Key Considerations for Nonclinical AAV Gene Therapy Development

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THE IMPORTANCE OF ADENO-ASSOCIATED VIRUS (AAV) GENE THERAPY

The first AAV-based therapy was developed for patients with an inherited disorder causing progressive blindness. This milestone paved the way for a surge in clinical trials exploring AAV-based gene therapies, which use the cellular functions of target cells to incorporate the AAV capsid through the cell membrane via endocytosis. By adding a functional gene to compensate for a mutated or absent one, AAV-based gene therapy offers an innovative approach to restoring functional protein production.

Benefits of AAVs for Your Gene Therapy Studies

- Derived from non-pathogenic human parvovirus
- No known diseases associated with AAV infection
- Low toxicity
- Replication-defective, ensuring controlled and safe use
- Long-term expression
- Cell-type specificity

Read on to discover

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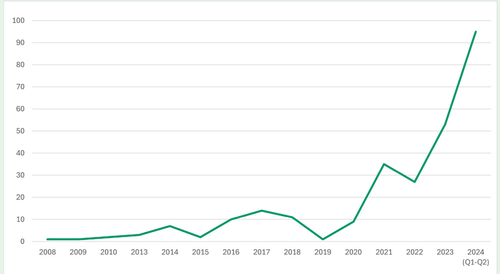
ALTASCIENCES' PROVEN TRACK RECORD IN GENE THERAPY STUDIES

Our capabilities and wealth of knowledge allow us to seamlessly navigate your gene therapy program.

With over five years of expertise, we employ CRISPR gene-editing techniques in nonhuman primates (NHPs) across various routes of administration, using our comprehensive experience in bioanalysis to conduct biodistribution studies.

We perform *in vivo* nonclinical studies of cell and gene therapies, such as on- and off-target activity, and immune responses—all of which necessitate careful monitoring and rigorous assessments.

Number of Gene Therapy Studies Initiated at Altasciences' Preclinical Site in Seattle by Year



YEAR	STUDIES PERFORMED
2008	1
2009	1
2010	2
2013	3
2014	7
2015	2
2016	10
2017	14
2018	11
2019	1
2020	9
2021	35
2022	27
2023	53
2024 (Q1-Q2)	95

Altasciences' Gene Therapy Study Experience

GENE DELIVERY SYSTEM	SPECIES		2022	2023
AAV (AAV1, AAV2, AAV8, AAV9, AAVrh74, engineered AAV)	NHPs	No. of studies	8	22
		No. of animals	213	262
	Rodents	No. of studies	3	0
		No. of animals	131	
LNP	NHPs	No. of studies	19	34
		No. of animals	556	829
	Rodents	No. of studies	0	5
		No. of animals		476
Oligonucleotides (ASOs, RNAi)	NHPs	No. of studies	8	6
	Rodents	No. of studies	15	11

NAVIGATING THE REGULATORY LANDSCAPE

Study Design Compliance Requirements

Altasciences' scientific and regulatory teams have decades of experience designing tailored research programs and conducting the studies required to ensure that your IND/NDA-enabling toxicology, safety pharmacology, and laboratory studies meet global regulatory standards.

As per the FDA guidance titled **"**S12 Nonclinical Biodistribution Considerations for Gene Therapy**"**, biodistribution studies are performed in the early phases of nonclinical studies so that the data is available when evaluating pharmacology and toxicology findings. Stand-alone biodistribution studies not conducted under GLP conditions are acceptable. When biodistribution is evaluated as part of a GLP study, the sample analysis can be conducted under non-GLP conditions, but all in-life evaluations and sample collection procedures must remain GLP-compliant.

Biodistribution studies require appropriate analytical methods to detect gene therapy products and identify transferred genetic material in collected samples. In some cases it may also be necessary to detect the expression products of the transferred genetic material. Insights gained from biodistribution studies are essential for designing and informing other nonclinical safety assessments, such as toxicology studies and immunogenicity evaluations. These insights help determine potential target tissues, establish appropriate sacrifice intervals, and set study durations, thereby contributing to a comprehensive evaluation of the gene therapy product's safety.

We can complete your large molecule program in approximately six months for the *in vivo* portion of the IND/CTA program.

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What You Need to Know About Conducting Animal Studies

In addition to the ICH S12 guidelines, the FDA guidance titled **"Preclinical Assessment of Investigational Cellular and Gene Therapy Products"**, emphasizes the importance of selecting a suitable animal model for successful clinical translation, and provides direction on species selection in gene therapy studies.

The FDA states that the chosen species must be biologically active to the investigational gene therapy product, and should:

- have comparable physiology and anatomy similar to humans;
- show similar pharmacological responses to the expressed transgene;
- be compatible with feasible delivery methods for the planned clinical system;
- be susceptible to viral or microbial vectors used in the gene therapy, and;
- exhibit immune tolerance to the human gene product or transgene.

In some cases, non-standard test systems may be considered.

These requirements make NHPs a preferred model in gene therapy studies. Altasciences' researchers specialize in conducting *in vitro* and *in vivo* feasibility studies with NHPs to determine the biological relevance of your investigational product.

Our experts design studies with precision to assess vector distribution in both target and non-target tissues and biological fluids, providing you with the data needed to make informed decisions.

Three Key Factors in Designing Gene Therapy Studies

1. Biologically relevant species must:

- support transfer and expression of the genetic material
- demonstrate competence in tissue tropism, gene transfer efficiency, and transgene expression

2. Biodistribution—vector presence:

- in desired target tissues/biological fluids
- in non-target tissues/biological fluids
- in the germline

3. Safety considerations:

- potential for random integration into host DNA
- potential immune response to the capsid or transgene proteins
- biodistribution data, coupled with clinical pathology and histopathology, to determine correlations with tissue-specific detrimental effects

Route of Administration

The route of administration is highly dependent on the intended site of transgene expression. Altasciences primarily uses intravenous administration for gene therapy studies, including methods like IV bolus injection and IV infusion (ranging from 15 minutes to an hour). This approach is favored for various reasons, including precise dosage control and faster delivery. Alternative routes, such as subcutaneous, intrathecal, intravitreal, and suprachoroidal, are also available.

It is critical that the intended clinical route of administration is used for gene therapy product delivery, though a product may require the need to be administered intravitreally for an ocular indication.

	ALTASCIENCES' TOTAL NO. OF INJECTIONS	
	Intrathecal (IT)	Intracisternal Magna (ICM)
NHPs (2021 to 2023)	229	16

Serotypes

There are at least 13 AAV serotypes routinely used in drug development. These serotypes differ in their ability to infect certain tissues and cell types, and depend on their target cell's surface receptors, and corresponding binding sites present on the capsid.

The benefits of AAV vectors include broad tissue tropism, the ability to transduce non-dividing cells, and long-term gene expression. The diversity of these variants allows us to choose the most suitable serotype when targeting specific organs or cell types. It also contributes to the versatility of AAV-based gene therapy, so we are able to optimize the delivery of therapeutic genes to achieve desired outcomes.

Tissue Tropisms

PREFERRED TISSUE TYPES (TARGET)	SEROTYPES
CNS	AAV1, AAV2, AAV5, AAV8
Heart (cardiac muscle)	AAV1, AAV8, AAC9, AAVrh.74
Intestines and lymph nodes	AAV10
Kidney	AAV2
Liver	AAV3B, AAV8, AAV9, AAV10
Lungs	AAV5, AAV9
Pancreas	AAV1, AAV8
Photoreceptor cells	AAV2, AAV5, AAV8
Retinal pigmented epithelium (RPE)	AAV1, AAV2, AAV5, AAV8
Skeletal muscle	AAV1, AAV8, AAV9, AAVrh.74

HOW ALTASCIENCES OPTIMIZES THE SAFETY AND EFFICACY OF AAV-BASED THERAPIES

Preexisting antibodies against AAV vectors pose a significant challenge. Many patients naturally carry antibodies from exposure to the wild-type virus and, similarly, NHPs can develop antibodies and cell-mediated immune responses due to environmental exposure to AAVs. Targets for these responses include the AAV capsid protein and vector DNA. Additionally, impurities from vector manufacturing can trigger immune reactions. Because of this, managing the impact of preexisting antibodies against AAV vectors is paramount.

Strategies and Considerations for AAV Immunity Challenges

Altasciences takes several factors into consideration to address these challenges, which we will explore in-depth on the following pages:

- screening
- evaluating seronegativity rates and serotypes
- AAV pre-screening
- seroconversion
- immunomodulation
- biodistribution
- tissue collection
- PCR analysis, design, and qualification
- vector shedding assessments
- inadvertent germline integration
- immunogenicity assessment
- expressed protein quantitation

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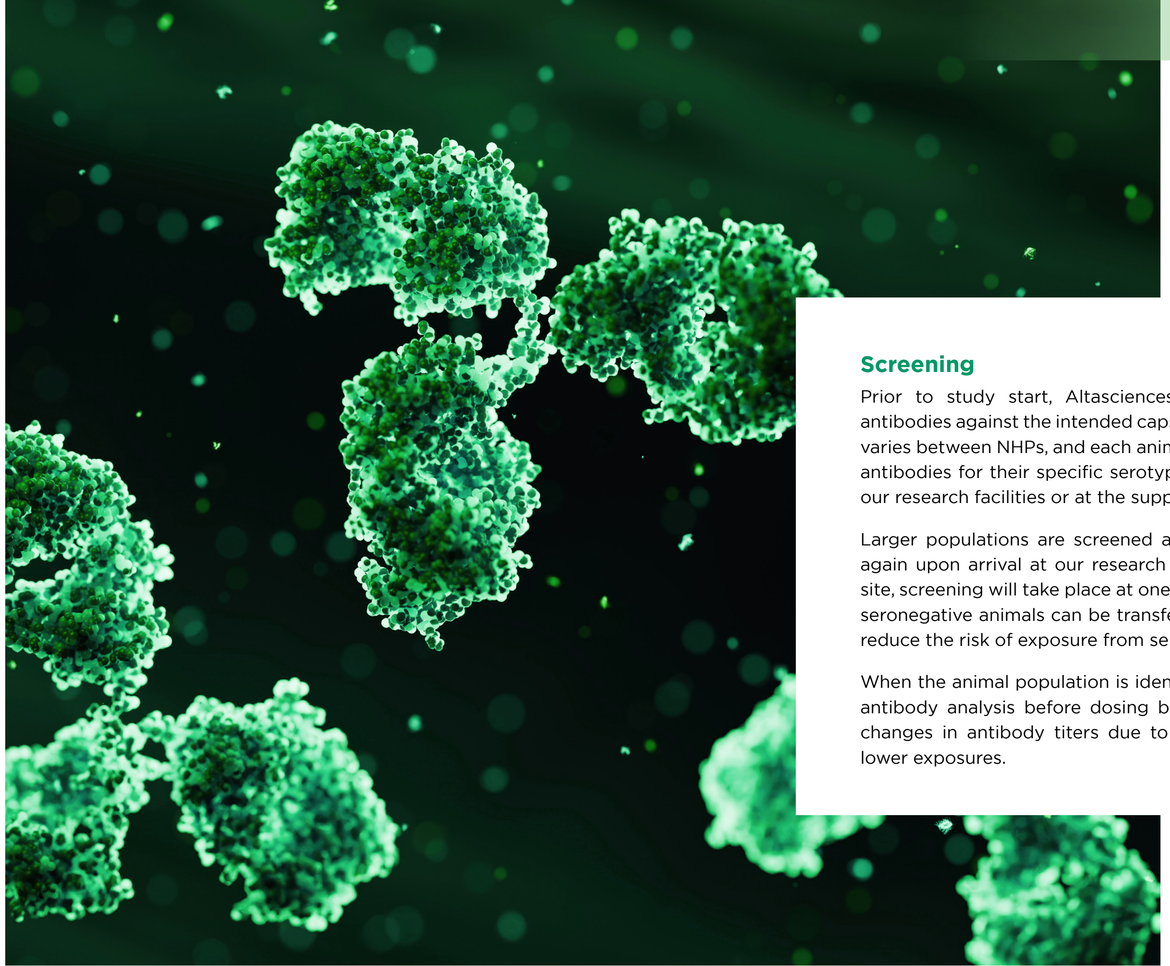


Screening

Prior to study start, Altasciences screens each NHP for preexisting antibodies against the intended capsid. The degree of preexisting immunity varies between NHPs, and each animal will be screened for the presence of antibodies for their specific serotypes. Screening is done either at one of our research facilities or at the supplier's site.

Larger populations are screened at the supplier, before being screened again upon arrival at our research facility. If the animals are already on-site, screening will take place at one of our research facility colonies, where seronegative animals can be transferred to their assigned study rooms to reduce the risk of exposure from seropositive animals.

When the animal population is identified, we collect samples for potential antibody analysis before dosing begins to account for any unexpected changes in antibody titers due to seroconversion—which could lead to lower exposures.

Evaluating Seronegativity Rates and Serotypes

Understanding seronegativity rates and serotypes in gene therapy is crucial for evaluating the immune response. Seronegativity rates indicate the absence of preexisting antibodies against viral vectors, while serotypes represent their distinct variations. Analyzing these factors optimizes patient eligibility, enhances therapeutic outcomes, and reduces adverse reactions.

Data collected at Altasciences over a two-year period shows that the seronegativity rate varies between serotypes but exhibits less variation based on origin.

AAV8 is a notable standout in the data, showing that only 36% of 1,219 NHPs screened were negative. For AAV8 or AAV9 serotypes, our scientists need an additional two months before your preferred study start date to ensure sufficient research animals are available.

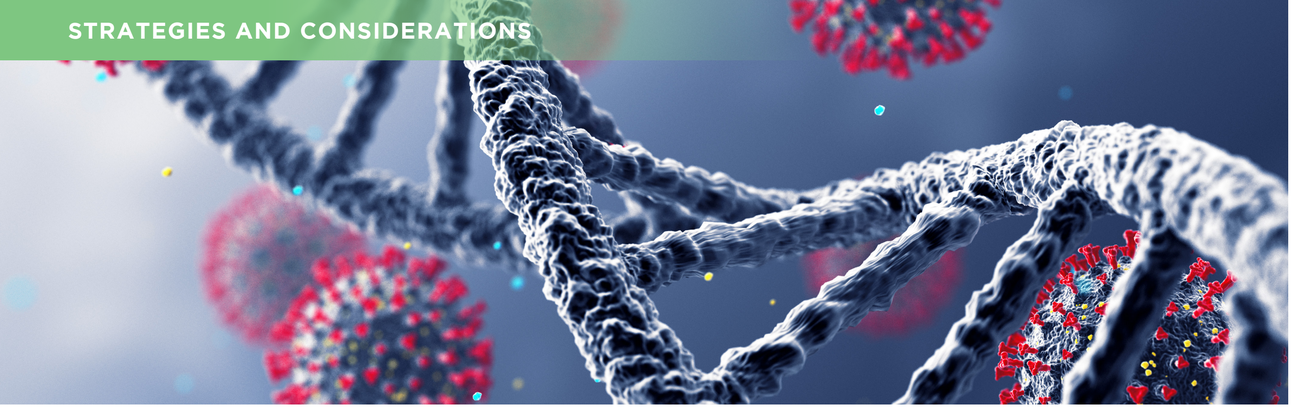
AAV SEROTYPE/ORIGIN	% NEGATIVE (N= NUMBER OF NHPS SCREENED)		
	CAMBODIAN	MAURITIAN	PHILIPPINE
AAV1	81 (n=30)	-	-
AAV2	77 (n=30)	-	83 (n=24)
AAV3	73 (n=30)	-	-
AAV5	100 (n=100)	-	97 (n=34)
AAV6	100 (n=30)	-	-
AAV7	67 (n=30)	-	-
AAV8	36 (n=1219)	44 (n=25)	-
AAV9	61 (n=160)	40 (n=25)	79 (n=34)
AAV10	80 (n=30)	-	-

AAV Pre-Screening

We have extensive expertise in developing, optimizing, and validating custom assays, including immunogenicity testing and biomarkers using ligand binding platforms.

To account for the high prevalence of preexisting humoral immunogenicity, research animals are screened for the presence of serotype-specific antibodies. We use two primary assays for AAV: (1) ligand binding assays, aimed at detecting total antibodies (TAbs), and (2) neutralizing antibodies (NAb), which detect antibodies that bind to the viral capsids, and block viral entry into cells.

We can develop ligand binding platforms for AAV serotype 8 for AAV-based therapies.



Seroconversion

The process of seroconversion involves the initial absence of detectable antibodies, followed by production and subsequent detection in the blood stream. The timing for this step is key. Therefore, we will begin such studies no more than a month after the animal species is selected to reduce the risk of seroconversion. Our experts will work with you to modify or redesign your study requirements, if necessary, to accommodate specific limitations.

Immunomodulation

Preexisting immunity can be mitigated by excluding seropositive animals for specific AAV antibodies. Immune responses can also be reduced by using immunosuppression—though it should be noted that immunosuppression is not recommended for the sole purpose of evaluating biodistribution, other than in particular cases, such as the species-specific nature of transgenes, or an increase in cell-mediated or humoral immune response to the therapeutic.

Immunosuppression may be necessary to mitigate a reaction that would otherwise be rate-limiting for the development of the gene product.

Below are the most common pre-treatment drugs chosen by investigators at Altasciences in 2022 and 2023, and their selected drug combinations.

COMMON PRE-TREATMENT DRUGS AND SELECTED DRUG COMBINATIONS	
Alone	• Dexamethasone, prednisolone, diphenhydramine, or tocilizumab
Combination	• Prednisolone, diphenhydramine • Tocilizumab, tacrolimus • Prednisolone, rituximab, diphenhydramine, sirolimus

AGENTS	CLASS	MECHANISM OF ACTION
Dexamethasone or Prednisolone	Corticosteroids	Reduction of proinflammatory cytokines and chemokines results in global anti-inflammatory and immunosuppressive effects.
Diphenhydramine	Antihistamine	Acts as an inverse agonist at the H1 receptor, thereby reversing the effects of histamine on capillaries, reducing potential anaphylactoid-like reactions.
Sirolimus (Rapamune, Rapamycin)	Immunosuppressive agent	Inhibits T-lymphocyte activation and proliferation that occurs in response to antigenic and cytokine stimulation.
Tacrolimus	Immunosuppressive agent	Calcineurin inhibitor—inhibits T-lymphocyte activation and proliferation, as well as T-helper cell-dependent B-cell response.
Rituximab	Monoclonal antibody	Targets CD2-, a specific B-cell surface antigen, and results in destruction of the lymphocyte.
Tocilizumab	Monoclonal antibody	Binds IL-6 receptors that prevent IL-6 mediated inflammation.

Biodistribution

We employ various strategies to ensure sample quality, such as using disposable scalpels and regularly sterilizing and switching tools to maintain clean collections. From the outset, we consider biodistribution sampling and tissue collection procedures to prepare for downstream sample analysis in gene therapy studies. All collections follow stringent procedures to minimize contamination, and compromise the stability of DNA.

Tissue Collection

While most studies use flash freezing at -80 °C with liquid nitrogen for DNA and RNA tissues, this method can sometimes contaminate samples. To mitigate this when feasible, we use preservatives (e.g., liquid or blood) for blood collection to reduce RNase activity.

We employ column-based methods for DNA and RNA isolation due to their reliability, cost-effectiveness, and low contamination risks. Various column-based kits are available, and are tailored to different tissue types and sizes, including specific ranges for cell numbers, bone marrow, and blood isolations.

Additionally, we employ high-throughput isolation methods if needed, such as QIAcube HT®, and the KingFisher™ purification system bead-beating procedures combined with lysis steps.

Below are our tissue processing capabilities, listed in order of sequence.

- **Homogenization**
  - Tissue Processing
- **Extraction/Isolation**
  - Column-based Isolation Preparation
    - Tissue
    - Blood
- **High-Throughput Isolation Preparation**
  - QIAcube® HT
  - KingFisher™
- **Qualification/Dilution**
  - DNA
    - Quantification and dilution
    - Stored at -20 °C
  - RNA
    - Quantification and dilution
    - Stored at -80 °C

PCR Analysis, Design, and Qualification

All tissue processing, isolations, and PCR assay preparation and instrument analysis are conducted in dedicated rooms. Our facilities offer cutting-edge equipment and tools to develop, optimize, and validate your complex molecular assays—such as Airclean 600 PCR Workstations, and capabilities for quantitative PCR (qPCR) and digital droplet PCR (ddPCR).

PCR instrumentation includes:

- QIAGEN QIAcube® HT nucleic acid purifier
- KingFisher™ purification instrument
- NanoDrop™ One spectrophotometer
- NanoDrop™ Eight
- QuantStudio™ 7 Pro real-time PCR (qPCR) systems
- Bio-Rad™ Automated Droplet Generator
- Bio-Rad™ QX200 droplet reader

We conduct biodistribution analysis of vector and target DNA, and transcript analysis of vector or targets. Established parameters for bioanalysis are used as guidelines for all of our PCR validations—with the one exception being linearity, as it pertains to qPCR only; ddPCR does not use a standard calibration curve. For all targets we assess accuracy, precision, sensitivity, specificity, matrix effect (sometimes referred to as selectivity), and stability for validations where necessary.

Altasciences has a proven track record in rigorously validating assays for biodistribution and transcript analysis. This step is not required by the FDA, but it adds an extra layer of oversight to ensure the best possible data for the assurance of our clients and regulatory bodies—in addition to meeting our own high standards.

PCR Workflow

METHOD TRANSFER
previous working method, established primer/probes
10-15 WORKING DAYS

OR

METHOD DEVELOPMENT
untested primers/probes, no master mix established, full assay design
1-3 MONTHS

METHOD QUALIFICATION
to support non-GLP studies
14 WORKING DAYS

METHOD VALIDATION
to support non-GLP studies
14 WORKING DAYS
plus long-term stability

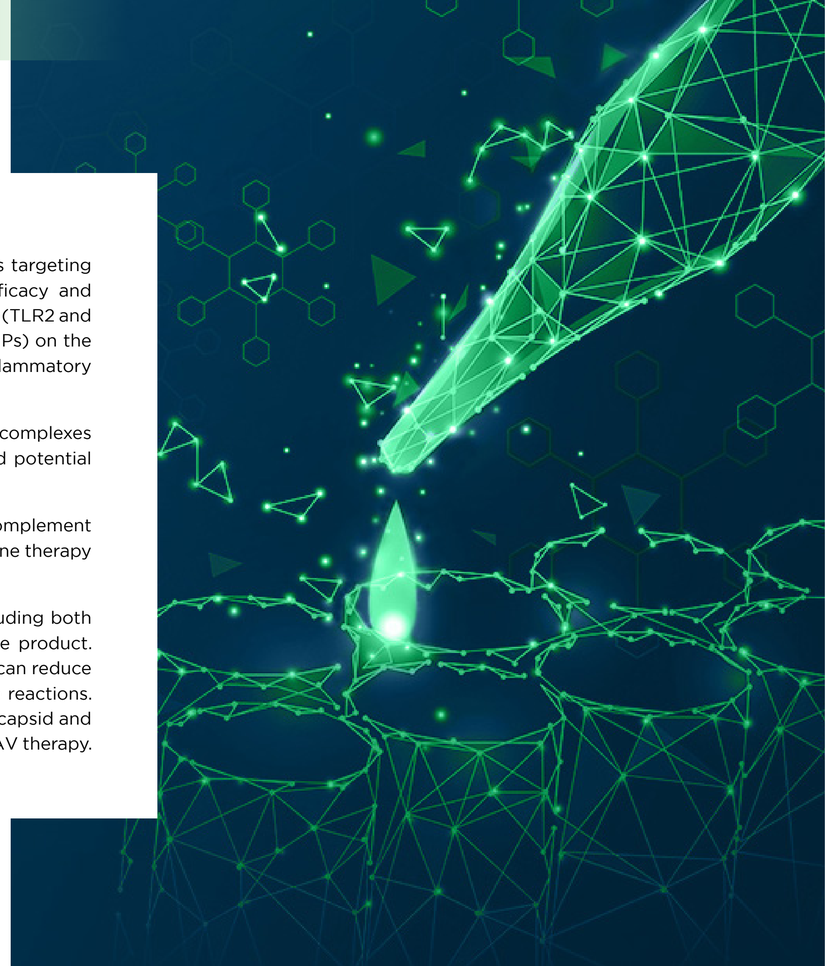
Immunogenicity Assessment

Administration of AAV for gene therapy can trigger immune responses targeting viral components or the transgene product, impacting treatment efficacy and safety. Innate immune responses are activated through toll-like receptors (TLR2 and TLR9) upon recognizing pathogen-associated molecular patterns (PAMPs) on the AAV capsid or genome. This activation leads to the production of pro-inflammatory cytokines like TNF-α and IL-6.

Additionally, preexisting anti-AAV capsid antibodies can form immune complexes with AAV capsids, triggering complement activation, inflammation, and potential damage, which can affect the safety and efficacy of the gene therapy.

We address these concerns by using pro-inflammatory panels and complement assays (Bb, C3a, C4d, C5b-9) on NHP serum to evaluate the impact of gene therapy on immunological endpoints.

AAV administration can also provoke adaptive immune responses, including both humoral and cellular responses against the AAV capsid and transgene product. Humoral responses from preexisting or treatment-emergent antibodies can reduce transduction efficiency, block transgene activity, or cause autoimmune reactions. Some AAV serotypes can induce T-cell responses against both the viral capsid and transgene product, potentially reducing the long-term persistence of AAV therapy.



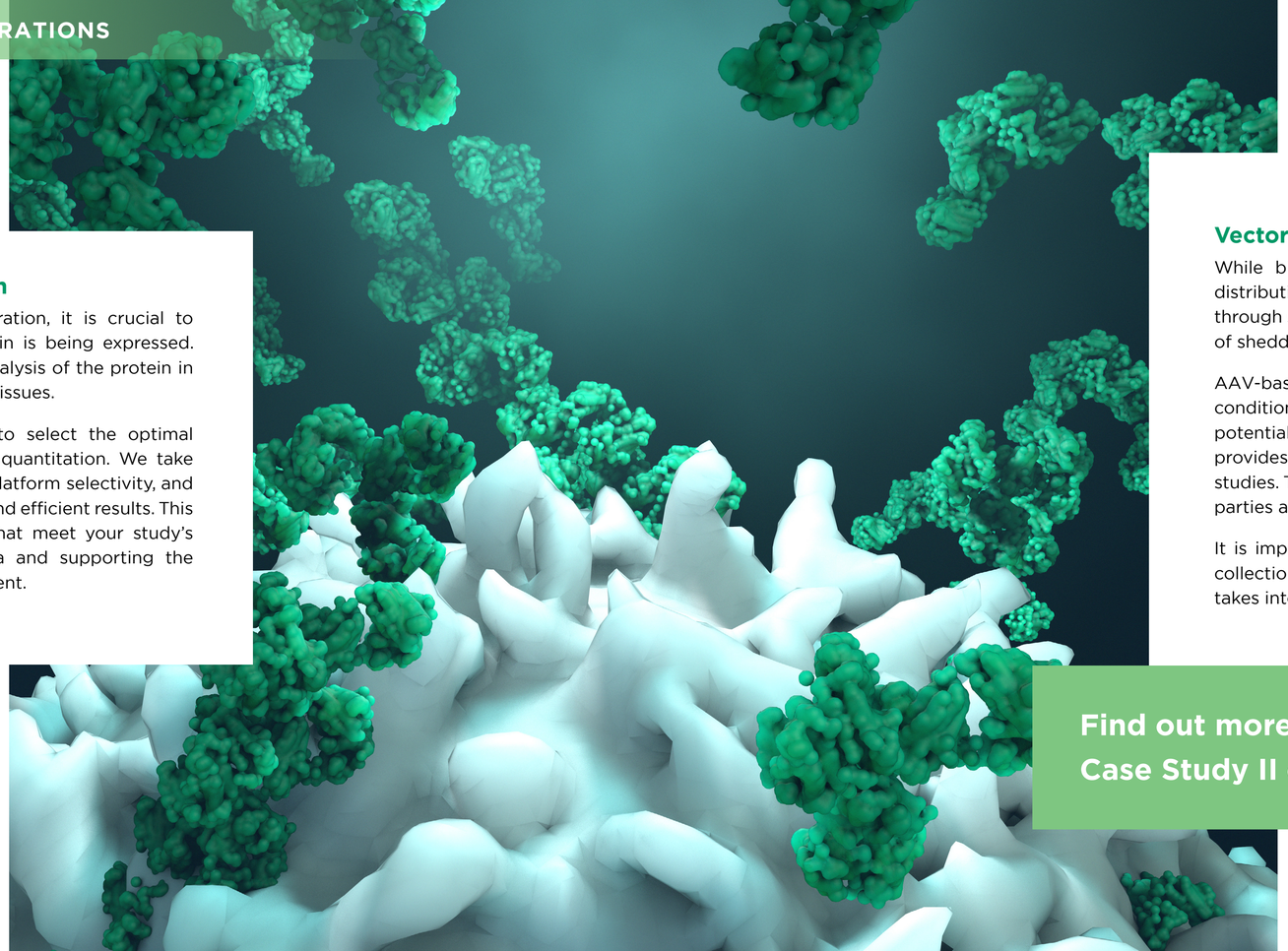
Altasciences' Bioanalytical Assays for Gene Therapies

ASSAY	PLATFORM	CHARACTERISTICS
Anti-AAV capsid tABs	Immunoassay	Tiered approach (screen, confirm, titer)
Anti-transgene protein tABs	Immunoassay	Tiered approach
Anti-AAV-capsid nAB	Cell-based assay	Inhibition of activity
Anti-transgene protein nAB	Cell-based assay	Inhibition of activity
Anti-AAV capsid or anti-transgene	ELISPOT Flow cytometry	Cytokine responses Immunophenotyping
Anti-transgene antibiotics	Immunoassay	Tiered approach

We will help you select the most relevant bioanalytical methods for your nonclinical study based on your AAV serotype and transgene product.

We offer a comprehensive range of advanced techniques tailored to evaluate the safety and efficacy of AAV gene therapies. Our expertise means that the selected methods accurately address the species-specific immune responses that may arise in animal models, even though these responses might not fully translate to human risk assessment.

Our data is essential for interpreting study safety observations and guiding the development of effective gene therapies. With our cutting-edge capabilities, we deliver critical insights that support the successful progression of your clinical applications.



Expressed Protein Quantitation

In addition to verifying gene incorporation, it is crucial to determine whether the intended protein is being expressed. This involves traditional bioanalytical analysis of the protein in plasma/serum and potentially in target tissues.

Our scientists possess the expertise to select the optimal bioanalytical methodology for protein quantitation. We take into account study endpoints, budget, platform selectivity, and sensitivity to ensure the most accurate and efficient results. This approach provides tailored solutions that meet your study's specific needs, delivering reliable data and supporting the success of your gene therapy development.

Vector Shedding Assessments

While biodistribution assays are performed to determine the *in vivo* tissue distribution of the viral vector DNA, shedding is the elimination of the viral vectors through secretions from the animal model or participant. We perform assessments of shedding to estimate the likelihood of transmission of vectors to others.

AAV-based therapy vector shedding is mostly replication incompetent, or conditionally replicative. The manufacturing processes for the assessment of potential replication-competent recombinants generated during production provides valuable data for assessing the need for nonclinical vector shedding studies. These studies are informative on the potential risk for transmission to third parties and the environment, but may not be required for all nonclinical programs.

It is important to note that when collecting DNA and RNA downstream, sterile collections in NHP shedding matrices are not a viable option. Altasciences always takes into account collection considerations, as they can impact your results.

Find out more by reading Case Study II on page 15 »

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Inadvertent Germline Integration

In germline gene therapy, targeting cells that produce eggs or sperm can lead to the effects being passed to future generations, unlike somatic gene therapy, which only affects non-reproductive cells and does not result in inherited changes. A significant concern with AAV vectors is the risk of random integration into host DNA, which can cause insertional mutagenesis and adverse effects, particularly if this occurs in germline cells.

To mitigate this risk, we include prospective biodistribution assessments in our studies involving both sexes. We analyze vector dissemination in gonads, testes, and ovaries, and if genetic material is detected in these tissues, further assessments are conducted to evaluate potential germline transduction.

Our study techniques involve monthly sperm collections from mature males and oocyte isolation from females after six to 12 months. PCR is used for downstream analysis to assess gene therapy target biodistribution within reproductive tissues. Ovarian tissues are also evaluated for vector presence, with oocyte collection from previously frozen or preserved ovaries conducted if the vector target is detected.

Testicular tissues are also assessed for biodistribution, and follow-up analyses determine target integration in sperm, ensuring a thorough evaluation of gene therapy biodistribution and potential germline integration.

HOW ALTASCIENCES SUPPORTS YOUR GENE THERAPY STUDIES

With scientific experts and state-of-the-art instrumentation, we provide gene therapy-related services for every phase of development. We have vast experience in not only common AAVs, PCR, ligand binding (ELISA) assays, but also in all related genetic therapy services.

We offer advanced *in vitro* and *in vivo* toxicology and safety assessments, ensuring a thorough evaluation of gene therapy products, with comprehensive evaluations in NHPs and potential off-target effects of gene therapy vectors.

The quality of bioanalytical methods is integral to the completion of your studies, and our robust methods and assays enable targeted quantification of DNA or RNA transgenes, vectors, and expressed gene moieties, as well as the assessment of their immunogenicity.

ADDITIONAL RESOURCES

Publications

- Issue 36 of *The Altascientist* — Nonclinical Studies in Cell and Gene Therapy
- Issue 37 of *The Altascientist* — Quantitative PCR (qPCR) and Droplet Digital (ddPCR): Leading-Edge Analysis for Your Gene Therapy Programs

Scientific Posters

- Incidence of Neutralizing Adeno-Associated Viral Antibody Subtypes in Cynomolgus Monkeys
- Historical Review of In-Life Data From Studies Utilizing AAVs for Gene Therapy

Blog

- Q&A With Dr. Norbert Makori and Pierre Jolicoeur: The Benefits of Combining Preclinical and Bioanalytical Solutions for Your Gene Therapy Studies
- Three Platforms Used to Monitor Cytokines and the Complement System
- Choosing the Best Bioanalytical Platform for Your Program

Webinars

- Nonclinical AAV Gene Therapy Development
- Nonclinical Safety Assessment for Gene Therapy Products—Key Considerations

Case Study I: Biodistribution and Transcript Quantification in Mouse Tissues Using ddPCR

Summary

A preclinical study in mice was completed for an AAV vector-delivered drug compound with a fully validated method in dPCR and RT-ddPCR. ddPCR and RT-ddPCR were both utilized to assess vector copy number and vector-derived transcript copy number, respectively, in the following mouse tissues: blood, bone marrow, brain, heart, kidney, liver, lung, lymph node, spleen, and gonads.

Methods

The sponsor provided primers and probe sequences and linearized plasmid reference standards, and ddPCR and one-step RT-ddPCR assays were optimized for the Bio-Rad Droplet Digital PCR system. Quality controls (QCs), positive controls, and negative controls were prepared in a background of 1 µg genomic DNA using naïve tissues supplied by Altasciences. Both assays were validated to a sensitivity of 50 copies of AAV vector target.

All study samples were received from the sponsor's designated testing facility and processed on-site at Altasciences in Seattle according to site-specific SOPs. Samples were cut frozen to comply with isolation kit input and subsequently homogenized using a bead-beating procedure. After tissue DNA and RNA were isolated using Qiagen DNeasy® and RNeasy isolation kits, respectively, all samples were quantified using UV spectrophotometry and diluted to working concentrations for downstream ddPCR analyses. Blood for RNA analysis was collected in PAXgene® reagent to preserve RNA sample; the coordinating PAXgene RNA isolation procedure was utilized to obtain RNA, which was then quantified and diluted as other sample isolations, prior to RT-ddPCR analysis.

Results

Validated range of detection was up to 180,000 copies of vector target. No vector copy number or vector-derived transcripts were quantified in vehicle control animals. Two exposure timepoints were assessed, and at both timepoints all tissue types reported quantifiable vector and vector-derived transcript copy numbers.

Case Study II: Vector Shedding Analysis via qPCR From Nonhuman Primate Shedding Matrices

Summary

A preclinical qPCR study was conducted in NHPs to assess vector shedding of a gene target of a recombinant AAV drug compound. Shedding matrices included blood, urine, feces, saliva, nasal swabs, and tears. Primers and a fluorescent probe specific to the gene target contained within the drug were designed by Altasciences and supplied by an outside vendor. The assay developed selectively quantified the vector copy number of a specific DNA target-sequence after the drug was administered and the assay was validated for use on GLP studies.

Methods

The reference material was a linearized DNA plasmid containing the TA-specific target sequence. Standards, QCs, spike-positive controls, and no-template control (NTC) samples were prepared in a DNA background obtained from Altasciences' naïve NHP tissues.

Shedding samples were aliquoted and DNA was isolated using Qiagen, column-based isolation kits. Fecal sample DNA isolation was performed using the automated QIAcube HT isolation system via the QIAamp® Fast DNA Stool Mini Kit. Blood, urine, tears, saliva, and nasal secretions were isolated using QIAamp® Mini DNA Kits. All sample DNA was quantified using UV spectrophotometry and diluted to working concentrations as appropriate for downstream PCR analysis. Amplification and PCR fluorescence data acquisition was performed using a ThermoFisher QuantStudio™ 7 Pro qPCR system.

Results

The vector copy number means were less than lower limit of quantification (LLOQ) for all control and acclimation study samples. Vector copy number was quantifiable in all blood samples after dosing for treated groups. Vector copy number means were quantifiable in fecal and urine samples for a limited time interval.

Case Study III: AAV Vector Delivery for Rare Disease Indication in Juvenile NHPs

Altasciences' team worked closely with the sponsor to manage timelines and study design requirements in a situation where obtaining sufficient seronegative juvenile NHPs presented a significant challenge to the product development.

The initial request was for 36 animals, aged 12 to 15 months, for a rare disease indication.

January

- Animal sourcing
- Screening and selection

April

- 50 juvenile NHPs identified; blood samples collected
- Results: 25/50 NHPs tested negative for AAV NAbs (50%).
- More animals identified and screened

May

- 48 juveniles located, bled, and tested for AAV Nabs

June

- Results: 16/48 NHPs tested negative for AAV NAbs (33%).
- Retained 16, released remaining 32
- Retested all 41 animals (25 from April, 16 from June)
- Results: Negatives were reduced to 28 total

July

- Shipped 28 NAb negative animals to our site
- Age was 2.5 months older than original request

August

- Study starts
- More than seven months since award

Learnings

- It is important to plan for more than six months in lead time to manage unique requirements and source NAbs negative animals.
- The percentage of juvenile NHPs with AAV NAbs is not always as low as expected.
- NAb lab availability and immune status of NHP populations vary significantly.
- Often, the number of animals suitable for study is less than planned. It is crucial to be flexible and decrease animals in selected dose groups, if required.

ABOUT ALTASCIENCES

Altasciences is a forward-thinking, mid-size contract research organisation offering pharmaceutical and biotechnology companies a proven, flexible approach to preclinical and clinical pharmacology studies, including formulation, manufacturing, and analytical services. For over 30 years, Altasciences has been partnering with sponsors to help support educated, faster, and more complete early drug development decisions. Altasciences' integrated, full-service solutions include preclinical safety testing, clinical pharmacology and proof of concept, bioanalysis, program management, medical writing, biostatistics, clinical monitoring, and data management, all customizable to specific sponsor requirements.

We help sponsors get better drugs to the people who need them, faster.

CONTACT US

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