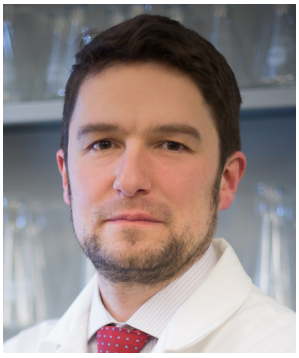
CELL & GENE THERAPY INSIGHTS

ANC-80: LATEST UPDATES ON THE NOVEL ANC-AAV GENE THERAPY VECTOR

SPOTLIGHT

INTERVIEW

# Addressing the Limitations of AAV Vectors through Evolutionary Guided Vector Design



**LUK H VANDENBERGHE, PHD,** is an Assistant Professor at Harvard Medical School and Associate Member of the Broad Institute of Harvard and MIT in Boston, MA, USA. He directs the Grousbeck Gene Therapy Center at Massachusetts Eye and Ear Infirmary in Boston, USA, a part of the Ocular Genomics Institute, a bench to bedside research program to study, diagnose, and develop treatments for diseases of the eye. His previous work led to the discovery of novel AAV serotypes such as AAV9, novel insights into AAV structure-function, and vector immunobiology. His laboratory at Harvard addresses mechanistic questions on AAV virology, develops technologies aiming to overcome hurdles to gene therapy clinical applications, and actively translates gene therapy programs in hearing and vision. His research focuses on delivery questions, specifically on the adeno-associated virus (AAV) for therapeutic gene delivery. Recent studies leverage structural and evolutionary information on AAV as a starting point for the design of synthetic viral vector systems, a first generation of which is referred to as AncAAVs which are now progressing to the clinic for a number of indications. Dr. Vandenberghe previously co-founded GenSight Biologics and Akouos. He also is a founder, board member, and advisor to Odylia Therapeutics, a non-profit catalyzing translation for gene therapies within the challenging field of ultra-rare disorders. Dr. Vandenberghe has over 50 peer reviewed publications and more than a dozen licensed patents, mostly related to gene therapy methods, technologies, and applications.

**Q** What characteristics of Adeno-Associated Virus (AAV) make it a favorable vector for enabling gene transfer?

"AAVs are overall safe, potent, and allow for modifications that enable us to select for or modulate vector phenotypes relevant to particular gene transfer applications"

**LHV:** AAVs have several favorable characteristics for gene delivery, but probably their most attractive feature is the fact that they are non-pathogenic and safe to use in patients. Over the past four decades, and specifically in the last 30 years, molecular engineers have worked to enhance the safety of AAVs by eliminating all viral genes out of this particle. This allows us to use recombinant vectors *in vivo* to target specific tissues and cell activities with a high degree of potency. Another advantageous characteristic of AAVs is the availability of multiple natural serotypes. Utilizing specific natural serotypes, and/or further engineering them, allows us to select specific functionalities or phenotypes fitting to the indication, modulate tissue targeting, and modify manufacturability.

In short, AAVs are overall safe, potent, and allow for modifications that enable us to select for or modulate vector phenotypes relevant to particular gene transfer applications.

**Q** What are limitations of AAV as a gene vector?

**LHV:** There are two main limitations to AAV in my view. First, the DNA packaging capacity is innately restricted to 5KB. Beyond that limit, yields are reduced and DNA fragmentation causes the final preparation to lack homogeneity. For many of the current uses, the 5KB size limitation is sufficient but it limits our ability for larger cDNA constructs or the transfer of larger disease genes. This capacity is further reduced when you seek to incorporate e.g. regulatory required elements to refining where, when, and how much the gene therapy is expressed.

A second important limitation to the AAV platform is presented by the issue of pre-existing immunity; the Achilles heel of AAV. This means that if a patient has encountered an AAV prior to their treatment, they

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will likely have developed immunity against this AAV particle. These long lasting memory responses can thwart and in many cases fully blunt the therapeutic effect of the genetic drugs we are developing. This unfortunately means that while we are building transformative therapies based on AAV, pre-existing immunity permits only a subset of the population benefits from these transformative therapies – until we find a way to mitigate or overcome this issue. We, as a field, are compelled to offer these therapies to the entire patient population and need to address the limitation of pre-existing immunity.

**Q** What phenotypic changes are possible when the sequence of an AAV capsule is changed, and how might this aid gene therapy translation?

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**LHV:** This field started in a very descriptive fashion, where scientists mainly using bio-mining techniques, isolated variants of AAV that are naturally circulating, vectorized them, and used them in preclinical models, with some making it to clinical settings. These different variants were shown to result in a plurality of both quantitative and qualitative phenotypes. Remarkably, many of those variants were only different on protein component capsid by a few dozens of amino-acids. These changes altered how well these particles are manufactured, how much yield they have in a manufacturing process, how and how efficiently they target cells, how specific they are to these targets, and even how the host immune systems reacts to them. For example, when administered intravenously, most AAVs go to the liver, but some viruses are a hundred-fold better than other viruses or viral vectors for this task. A qualitative phenotype was shown in the fact that a few AAVs can cross the blood brain barrier systemically after IV administration, which is otherwise inaccessible to most drugs, let alone large particles like AAVs. It is this finding that was leveraged for the gene therapy under development by Avexis for Spinal Muscular Atrophy which is IV administered to reach motor neurons in the spinal cord to treat this fatal disorder.

**Q** What are some of the challenges associated with changing a capsid to fit a phenotype?

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**LHV:** That's an interesting question. That is the focus of our work, as well as others in the field.

When we study viruses that are slightly different in their capsids, we observe these qualitative and quantitative changes that I mentioned in the previous question. In our current work, we further combined capsid variations seeking to identify novel and/or improved phenotypes. The challenge with these approaches is that AAV is a highly complex biological molecule. There are 60 molecules that make up an AAV capsid that tile together in pristine symmetry to form an icosahedron, a 20-faceted structure, almost like a buckyball. Any change that we engineer in must be compatible with this architecture. So, the ability for us to design and build variation onto an AAV model is limited. This is further complicated by the fact that we lack a real structure-function blueprint. This remains one of the main challenges of engineering new capsids to seek phenotypic improvements.

**Q** Your lab uses ancestral sequence reconstruction to predict ancestral sequences of AAV capsids in order to grow and identify synthetic AAV particles, such as the novel virus Anc80. What are the specific properties of Anc80 that make it a desirable vector for gene transfer?

**LHV:** The challenges associated with engineering new particles served as the starting point for the approach of ancestral sequence reconstruction. We aimed at functionally exploring novel architecture and novel compositions that adhere to the icosahedron symmetry, but had no real roadmap on how to do this. One early idea was to go back to viruses from the past, since we knew they had originally adhered to this symmetry, model what they may have looked like, and use that as a roadmap to build new structurally sound capsids. Moreover, this allows us to interrogate the variation in currently used AAVs in smaller steps, and explore vector phenotypes at these various stages so we can map them more accurately. We believe that this type of data may lead us to a blueprint that in the future could allow for rationally designing these potent carriers for gene therapies. To that regard, one of our first successes was building Anc80L65, which approximated an ancestral vector at the root

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of vectors such as AAV1, AAV2, AAV3, AAV8 and AAV9, which are currently in clinical testing.

We were surprised that Anc80 ended up being an extremely robust vector, with its own set of phenotypic changes. For example, this ancestral virus' potency in targeting the liver of both small and large animals is similar to current vectors. More recently, we've shown that Anc80L65 following injection in the cochlea has a unique ability to target inner and outer hair cells. These findings, which may ultimately be relevant for gene therapy approaches in hearing loss, were published in a 2017 issue of Nature Biotechnology.

Overall, Anc80 is a robust vector with a host of applicable phenotypes. Some of those are analogous to current vectors, yet, in some ways, Anc80 seems to be superior. We are now moving forward with translational studies to build out further programs on this particular viral capsid.

**Q** In response to the challenge of pre-existing immunity to AAV, is there any evidence that Anc80 will not have these issues?

**LHV:** As mentioned, pre-existing immunity is the Achilles heel to the entire field, and particularly relevant in systemic administration. One approach to overcome this issue is to develop a vector technology that is as far removed from agents that are circulating in human populations. We felt AAV is more than an adequate starting point in terms of its safety and efficacy profile for various targets. In that we aimed to move AAV away from those naturally circulating AAVs, and hypothesized that an ancestral AAV was that 'goldilocks' vector – far removed from an immunological perspective, but not too far in terms of potency and safety. Indeed, the diversity we generated in our initial approach is scattered all over the capsid, potentially resulting in a disrupted binding of the epitope with the antibody. We have modeled this in animals by vaccinating them with one virus, then coming back with Anc80; we see the response is either non-detectable or greatly minimized. Interestingly,

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these responses, especially in humans, are rather broad. Anc80 experiences cross-reactivity, which may cause these epitopes, even when disrupted, to still be recognized by antibodies, meaning that there still is work to to and Anc80 does not fully address this issue.

**Q** What, if any, additional capsid changes will make AAV vectors even better for gene transfer?

**LHV:** There's a long exciting path ahead of us.

One example of how scientists can improve phenotypes and diversity of the existing structure is in the insertion of small peptides into the capsid, carried out by another group on a vector called AAV-PHP.B. This insertion is tolerated architecturally by the virus. The work of the Deverman group at Caltech has shown that we can improve a particular function by way of this directed evolution modality, such as the blood-brain barrier transfer function in the mouse model. Unfortunately, this approach is restricted to the specific mouse model in which it was developed, but this example, alongside our group's example of the Anc80 improvements in transduction of the inner ear, shows additional opportunities for advancement of the field. We are working to further understand the complexity of the capsid which I strongly believe will open up opportunities to target more diseases for more patients.

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**Q** What are the next steps in the commercial development of the Anc80 vector, and how did you come to start working with Lonza?

**LHV:** Many of these technologies have a number of opportunities. The primary reason for our lab to partner with Lonza stemmed from the fact that there's only so many things one group, whether academia or industry, can pursue. We were interested in making our technology broadly available since a multitude of potential disease applications in many fields may be possible, and none of these can be pursued by a single entity. Lonza


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worked with us on a creative model to enable our vector technology and make it broadly available.

In this model we try to address two key limitations. First, the ability to manufacture AAV vectors, especially at scale and for clinical use, is a bottleneck for the field. We recognized that we were not ideally positioned by expertise or bandwidth to develop processes and manufacture our vector technology. In that sense, Lonza, as one of the senior players in the business of manufacturing viral vectors, was a natural fit. From a strategic perspective, that was clearly our main interest in the partnership. The second limitation was how to distribute this technology to all the entities to make the most use of it. Lonza was another good fit as they can provide licenses to compelling translational programs.

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