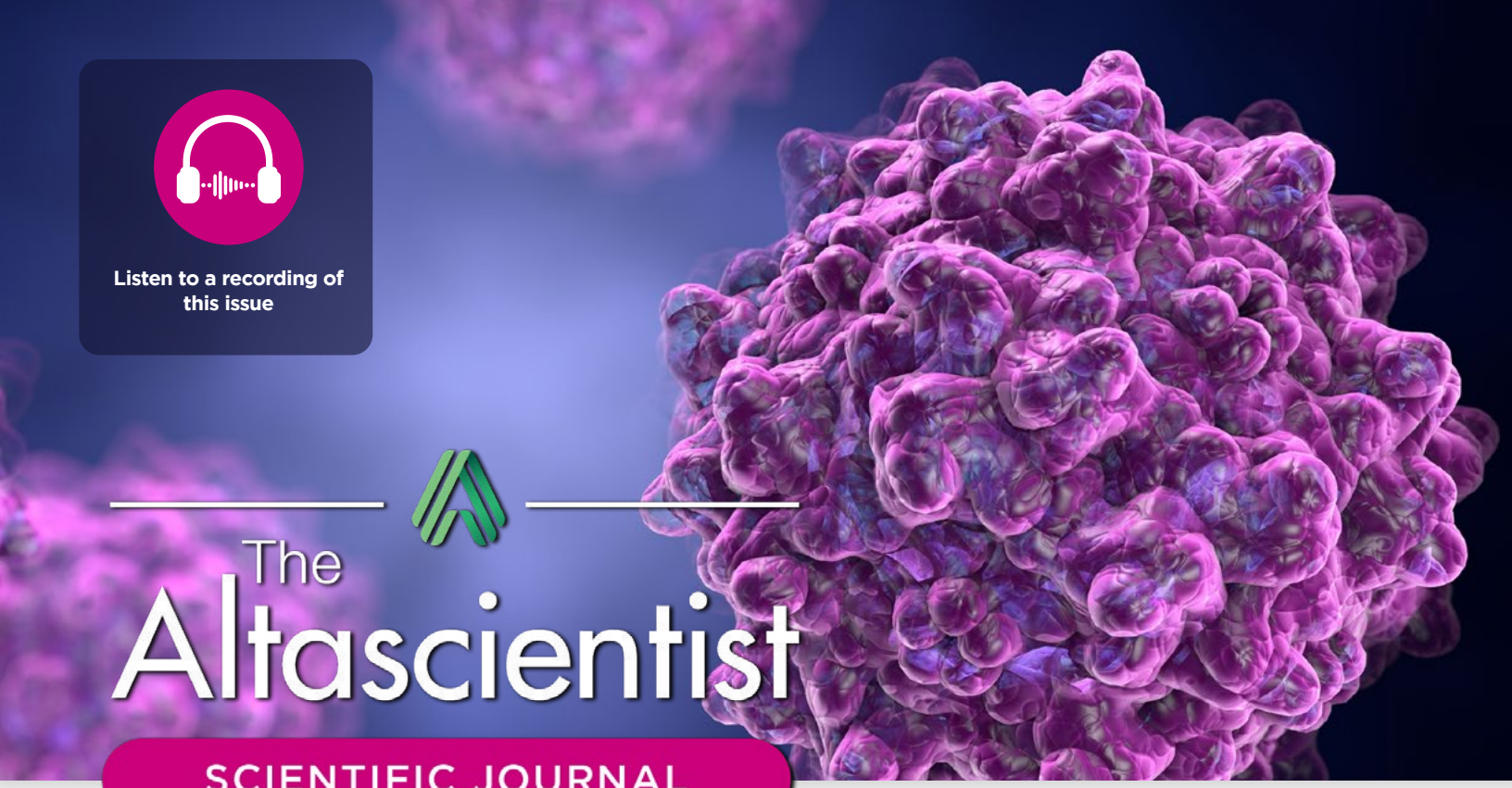

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NONCLINICAL STUDIES IN CELL AND GENE THERAPY – KEY CONSIDERATIONS AND REGULATORY GUIDANCE

Traditional approaches modulate the course of disease but do not provide a cure, particularly in the case of monogenic diseases, which are caused by mutations in single genes that a person is born with. It is estimated that there are greater than 6,000 monogenic diseases, affecting over 350 million people worldwide.[1] For these diseases, cell and gene therapy may provide hope for a cure.

There are significant challenges associated with the successful development of these complex, leading-edge therapies. Challenges involved in the *in vivo* preclinical study of cell and gene therapies include understanding on- and off-target activity, immune responses, and other serious adverse events (AEs). All of these must be carefully monitored, rigorously assessed, and managed to the highest extent possible.

> *"The biggest challenges for … new genome editing therapeutics are the specificity of delivery, control of their activity, detection of potential off-target mutations, and their inherent immunogenicity. The goal of an efficient gene editing therapy is to show perfect specificity for the target sequence without mutations introduced to any other region of the genome."*
>
> - Catherine Jomary, ATMP Lead, IPS-Integrated Project-Services. The Medicine Maker.com

In this issue, we review considerations for nonclinical cell and gene therapy development, including expert approaches to mitigating complex challenges, improving study efficiency, and maximizing translational opportunities to first-in-human trials.

INTRODUCTION

Recent advances in cell and gene therapies offer a fundamentally different approach to disease than medicines and surgery—they are "living drugs" that can provide cures for a range of conditions by terminating the disease process at the cellular or genetic level. This is achieved by replacing a disease-causing gene with a healthy copy, inactivating a defective gene that is not functioning properly, or introducing a new or modified gene into the body, usually by use of a non-pathogenic virus vector.

Cell and Gene Therapy—Similar, but Different

Gene and cell therapies are often discussed together, but they are not the same; and some therapies are considered both cell and gene therapies, as they alter genes in specific types of cells.

Gene therapy is a technique that adds or replaces a defective gene. Three vector types are used in the vast majority of gene therapy: adeno-associated virus (AAV), adenovirus, or lentivirus vectors.

Cell therapy injects living cells (either autologous or allogeneic) into a patient. Cell therapies include immunotherapy, oncology, and regenerative medicine. Some common types of cell therapies include CAR-T and natural killer (NK) cell therapies.

ICH S-12 Guideline on Biodistribution Studies

The ICH S-12 Guideline, Nonclinical Biodistribution Considerations for Gene Therapy Products, provides clear direction on the additional components required of nonclinical gene therapy drug development programs. Biodistribution (BD) studies can be conducted as standalone, or as part of the standard pharmacology and toxicology studies. All studies must be conducted in a relevant animal species or model, using a route of administration that reflects the intended clinical route, and dose levels that provide sufficient characterization of the BD profile to inform design aspects of first-in-human (FIH) trials.

If the BD studies are conducted as part of a GLP study, all procedures must remain in compliance with GLP. As standalone, regulatory authorities will accept non-GLP BD studies that are supported by robust scientific principles. Sample analysis can also be non-GLP, with qualified versus validated methods, if all practices adhere to good scientific principles.

BD analysis determines the distribution and persistence of the vector to target and non-target tissues following direct *in vivo* administration in animals. This assessment is a critical element in understanding the safety profile of the gene therapy product, and insights gained are applied to the toxicology study design (i.e., possible target tissues, sacrifice intervals, and study duration).

Generally, BD analysis is conducted at the molecular level using a high-sensitivity bioanalytical methodology. The current "gold standard" for BD studies utilizes a quantitative polymerase chain reaction (qPCR) assay to assess vector/virus genomes in biological fluids and tissue samples.

Non-terminal imaging techniques also offer an effective modality for monitoring the BD of a gene therapy product in both animals and humans. Consult Issue 33, page 8, of ***The Altascientist*** for an in-depth look at one of Altasciences' nonhuman primate (NHP) studies with non-terminal imaging.

SPECIES SELECTION

According to FDA guidance—Preclinical Assessment of Investigational Cellular and Gene Therapy Products:

The animal species selected … should demonstrate a biological response to the investigational cell and gene therapy (CGT) product similar to that expected in humans… Some factors that should be considered when determining the relevant species include:

- comparability of physiology and anatomy to that of humans;
- permissiveness/susceptibility to infection by, and replication of, viral vectors or microbial vectors for gene therapy;
- immune tolerance to a human CT product or human transgene expressed by a GT product; and
- feasibility of using the planned clinical delivery system/procedure.

Due to their similarities to humans on genetic, physiologic, immunologic, and developmental levels, NHPs are usually the species of choice for gene therapy programs. Mice, rats, rabbits, dogs, and swine are also relevant test animals, depending on the specifics of the test article and the requirements of the program.

Choosing an Appropriate Vector

AAV-based vectors are the most commonly used vectors for gene therapy. They are non-pathogenic viruses, and vectors derived from them can drive long-term transgene expression without integration of the vector DNA into the host genome. Also supporting their use is the broad tropism (the ability of different viruses to infect different cellular types) of the AAV serotypes and the more than 100 AAV variants described in the literature (Gao et al., 2002, 2004; Asokan et al., 2012; Nonnenmacher et al., 2012).

AAV2 is the most commonly used serotype, and different AAVs are optimal for transduction of given organs, as demonstrated in Table 1 below.

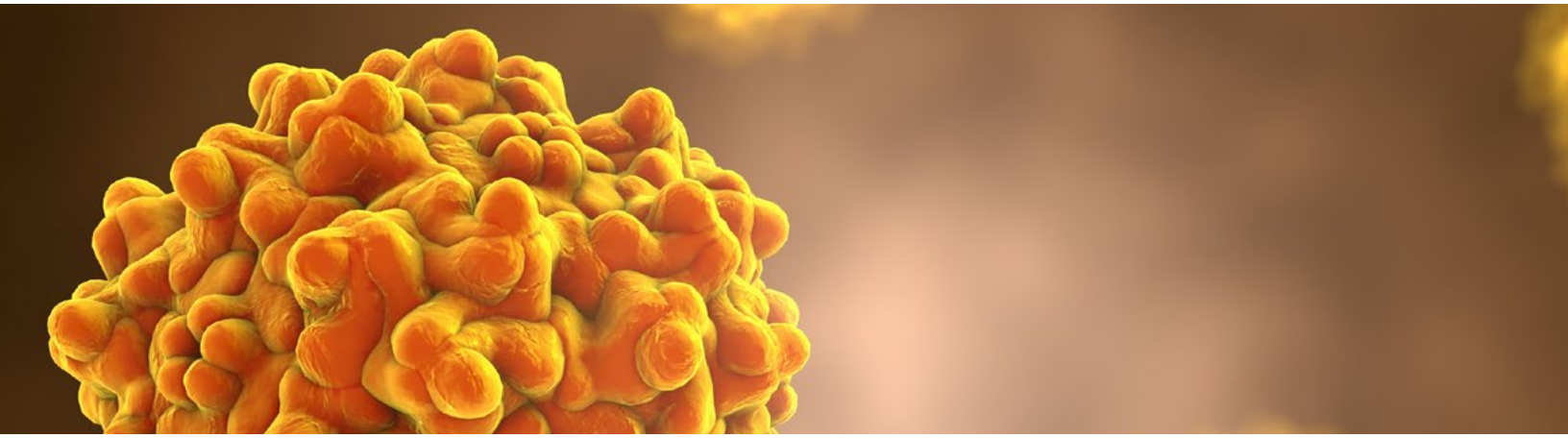
Table 1. Optimal Serotypes for Different Tissues

TISSUE	OPTIMAL SEROTYPE
CNS	AAV1, AAV2, AAV4, AAV5, AAV8, AAV9
Heart	AAV1 AAV8, AAV9
Kidney	AAV2
Liver	AAV7, AAV8, AAV9
Lung	AAV4, AAV5, AAV6, AAV9
Pancreas	AAV8
Photoreceptor cells	AAV2, AAV5, AAV8
RPE (retinal pigment epithelium)	AAV1, AAV2, AAV4, AAV5, AAV8
Skeletal muscle	AAV1, AAV6, AAV7, AAV8, AAV9

Despite the significant number of serotypes and variants with diverse capsids, certain tissues and cell types remain refractory to transduction. An AAV pseudotyping strategy can be employed to improve transduction, which involves mixing the capsid and genome from different viral serotypes. For example, we can replace the capsid of AAV2 with the capsid of another AAV serotype, denoted by a slash. As such, an **AAV2/5 vector** has the **genome of AAV2** and the **capsid of AAV5**.

For some gene therapy applications, the goal is to transduce only a specific tissue, and all current AAV vectors transduce more than one tissue, to varying degrees.

Viral tropism can be affected by pseudotyping, and also by the use of hybrid capsids, which are derived from multiple different serotypes. A common example of a hybrid capsid is AAC-DJ, which contains a hybrid capsid derived from eight serotypes.



GERMLINE MITIGATION STUDIES

In **germline gene therapy**, the therapy is targeted to cells that create eggs or sperm. The gene therapy's effects will be passed down to the children and future generations. In **somatic gene therapy**, the target is a bodily cell that does not make sperm or eggs. Gene therapy off-target or unanticipated effects will remain with the recipient, and not be passed down to any future generations.

When developing a somatic gene therapy, there is a risk that the vector will **inadvertently integrate to the germline**. This can be identified by BD assessment to confirm presence or absence of the gene therapy product in the gonads of both sexes of animals. If an appropriate analytical method does not indicate persistence, further evaluation may not be necessary.

If the analysis demonstrates continued presence of the introduced genetic material in the gonads, additional studies need to be conducted to assess gene therapy product levels in germ cells (e.g., oocytes, sperm) or non-germline (somatic) cells in the test animals. Generally, these studies involve three male and three female sexually mature NHPs, dosed once, and analyzed as follows:

Females

- Collect ovaries.
- Isolate oocytes after six to 12 months.
- Conduct qPCR analysis of oocytes for gene editing/integration.

Males

- Collect semen pre-dose and once monthly for six to 12 months.
- Conduct qPCR analysis of semen for gene editing/integration.

Detection of Off-Target Mutations

With gene therapy, there is always a risk that the newly introduced gene will have impacts beyond the intended target. **Off-target effects can be defined as unintended cleavage and mutations at untargeted genomic sites,** which may show a similar but not identical sequence relative to the target site, or may be entirely unrelated. Disruption of the function or regulation of non-targeted genes can be some of the serious consequences of off-target mutations.

Exaggerated On-Target Effects

Larger structural changes of the genome sequence, occurring at the intended on-target editing site, are another cause of concern. There can be exaggerated impacts at the target that result in serious AEs or toxicity in the test system.

Immunogenicity

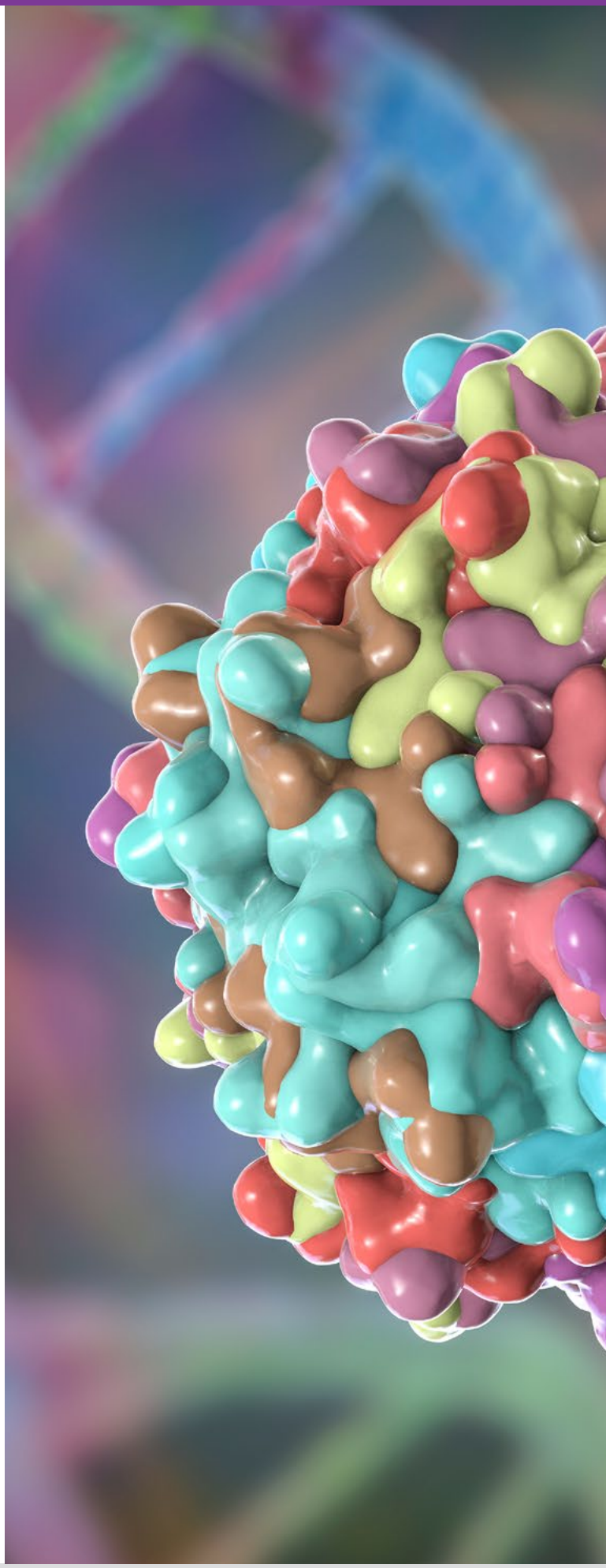
Although AAVs are not strongly immunogenic, they can elicit both a cellular and humoral immune response. As even very low levels of antibodies can prevent successful transduction, antecedent anti-AAV antibodies can be a serious concern when screening animals for inclusion in a nonclinical study.

Immunosuppression is not recommended for the sole purpose of evaluating the BD profile. However, if product- or species-specific immunosuppression is otherwise warranted, it may be utilized with appropriate justification. Pre-treatment with 2 mg/kg of dexamethasone, approximately one to two hours prior to AAV administration, is typically adequate to mediate immune related responses. Immunosuppression can also be used to rule out toxicity due to an immune reaction.

Many animals may have to be screened in order to arrive at the required study numbers. Generally, a study would consist of:

- five* rodents per sex/group/timepoint; or
- three* non-rodents per sex/group/timepoint.

*Justification must be provided for the number of animals used, including number per sex.



In our experience, the negative percentage rate for AAV neutralizing antibodies (NAbs) can vary widely, as shown in Table 2 below.

Table 2. Naïve Screening Initial Pre-study Negativity Rate

AAV Serotypes	Number of Animals Screened	% Positive	% Negative
AAV1	152	19	81
AAV2	30	23	77
AAV3	30	27	73
AAV5	30	0	100
AAV6	30	0	100
AAV7	30	33	67
AAV8	1,119	62	38
AAV9	60	27	73
AAV10	30	20	80

Immunogenicity evaluations may require multiple assays, as demonstrated in Table 3.

Table 3. Immunogenicity Assays and Platforms

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 transduction
Anti-transgene protein NAb	Cell-based assay	Inhibition of activity
Anti-AAV capsid of anti-transgene protein T cell response	ELISPOT Flow cytometry	Cytokine responses Immunophenotyping
Anti-transgene antibodies	Immunoassay	Tiered approach

When planning your gene therapy study, it is important to keep these data in mind, as they can affect study start-up, timelines, and budget.

PLANNING YOUR NONCLINICAL STUDIES

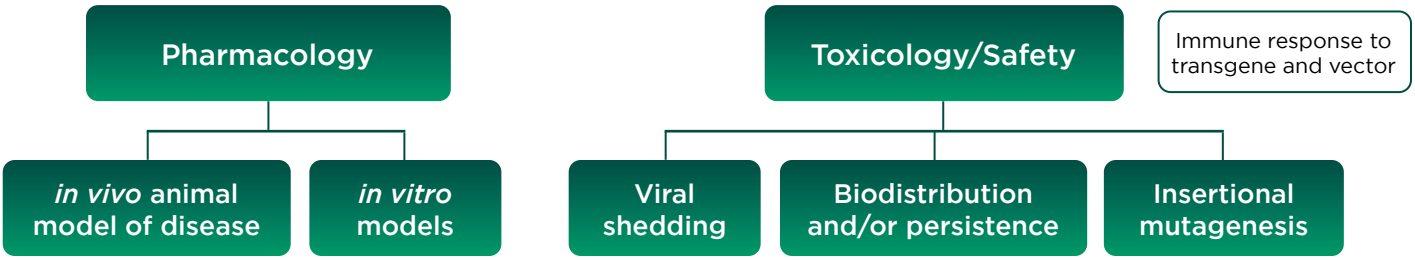
Early planning is essential, as there are many elements to manage, and potential challenges to mitigate.

AAV NAbs assays are available and, based on the AAV serotype, a pool of NHPs is bled and held until NAbs results become available.

Once the results are available, animals that are negative to the AAV are promptly selected and isolated for study.

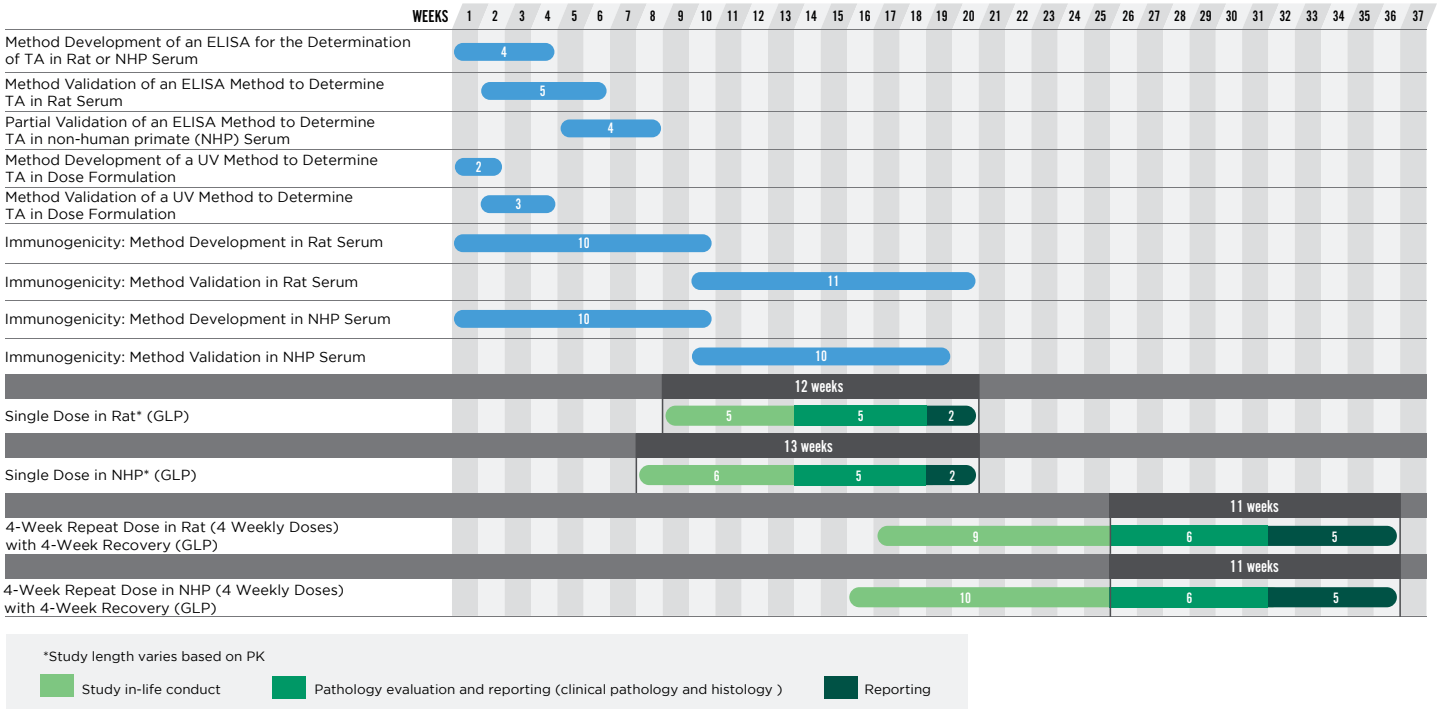
Nonclinical study designs for cell and gene therapy have unique characteristics relative to other modalities. As demonstrated in Figure 1 below, additional assessments specific to gene and cell therapies are required.

Figure 1. Unique Characteristics of Gene Therapy Studies



We can complete your large molecule program in approximately six months by using the program management approach proposed in the chart below for the *in vivo* portion of the IND/CTA program. **This chart represents only one proposed approach. Each program is different, and will be assessed to address its unique requirements.**

Figure 2.



ALTASCIENCES' CASE STUDIES

Case Study I

AAV Vector Delivery for Rare Disease Indication in Juvenile NHPs

In this case study, we demonstrate how 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.

Our flexibility and perseverance in sourcing and testing NHPs for this study allowed us to initiate the nonclinical testing with a relatively minor time delay and slight changes to the study design (fewer animals, age adjustment).

Case Study II

Gene Therapy Utilizing Adeno-Associated Viral (AAV) Vectors: Historical Data Review to Characterize Common Challenges and Identify Opportunities for Refinement

Consult the full poster: Gene Therapy Using AAV Vectors—Historical Data Review

The presence of naturally occurring NAbs in the primate population requires screening of many animals to obtain an adequate negative titer cohort for the study. Additionally, pre-treatment with corticosteroids prior to AAV vector administration is often needed to counter any adverse reactions.

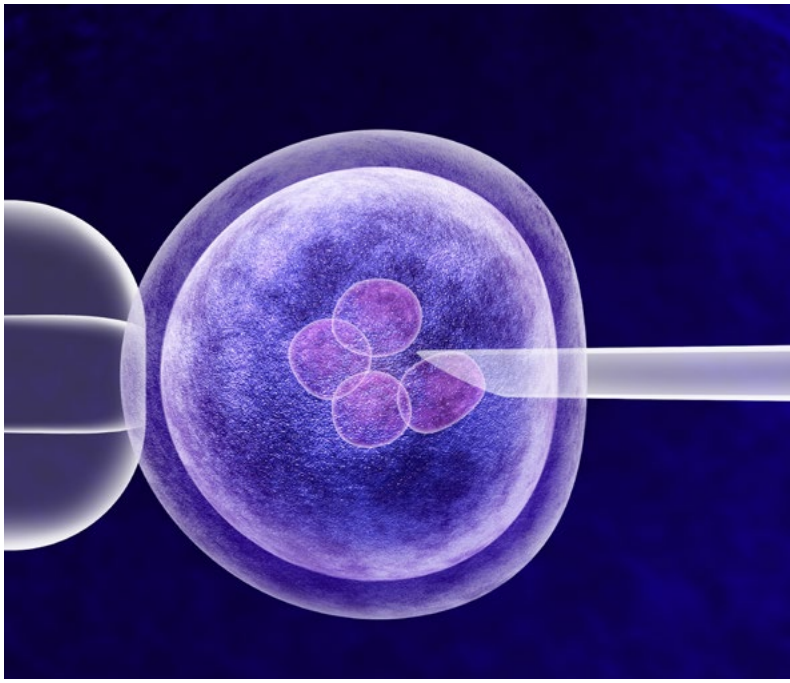
Given the unique challenges of working with AAV vector-based test articles, Altasciences reviewed data from 22 toxicology studies conducted in the past few years with the aims of:

- establishing ranges for the number of animals to screen for NAbs;
- establishing the dosage range for commonly used corticosteroid given prior to AAV vector administration; and
- describing the most common in-life findings.

Approximately 38% (430/1,119) of animals screened for NAb against AAV8 were suitable for study assignment based on established criteria for negative or low viral titers by AAV neutralizing antibody assay (≤ 5 NAb50 in HEK293 cells).

Pre-treatment with 2 mg/kg of dexamethasone at approximately one to two hours prior to AAV administration was adequate to mediate immune-related responses. There was no discernable effect of AAV administration on body weight, and most abnormal post-dose clinical signs were minor and not directly attributable to the AAV vector.

In conclusion, this historical data set serves as a guide for more informed study designs for AAV vector-based therapeutics, and allows for potential reduction and refinement of animal use in safety testing.



HOW ALTASCIENCES CAN HELP

Given the complexity of cell and gene therapy development, it is strongly advisable to engage with an experienced, knowledgeable partner that has well-rounded expertise in the many aspects of this leading-edge technology. Altasciences is that partner.

Our Sacramento and Seattle sites, in particular, are specialized in developing cell and gene therapies, such as AAV or lentivirus (lenti) products, and applying gene editing technologies like CRISPR/cas9.

Nonclinical Evaluations

- *In vitro* and *in vivo* toxicology/safety
- *In vivo* range finding
- MRI/Stereotaxic guided administration
- Bioanalysis/PK: biodistribution, persistence and shedding
- Biomarker strategy, assay development and testing
- Regulatory and strategic product development consulting
- Clinical development preparation and planning

Capabilities

- Extensive, in-house capabilities and experience (including historical background data)
- Operational experience with infant/juvenile NHPs for long-term studies
- Surgery expertise for biopsies, such as in gene editing studies requiring repeated liver sampling
- Special necropsy procedures to avoid contamination and ensure the integrity of genomic material in tissues
- GLP laboratory in Seattle to perform qPCR, RT-qPCR of vector and biodistribution and persistence of vector/transgene in tissues

Our Experience Works for You

- More than 130 in-life studies in the last five years, over 95% in NHPs
- Many administration routes, including intravenous, subcutaneous, intrathecal, intravitreal, and suprachoroidal
- Biodistribution analysis (PCR) conducted on-site
- More than nine years' experience in IND-enabling studies for rare diseases
- More than five years' experience with CRISPR (gene editing) therapeutics in NHPs

Early Discovery Research by the Numbers

Figure 2. Number of Gene Therapy Studies Initiated Per Year at Altasciences' Seattle Site

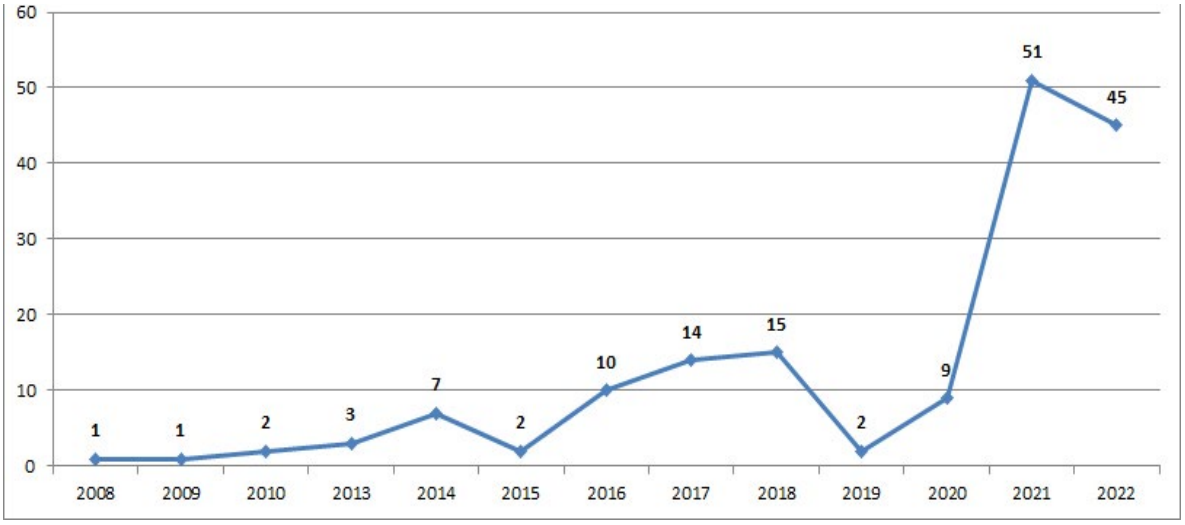


Table 4. Studies Completed per Organ System and Method

Organ System	Method	Studies Completed
Brain—putamen, thalamus, cerebellum, intracerebroventricular	MRI-guided	51
	Stereotaxic (non-MRI-guided)	2
Spinal cord	Intrathecal	14
	Cisterna magna	3
Ocular	Chronic CSF port (up to 4 years)	49
	Intravitreal	25

In-House Laboratory Sciences Support

Our bioanalytical teams are ready to support your cell and gene therapy programs with expertise, experience, and leading-edge capabilities for all the necessary assessments.

ANALYSIS	PLATFORM
PK	Ligand binding (LBA), polymerase chain reaction (PCR)
PD	LBA, flow cytometry, PCR, ELISpot
Immune response	LBA, NAb, TAb, cellular, flow cytometry, cytokines
Biodistribution	PCR, LBA
Viral shedding	PCR

We also have molecular biology capabilities on-site, supported by the latest instrumentation:

- Bio-Rad's Droplet Digital PCR system (QX200), with automated droplet generator
- Applied Biosystems QuantStudio™ 7 Pro real-time PCR systems
- Qiagen QIAcube – automated DNA and RNA isolation
- NanoDrop™ One spectrophotometers
- PCR dedicated hoods/chambers

GLOBAL GUIDANCE DOCUMENTS

- FDA guidance: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/preclinical-assessment-investigational-cellular-and-gene-therapy-products
- EMA guidance: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-non-clinical-studies-required-first-clinical-use-gene-therapy-medicinal-products_en.pdf
- ICH guidance: https://database.ich.org/sites/default/files/ICH_S12_Step4_Guideline_2023_0314.pdf

ALTASCIENCES' RESOURCES

Poster

Gene Therapy Using AAV Vectors—Historical Data Review

Article

A Novel Hybridization LC-MS/MS Methodology for Quantification of siRNA in Plasma, CSF, and Tissue Samples

Fact Sheets

Gene Therapy Bioanalysis

Purpose-Built Facilities for Cell and Gene Therapies

Webinars

Cell and Gene Therapy: Enhanced CNS and Ocular Delivery in NHPs—Overcoming Technical Challenges

Use of Droplet-Digital PCR (ddPCR) in Preclinical Research

REFERENCE:

1. Stanford University Center for Definitive and Curative Medicine. https://med.stanford.edu/cdcm/CGT.html Accessed July 31, 2023.

ABOUT ALTASCIENCES

Altasciences is an integrated drug development solution company offering pharmaceutical and biotechnology companies a proven, flexible approach to preclinical and clinical pharmacology studies, including formulation, manufacturing, and analytical services. For over 25 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. Altasciences helps sponsors get better drugs to the people who need them, faster.