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Voyager's journey to identify a new shuttle for bringing gene therapies into the brain

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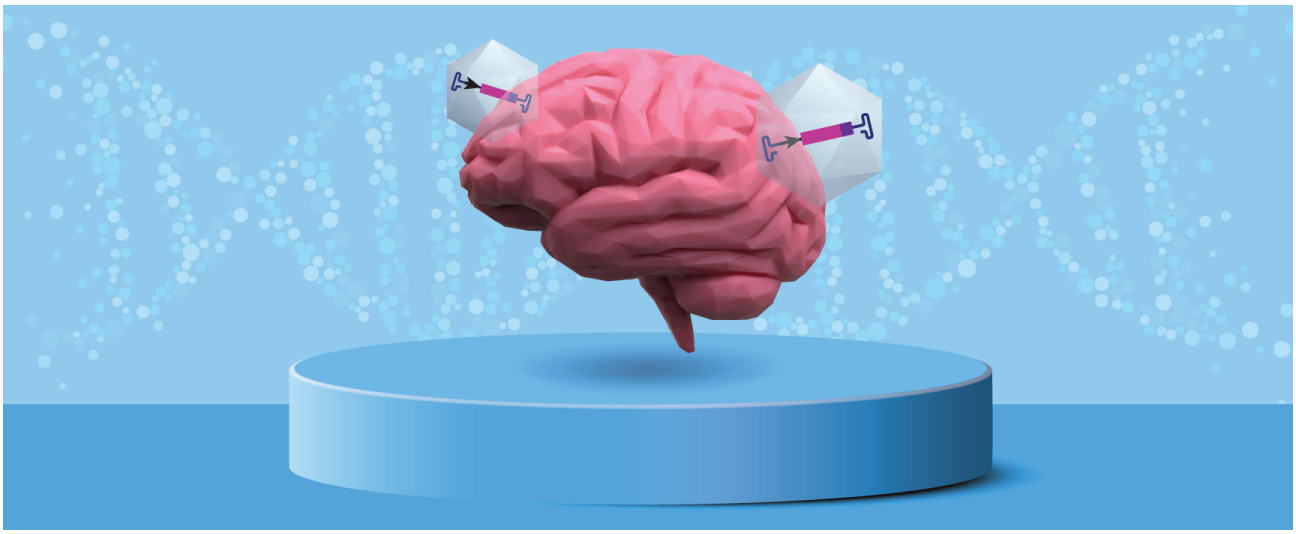

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Voyager has taken two steps toward safer and more effective systemic delivery of new CNS gene therapies. First, the company has found capsids that cross the blood-brain barrier while simultaneously de-targeting the liver; and second, it's worked backwards to identify the cell surface receptor used by the capsids for brain entry.

Voyager Therapeutics Inc. (NASDAQ:VYGR) reported at this year's American Society of Gene & Cell Therapy (ASGCT) annual meeting its method of finding a family of capsids that cross the blood-brain barrier (BBB) and how it identified the receptor — ALPL — that the capsids use to do so.

The company also reported preclinical data from two of its three wholly owned programs, one of which uses an ALPL-directed capsid.

One of the biggest issues adeno-associated viral (AAV) vectors are facing as vehicles for gene therapies, across indications and target tissues, is achieving an acceptable therapeutic index. The vector needs to sufficiently transduce the cells of interest while staying below a liver transduction threshold to prevent toxicity. That's challenging because the vectors naturally accumulate in the liver.

For CNS applications, the therapeutic index problem is complicated by the blood-brain barrier and the challenges of efficient uptake. Once across the barrier, AAVs have an additional toxicity problem if they reach too many of the dorsal root ganglion (DRG) sensory neurons.

Limited translation from preclinical animal models to humans has impeded innovation in AAV CNS delivery. For example, LY6A and LY6C1 have been identified as receptors present in the mouse brain that facilitate transduction of the AAV-PHP.B and 9P39 capsids, respectively, but it was later found that those receptors are not present in non-human primates.

More recently, CAIV was identified as a receptor that brain-tropic AAVs use to cross the blood-brain barrier, but Mathieu Nonnenmacher, Voyager's VP of novel capsid discovery, told BioCentury that while monkeys do express an equivalent gene, the protein is polymorphic and the capsids may not bind to the monkey form. "So far, the capsids that have been fully validated were validated in mouse. People are trying now to

make capsids that attach to the human form of CAIV, but this is very early work."

Now Voyager has designed new vectors that target an alternative receptor — the glycosylphosphatidylinositol (GPI)-anchored alkaline phosphatase ALPL — that's conserved across species. The distinction should help facilitate translation from preclinical experiments to human trials.

Voyager's vector discovery

The company's process for developing new CNS gene therapies involved using its TRACER platform to identify cross-species BBB-penetrant capsids, then identifying the capsids' cell surface attachment receptor.

TRACER uses directed evolution on a library of AAVs with a randomized six to seven amino acid peptide inserted into a pre-defined location on the capsid surface.

Voyager sought to identify new capsids with increased CNS tropism and decreased liver targeting compared with both its first-generation capsid VCAP-102 and the parental capsid AAV9, a widely used AAV serotype for CNS targeting. AAV9 has some natural brain tropism, but there is room to improve.

Voyager revealed at the meeting that its new VCAP-Gen2 capsid transduced at least 50% of cells in each of several brain regions in non-human primates. The capsid transduced both neurons and astrocytes and had 45x less expression in the liver relative to AAV9.

Even with higher transduction in the brain, the capsid did not have increased transduction of the dorsal root ganglion, which may bode well for the safety profile.

The company showed that its capsid works in mice as well, transducing similar numbers of cells, which Tyler Moyer, a senior scientist on Voyager's novel capsid discovery team, said is beneficial because many preclinical models of human diseases are in mice, and now there is no need to use a surrogate capsid for its preclinical studies.

"We have a capsid that is not only quantitatively comparable between the two species, but really appears to target the same number of cells of each type, which means we can really faithfully recapitulate the properties of those capsids between the mouse disease models that are currently used now and, hopefully, patients later," Nonnenmacher told BioCentury.

Nailing down the receptor

The cross-species aspect helped Voyager to identify the cell surface attachment receptor that its capsids, both first generation VCAP-102 and second generation VCAP-Gen2, utilize to cross the BBB.

> **"THE TRANSLATABILITY IS ALMOST COMPLETELY DE-RISKED."**
>
> MATHIEU NONNENMACHER, VOYAGER

"We felt pretty confident that it would work in humans, so that led us to just go right after human receptors, and we could take advantage of all the available human assays and reagents," said Brett Hoffman, a senior scientist II on the novel capsid discovery team.

The company took two orthogonal approaches to identify ALPL. The first was a capsid binding screen against over 6,000 human surface membrane proteins, which Hoffman said is "pretty good coverage of known, annotated proteins."

The second was a transduction-based screen in a stable cell line whereby each cell overexpressed a single human protein. The company screened about 17,000 proteins and then identified which cells were transduced with the AAV capsid.

Both methods identified ALPL, an alkaline phosphatase that is anchored to the membrane via a GPI linker. "This really fits with the other receptors that have been identified for novel capsids that have brain tropism," said Hoffman, as LY6A, LY6C1 and CAIV are all GPI-anchored.

Notably, in the transduction-based screen, the parental capsid AAV9 did not bind ALPL, suggesting the company's evolved capsids created a new interaction. At the same time, its new capsids can still use the endogenous AAV receptors, AAVR and galactose, to transduce cells.

"This shows that binding ALPL is a gain-of-function [effect] due to our peptide insert — so we've given it a new receptor to interact with — but we're not preventing the normal interactions of AAV9," said Hoffman.

The company found ALPL is highly expressed in brain endothelial cells, a major component of the BBB that mediates transcytosis across blood vessels into the brain, using mouse, monkey and human brain slices.

As far as the liver de-targeting aspect of the capsids, the company hypothesizes that it is through different mechanisms that are not currently understood.

"The translatability is almost completely de-risked. I won't say completely until we put it into a human, but it's de-risked as much as it can be. I really don't see what more we could do to de-risk those capsids," said Nonnenmacher.

Voyager's presentations at ASGCT 2024

Description	Abstract
Determining the likelihood that an AAV development candidate is manufacturable, safe and efficacious	65
Directed evolution to discover second gen BBB-penetrant AAV capsids with increased brain tropism and liver de-targeting	119
Multiplexed reporter protein tagging platform to analyze multiple AAV capsids in rodents and NHPs	511
New generation of AAV capsids bioengineered via the TRACER platform to evade pre-existing neutralizing antibodies	973
Machine learning to predict production fitness of capsid variants	974
Identification of the cell surface receptor utilized by BBB-penetrant AAV capsids	975
A predictive transcytosis model to recapitulate capsid-receptor interaction and phenotype of BBB-penetrant AAV variants	976
Internally developed HEK293 cell line for optimal production of capsids with brain tropism	1035
Comparing performance of methods for removing empty capsids during manufacturing	1037
First gen capsid VCAP-102 demonstrated higher biodistribution and widespread expression in the CNS compared with AAV9 in four species	1452
AAV gene therapy encoding anti-tau siRNA shows over 70% decrease in tau mRNA in multiple brain regions	1602
AAV gene therapy encoding anti-SOD1 siRNA shows over 80% decrease in SOD1 mRNA in spinal cord motor neurons	1647

Source: ASGCT 2024 abstracts

Programs in progress

Voyager has three wholly owned gene therapy programs in its pipeline, including an amyotrophic lateral sclerosis gene therapy program, VY9323, that delivers a vectorized anti-SOD1 siRNA using a second generation capsid.

The company showed at ASGCT that it can transduce over 80% of cells in spinal cord motor neurons in non-human primates after intravenous (IV) delivery.

Voyager plans to submit an IND application for the SOD1 program in mid-2025.

No gene therapies are approved for ALS, but a handful are in development. "To our knowledge, there aren't any other intravenous delivered capsids," said Nonnenmacher. "uniQure has a program with an intrathecally delivered gene therapy that we think ours compares quite well to" based on the route of administration.

AMT-162 from uniQure N.V. (NASDAQ:QURE) is in Phase I/II testing; it comprises a recombinant AAVrh10 vector expressing a SOD1-targeted miRNA. uniQure's website says the therapy produced "relevant SOD1 reduction in spinal cord motor neurons" in non-human primates and increased survival in a mouse model of SOD1-ALS.

Voyager also has two Alzheimer's disease programs, but the company did not disclose which capsids the therapies utilize.

One of the Alzheimer's gene therapies encodes anti-tau siRNA. At ASGCT, the company showed an over 70% decrease in tau mRNA and over 50% decrease in tau protein in multiple brain regions after IV administration in a mouse model expressing all six isoforms of human tau.

Voyager plans to submit an IND application for the tau program in 2026.

Its other Alzheimer's program is a vectorized anti-β-amyloid antibody. The company did not provide an update on this program at ASGCT, but vectorized biologics were a theme at the conference.

"Our story is a unique combination of equivalence across species, known mechanism of action, and a capsid that has been discovered spontaneously by an empirical assay and validated in all these different species, "said Nonnenmacher.

Voyager has 13 partnerships, two of the most recent are with Novartis AG (SIX:NOVN; NYSE:NVS) and Sangamo Therapeutics Inc. (NASDAQ:SGMO) for use of BBB-penetrant capsids derived from its TRACER platform.

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