene therapy product approvals have been granted by regulators including the US Food and Drug Administration (FDA) and European Medicines Agency. New technologies are helping to further refine the targeted delivery of therapeutic genes, pushing this promising branch of medicine towards its full potential.

The cause of many medical conditions, from cystic fibrosis to haemophilia and Duchenne muscular dystrophy (DMD), can be traced back to a faulty or missing protein. Gene therapy aims to deliver a functional version of that gene into cells. This exogenous gene then drives the production of a working version of the deficient protein to restore healthy cell function.

Most recently approved gene therapy products use an adeno-associated virus (AAV) as their gene delivery vector. For example, Elevidys, a new treatment for paediatric patients with DMD that was granted accelerated approval by the FDA on 22 June 2023, is an AAV-based gene therapy. AAV is a single-stranded DNA parvovirus that can be engineered to infect cells after 96% of the AAV genome has been replaced with a therapeutic DNA cargo. "Right now, AAVs offer the safest and best delivery method," says gene therapy researcher Ye Bu, director of research and development at PackGene, an AAV-focused contract development and manufacturing organization that was founded in the US and is currently headquartered in China. PackGene recently built, staffed and equipped a dedicated AAV cGMP facility in Houston, Texas that is scheduled to fully launch in early 2024.

WE HAVE ALREADY SCREENED MANY AAV CANDIDATES, SO WE HAVE A MAP OF EXISTING DATA THAT CAN HELP US DESIGN SMART AAV LIBRARIES THAT HAVE A BETTER CHANCE OF HITTING THE TARGET

AAVs have become the therapeutic gene delivery vehicle of choice because they are non-pathogenic, easy to produce, transduce efficiently across various cell types, and show low immunogenicity. However, the tropism (selective tissue targeting) of AAVs isolated from nature is one shortcoming, Bu notes. "The tropism of natural AAV serotypes is limited," he says. "They also tend to infect the liver, which can cause severe side effects in some cases."

Improving AAV tropism could offer multiple benefits, says Bu, who has led development of PackGene's π-Icosa AAV engineering platform. More efficient delivery of therapeutic gene payloads with higher tissue and cell specificity may enhance therapeutic efficacy and reduce required dosages. Reducing off-target delivery and required dosages may ultimately mitigate potential side effects, particularly serious liver injury. Reducing required dosages also cuts treatment costs, which currently can exceed a million dollars per dose.

ENGINEERING OPPORTUNITIES

Several AAV engineering approaches have been pioneered to enhance gene therapy delivery to target tissues, says Irene Song, senior director of global product at PackGene. Rational design leverages known structural features of naturally occurring AAV serotypes and their corresponding tropisms. By comparing structural features across AAV serotypes with shared or disparate patterns of tropism, scientists can design chimeric AAVs by combining features from multiple natural serotypes to generate unique tropism patterns.

Directed evolution is an alternate strategy. DNA replication techniques such as error-prone PCR or random peptide insertion can be used to generate large AAV libraries with random point mutations in their capsid structure. After screening an initial library, the best-performing capsids are subjected to one or more rounds of AI-directed mutagenesis that functionally evolve AAV tropism toward a desired pattern.

Potentially the most powerful approach — and the basis for PackGene's π-Icosa platform — is to use rational design and directed evolution in tandem, says Song. "Because the mechanism by which AAVs are targeted to specific cells is not fully understood, we combine rational design with directed evolution. This way we allow nature to help identify the ideal capsid for us."

One recent PackGene study was designed to engineer AAVs that target muscle cells and offers a case in point. The team combined a few natural capsid sequences to create novel serotypes using rational design. "We made changes in the receptor-binding region of the capsid to generate a chimeric capsid with reduced liver targeting," Bu says. The team passed the resulting chimeric capsids through two rounds of directed evolution to enhance muscle targeting while retaining the reduced liver targeting and toxicity observed in the parent chimeric serotype. The early data is very promising, Bu notes.

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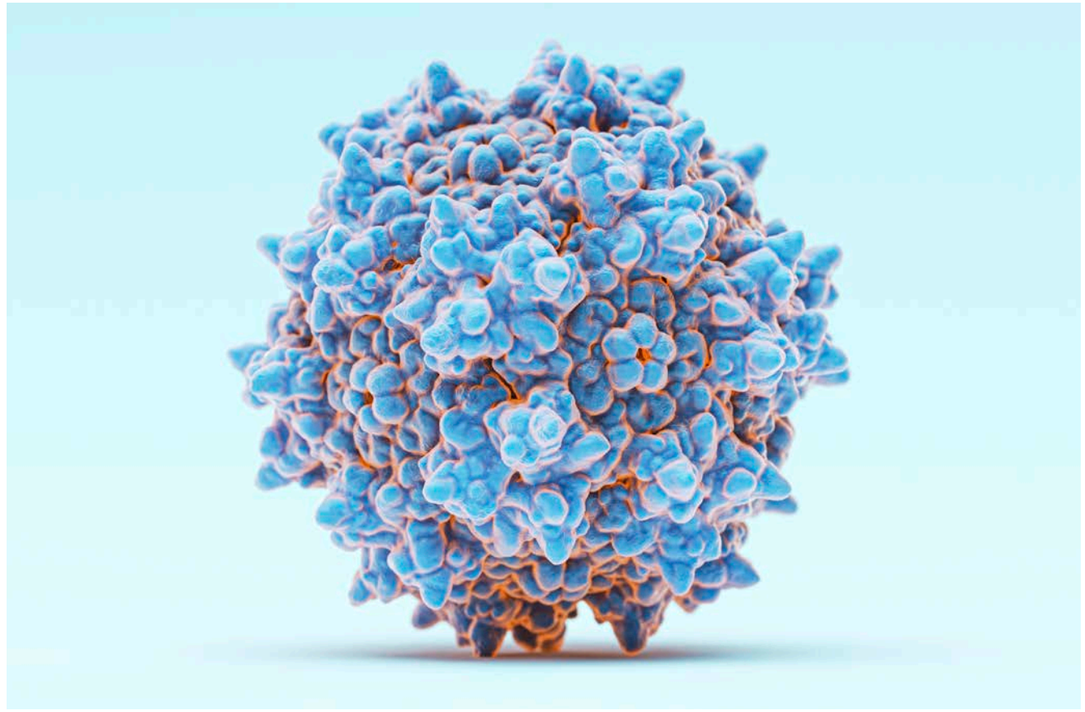

▲ **Adeno-associated viruses (AAVs) are the delivery vehicles of choice for gene therapies, but the selective tissue targeting of natural AAV serotypes is limited.**

HITTING TARGETS

Biological screening is a critical and complex aspect of AAV engineering. A common in the field is that the tropism of a particular AAV serotype can differ across species. AAVs engineered to target particular cells following systemic delivery in mice, for instance, will not necessarily function similarly in other species, including humans (Li, C. and Samulski, R.J. *Nat. Rev. Genet.* **21**, 255-272; 2020).

"We have seen examples of capsids that are very good at targeting brain tissue in mice, but not in non-human primates (NHPs)," Bu says. PackGene now places increased emphasis on screening in NHPs as they are physiologically much closer to humans, he adds. The goal is to identify candidates with a reproducible tropism across both NHPs and mice as an indicator of robust tropism patterns with improved translatability into humans. Ensuring consistent efficacy and toxicology test results across mice and NHPs can also benefit Investigational New Drug filings to regulators.

Engineering and screening AAVs for optimal tropism is an essential but tedious part of modern gene therapy development, says Song. "By outsourcing this process, a gene therapy company can free up their scientists' time for other important work such as verifying biological assays or refining the therapeutic gene cassettes," she says.

Outsourcing can also get the job done faster, Song adds. "We have already screened many AAV candidates, so we have a map of existing data that can help us design smart AAV libraries that have a better chance of hitting the target," she says. "We also have streamlined workflows for screening different tissues efficiently, making it easier for us to generate a viable construct quickly when compared to a gene therapy company doing it independently."

In addition to muscle-targeting AAVs with reduced liver off-targeting, the team has developed AAV serotypes that target human primary T cells, which could be of interest for developing CAR-T-cell therapies. Bu adds: "Right now, we are also screening for central nervous system tropisms in NHPs, and we have some promising initial readouts."

"The new serotypes we have developed could be a good starting point for gene therapy companies looking to target these cells," Song says. "They can alternatively get a head-start by taking what we have developed and building their own new serotypes upon it." ■

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