Roundtable report

Cerba Research

Mitigating immunogenicity risks factors in AAV gene therapy clinical trials

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Cerba Research

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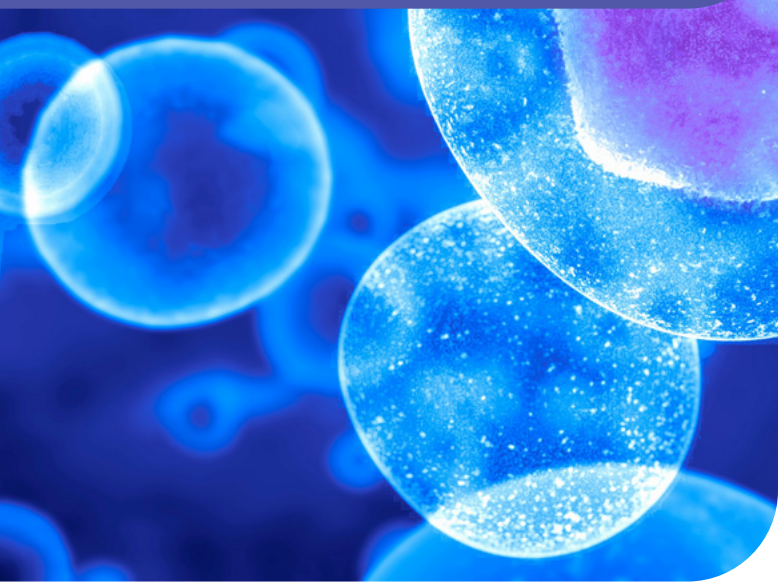

Key takeaways

- rAAV-based therapies immunogenicity risk factors span a wide range.
- Adopting the right bioanalytical strategies is crucial for accurately assessing AAVs' risk factors.
- In vivo and in vitro studies have limitations that can be addressed through industry collaboration.
- Laboratory services providers who view AAV virus-based therapeutics developers as partners can help navigate this challenging field.
- Guidance for developers and laboratory services providers needs to be in step with current and future developments.

Overview

The advent of adeno-associated virus (AAV)-based gene therapies using recombinant AAV vectors (rAAV) has propelled the gene therapy field forward. However, rAAV-based therapeutics pose significant challenges and limitations that require careful evaluation due to the associated immunogenicity both pre- and post-therapy.

Utilizing relevant immunoassays during in vitro and in vivo studies is a key part of adopting the correct bioanalytical strategies to assess the immunogenicity of rAAV-based gene therapies and thus work towards companion diagnostics (CDx) during clinical trials. As a function of those assessments, appropriate clinical mitigation strategies can be employed to alleviate their immunogenicity.

Specialized laboratory solutions providers like Cerba Research can help developers and manufacturers of rAAV-based therapeutics navigate the complex R&D, clinical, and regulatory environment that surrounds the immunogenicity assessment and CDx for these promising therapies.

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Context

The panelists discussed the evolving landscape for rAAV-based gene therapies, current views and approaches to clinical development that may be due for an update, regulatory barriers, and what the future holds for AAV developers, and laboratory services companies alike.

Key takeaways

rAAV-based therapies immunogenicity risk factors span a wide range.

rAAV-based gene therapies are a potent approach to treating many genetic disorders, including pediatric diseases such as spinal muscular atrophy and Duchenne muscular dystrophy. However, they may present serious risks to patient safety if they are not rigorously evaluated and administered.

One of the main risk factors of rAAV-based therapeutics is immunogenicity, (i.e., their ability to provoke an immune response in the patients against the intended treatments). The strength of the immune response depends on patients' own pre-existing immunity, in the form of neutralizing antibodies (NAb), against the rAAV capsid used for therapy. This calls for a robust screening assay for pre-existing antibodies (i.e., total antibodies (TAb) and NAb)) before therapies can be administered, noted Karthikeyan Devaraju, senior scientist, cell and gene therapy, at Cerba Research.

> "Immunogenicity is considered throughout the therapy's development, from production to the clinical phase. People have immunogenicity against certain AAV capsid serotypes, and this can impede therapy if using these serotypes. This needs to be factored in when designing the therapy and associated screening assays."
>
> **Karthikeyan Devaraju,** Cerba Research

There are many other immunogenicity-related challenges in rAAV gene therapy, including the immune response towards the therapeutic gene, and dose and route of administration.

Improving distribution and penetration into the target organs and cells in relation to dosage has a direct impact on the outcome of the therapy. Systemically administered rAAV-based therapies pass through the liver, the main organ for detoxification. This needs to be factored in when selecting the right serotype and dosage for the intended target organ/cells while avoiding unwanted transduction of non-target tissues.

Managing dose-related ratios of viral genome against empty viral particles is another measure that can help limit immunogenicity. Empty capsids will increase the immunogenicity risk and make the therapy ineffective. The rAAV production process plays a role in improving the ratio of packaged viral genomes within capsids.

> "Affinia and others are trying to develop novel capsids that improve efficacy, AAV transduction, and biodistribution tenfold or even one hundredfold, so that you can lower the dose and thus have less of an immune response."
>
> **Roberto Calcedo,** Affinia Therapeutics

> "In most therapies for genetic diseases, you have to look at the benefit-risk profile. To better manage the benefit-risk for patients is to think about how you can control the T-cell response."
>
> **Jean-Philippe Combal,** Vivet Therapeutics

Adopting the right bioanalytical strategies is crucial for accurately assessing AAVs' risk factors.

As the rAAV-mediated gene therapies are widely adopted, more immunogenicity risks come to light.

The right bioanalaytical strategies are needed to assess immunogenicity risks. The key assay is the pre-screening assay for TAb/NAb that is needed as a CDx to select the patients for rAAV-mediated gene therapy. The development of CDx needs specialty testing laboratories with regulatory knowledge.

"Identifying a corresponding CDx is essential for the appropriate use of rAAV-based gene therapies, due to the importance of risk-benefit analysis in applying those therapies. You only have one chance to treat the patient, and you cannot fail in your assay," said Jean-Philippe Combal, co-Founder and CEO at Vivet Therapeutics.

Combal also noted that there is a temptation when choosing a CDx to focus on the type of assay used (e.g., NAb, TAb, cell-based assay) while overlooking the need to set the right threshold for decision making based on the assay's output. Other panelists concurred that the lack of cell-based NAb assay standardization is an issue for selecting a CDx. As a result, many companies are gravitating toward the most accessible option, said Roberto Calcedo, VP of preclinical and immunology at Affinia Therapeutics.

> "A standard assay means you use the same cell line, the same multiplicity of infection (MOI), the same serum dilution. All these things are still not normalized and, because of the complexity of using neutralizing antibodies assays, what the field has done is favor total antibodies assays."
>
> **Roberto Calcedo,** Affinia Therapeutics

> "I've spent the last 20 years in the vaccine industry and neutralizing antibody assays are still the gold standard . . . Maybe that's the future for AAV serotype 8 or 9, where there will be a standard that comes out and you can compare assay to assay."
>
> **Keith Gottlieb,** Elpida Therapeutics

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In vivo and in vitro studies have limitations that can be addressed through industry collaboration.

One way that AAV therapy manufacturers are trying to get around the problem of lack of standardization is by using in vivo and in vitro modeling for neutralizing antibodies. However, these in vivo assays are complex and have important limitations, including very high variability and validation challenges, which most individual manufacturers do not have the resources to do by themselves.

> "It is very difficult to extrapolate thresholds from monkey data because the potency or the affinity in monkey models might be different."
>
> **Jean-Philippe Combal,** Vivet Therapeutics

The way to go is with in vitro and laboratory services providers who have the capabilities to do this, Calcedo said. He added that there is an even better solution, if the industry can organize itself and come to an agreement.

> "Having laboratory services organizations that have an AAV-neutralizing antibody assay validated that can be used by everyone contracting their services to test their sample would be ideal."
>
> **Roberto Calcedo,** Affinia Therapeutics

"From a specialty laboratory solutions perspective, the knowledge and technology to develop such an universal assay is out there and Cerba Research is already building on it," said Devaraju. "We are evolving with the technologies as they are being adopted for the therapies."

Laboratory services providers who view AAV virus-based therapeutics developers as partners can help navigate this challenging field.

"Because of the multiple clinical development, clinical application, and regulatory challenges that surround AAV virus-based gene therapies, finding the right specialty laboratory partner is paramount for developers. The best relationships are those rooted in a collaborative mindset," said Hanna Lesch, chief technology officer, at Exothera, a custom development and manufacturing organization (CDMO) active in the field.

> "A client relationship is different to a partnership. The way I see it, customers are partners for us because we are developing something for them together."
>
> **Hanna Lesch,** Exothera

"Having a partner-level working relationship with laboratory services providers and CDMOs is especially critical for AAV therapy developers because of the singularity and novelty of the therapies being developed and manufactured, and the discovery mindset needed to traverse that terrain. The early phase of AAV clinical trials is a bit of a learning curve for both sides," said Keith Gottlieb, head of program management and operations at Elpida Therapeutics.

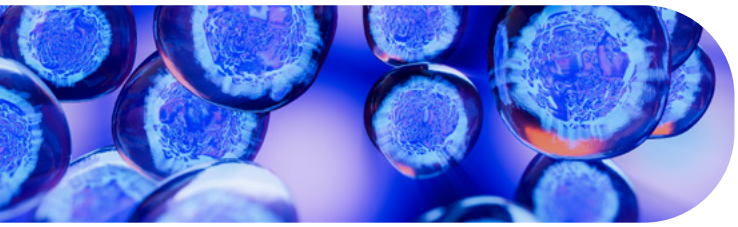
Gottlieb further explained that this mutual reinforcement extends to collaboratively teaching regulators that the conventional methodology of conducting clinical trials cannot necessarily be replicated in trials for genetic disorders or other rare diseases. "We deal with ultra-rare populations, so what's always been done does not apply. You can't run placebo-controlled trials or double-blind trials when you only have eight patients," he said.

> "CDMOs, specialty laboratory solutions providers, and therapy developers make a perfect triangle to ensure that the product gets an approval fast."
>
> **Hanna Lesch,** Exothera

Guidance for developers and laboratory services providers needs to be in step with current and future developments.

Having an authentic partnership between therapy developers on one side and CROs, specialty laboratory solution providers, and CDMOs on the other is essential for all parties, particularly when dealing with regulators.

Since the latter are not allowed to engage directly with regulatory agencies for methodological or technical issues related to the clinical trials they conduct, they depend on their sponsors as intermediaries, even though they carry the responsibility for justifying the final product to regulators.



"It is very important for the person responsible for product development to closely follow the regulatory landscape. Guidelines tends to lag behind the rapid advancements in the field, so staying informed about new technologies that might enable innovative solutions is critical," Lesch said. "At the end, I'm the one who's justifying my product to the regulatory bodies, so I need to be on the front line and know what's happening."

Other panelists agreed that they always had to stay a step ahead of regulators or plan engagement with them strategically. They mentioned multiple other challenges stemming from unharmonized or ever-changing regulations in the US and Europe.

"The first regulatory complexity is to ask where I should do my clinical trial: in Europe or in the US?" Calcedo said. He acknowledged that the vast amount of regulatory red tape in Europe, which is compounded by individual EU countries having their own regulations on top of EMA regulations, often pushes sponsors toward running trials in the US.

"We've been trying to run trials in different countries in Europe and it's not just the EMA requirements; you have to go to each country. Italy, Spain, and Germany each have their own requirements, and some are easier than others," Gottlieb observed. "That's definitely been a challenge."

Another recent regulatory hurdle stems from the fact that CDx, whose modality is in the form of assays, are now governed by a regulation that regards them as medical devices, Calcedo observed. That puts a completely different standard to what an assay has to be, bringing the burden of developing an assay to a standard that is only achievable by large companies. (Most AAV gene therapy developers are small biotechs with limited resources.)

"Assays becoming a medical device is an unknown trajectory for many of us, so we want to work with sponsors and regulators as to how to address this," said Devaraju. "At the end, we want to ensure that sponsors and ultimately patients are benefited."

> "What I look forward to with a lot of hope is the evolution of regulatory guidelines and development approaches toward therapeutic platforms where an AAV combined with a specific effector that gets modulated through multiple targets and multiple diseases will enable synergies. From a regulatory point of view, I also hope that learning will be shared, which is especially important for rare diseases."
>
> **Letizia Goretti,** Alia Therapeutics

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Conclusion

As progress in the field of AAV gene therapies continues, the next few years will be key for developing and evaluating AAV modalities that deliver therapies at lower doses but with better distribution, to minimize immune response in patients. "I think the future will come from new AAV capsids. There's a lot of engineering regarding novel capsids and the goal is to reduce the dose," Calcedo said.

> "Having a super strong capsid that can reduce the dose threefold, fourfold, or even greater will tell us if AAV development is moving to the next phase."
>
> **Roberto Calcedo,** Affinia Therapeutics

"We should not forget the correct construct design with right promoter selection. From a manufacturing point of view, we will have better tools in our hands," Lesch added. "We will have super producer cells that produce high titers, better full/empty ratios from the start, and better purification tools with better recoveries. We will be putting better-quality vectors into patients."

> "My hope is that prices come down, that the regulatory aspects will become a little better...and that regulators will have a better understanding that creating hurdles is preventing innovative therapies from getting to market when they cause companies to just drop them. In terms of the immunogenicity aspect, the idea of biomarkers needs to be pursued a bit more."
>
> **Keith Gottlieb,** Elpida Therapeutics

Devaraju concurred with the outlook that not only prices but also costs for AAV gene therapy development will likely come down. As we progress, the costs of AAV trials will come down, as we have seen with CAR-T trials.

Finally, the development of AAV therapies together with specialized testing solutions and TAb/NAb screening is expected to move towards a platform approach. Standardization, in combination with innovative approaches towards a platform approach for immunogenicity in both pre-screening and post-therapy, will aid the development and accessibility of safe and efficient rAAV therapies.

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Biographies

Dr. Roberto Calcedo

VP, Preclinical & Immunology, Affinia Therapeutics

Robert Calcedo was instrumental in the discovery and characterization of AAV7, AAV8, AAV9, AAVrh10, and other AAVs with significantly improved transduction profiles and reduced immune responses. He also worked on developing adenovirus vectors as genetic vaccines: he was critical in discovering that the adenovirus is part of the gut microbiota where it resides, even in healthy individuals, and that the biology of adenoviruses and immunity to them are very different between humans and primates. Calcedo is the co-inventor of several patented products and the author of more than 75 peer-reviewed publications. He holds a PhD in biological sciences from the University of the Basque Country (Universidad del Pais Vasco) in Spain, where he was a pre-doctoral fellow.

Jean-Philippe Combal

Co-Founder & CEO, Vivet Therapeutics

Jean-Philippe Combal has more than 27 years of experience in the pharma and biotech industries. He has a broad range of experience leading global development and a successful track record of innovation and development in the area of orphan drugs and gene therapy. As chief operating officer of GenSight Biologics from 2014 to 2017, he was instrumental in an $80 million investment round, including a successful IPO, and he piloted the advance of two major gene therapy programs from non-clinical to Phase III pivotal trials, as well as regulatory and launch readiness strategies. He is also the chair of the board of directors at SpliceBio.

Dr. Karthikeyan Devaraju

Senior Scientist, Cell & Gene Therapy, Cerba Research

Karthikeyan Devaraju joined Cerba Research in 2022 as a cell and gene therapy R&D scientist. In his current role, he has been instrumental in expanding the company's molecular clinical trial solutions for cell and gene therapy. Devaraju has conducted research on neuroregeneration, induced pluripotent stem cells, and cell fate engineering at major institutions, such as Lund University in Sweden, the University of Geneva in Switzerland, and the Max Planck Institute in Nijmegen, Netherlands. His expertise spans stem cells, viral vectors, product development, process optimization, and analytical development. He holds a PhD in stem cells and neurology from Lund University.

Dr. Keith Gottlieb

Head of Program Management & Operations, Elpida Therapeutics

Keith Gottlieb brings over 20 years of experience in clinical immunology and vaccine development to gene therapy, specializing in the development and validation of correlates of immunity and identifying correlates of protection. He has held senior leadership roles at Vaxart, Emergent BioSolutions, and Focus Diagnostics, contributing significantly to advancing various vaccine programs for norovirus, influenza, RSV, COVID-19, Lassa fever, Ebola, and Chikungunya, among others. Gottlieb earned his PhD with a focus on polyomavirus and completed post-doctoral research on hantavirus, concentrating on virus biology in host species. He is now dedicated to advancing gene therapy programs at Elpida Therapeutics, with the goal of treating as many children as possible.

Letizia Goretti

CEO, Alia Therapeutics

With over 25 years of international experience, Letizia Goretti has a broad background in strategic, operational, R&D, and commercial activities in the life sciences sector. She served as the senior director of transactions and alliances at the Johnson & Johnson Innovation Centre in leading strategic partnerships focused on genomics initiatives such as the UK Biobank Whole Genome Sequencing Consortium and Our Future Health. At Johnson & Johnson in Belgium, she played key roles in launching the hepatitis C and HIV portfolios and managing a $1.2 billion mature brand portfolio. She is now the CEO of Alia Therapeutics, a start-up founded by Professor Anna Cereseto and her team at Trento University that uses innovative genome editors to expand the range of targetable genomic sites and enhance the precision of genetic modifications.

Dr. Hanna P. Lesch

Chief Technology Officer, Exothera

Over the years, Hanna Lesch has held multiple directors positions, leading R&D at FKD Therapies, Finvector, and the Kuopio Center for Gene & Cell Therapy. Her research has focused on gene therapy and translational development, including early-stage analytics and the development of scalable, robust manufacturing processes under current regulatory guidelines. Lesch's PhD in Molecular Medicine was obtained from the University of Kuopio in Finland, with post-doc work there and at the University of California San Diego. She has several patents related to vector manufacturing. As chief technology officer at Exothera in Belgium, her main responsibilities are technology development and innovation in bioproduction platforms.

Dr. Andrew Warmington

Manufacturing Editor, Citeline (Moderator)

Dr. Andrew Warmington has been writing about pharmaceuticals and related industries since becoming editor of Speciality Chemicals Magazine in 2002. His particular area of expertise is in the C(D)MO drug substance and CRO markets, plus the regulatory side, which was developed during a stint with Chemical Watch. He joined Citeline as Manufacturing Editor in 2022, since when he has authored numerous white papers and chaired roundtables for clients.