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DISCOVERY STUDIES—AN AGILE APPROACH TO EARLY DATA FOR QUICKER DECISIONS

The early identification of toxicity, and flexibility to explore parameters and characteristics of drug candidates *in vivo* via early discovery non-GLP studies, allow for improved drug development strategy and faster go/no-go decisions. This data from animal models, and particularly nonhuman primates (NHPs) where their genetic, immunologic, and physiologic similarities to humans make them the model of choice, is an important part of informing decisions about candidate molecules. Access to early discovery studies' data accelerates the process of refining candidate molecules and identifying promising therapeutic targets, novel delivery methods, and pharmacodynamic markers.

IN THIS ISSUE

We explore the characteristics and advantages of non-GLP discovery studies, along with emerging trends in this important early phase of drug development research.

We also present **two case studies** from our Sacramento site, highlighting discovery studies that assessed the biodistribution of the test article under specific experimental conditions.

Gene therapy and other advanced biologics are rapidly evolving fields that rely heavily on early-phase research to assess the feasibility, safety, and distribution of novel delivery platforms. One of the critical challenges in this space is the ability to quickly generate preliminary data that inform decisions on whether and how to proceed to more rigorous investigational stages, including GLP-compliant safety assessments.

Non-GLP exploratory studies play a pivotal role in this process. These studies allow for the evaluation of key characteristics such as biodistribution, immunogenicity, pharmacokinetics (PK), pharmacodynamics (PD), proof-of-concept (POC), and mechanistic insights. They are instrumental in addressing several early development objectives: determining whether a drug candidate should proceed to more advanced stages—thereby

- minimizing investment in costly safety assessments when early data is unpromising;
- generating data that inform the design of more efficient and targeted pivotal toxicology studies;
- characterizing a compound's mechanism of action or potential toxicity;
- assessing the feasibility and potential efficacy of novel compounds, delivery methods, or therapeutic strategies.

Non-GLP discovery studies enable more agile and cost-effective decision-making, ultimately accelerating the development of promising therapies.

THE VALUE OF CONDUCTING DISCOVERY STUDIES

Flexibility

Early discovery non-GLP studies offer tremendous flexibility in study design, execution, and documentation. As the study advances and based on results, test groups can be added or modified in real time and dosages or route of administration changed. Prior approval of such mid-stream modifications is not necessary, although detailed documentation and record-keeping remain crucial for scientific integrity.

For example, a sponsor developing a new medication can test different routes of administration very early in their program and choose the most likely drug candidate to proceed to GLP studies and further development. Oral, injectable, transdermal, depot, or other potential methods of delivering the medication could be assessed in different groups of animals. Dosing levels and frequency can be adjusted during the course of the study to deliver relevant toxicity data for future consideration.

Study designs can vary from single-dose, single-draw studies to extended studies lasting six months or longer. Early discovery work may include single or multiple group studies with different dose ranges, routes of administration, and other parameters. These studies typically involve using between 3 and 16 animals, with the option to include either a single gender or a mix of both genders.

Cost and Time Efficiency

Discovery studies are generally fast and cost-effective. These studies do not have to undergo the rigor of regulatory requirements, allowing for faster start-up, shorter testing durations, less detailed and more flexible protocols, smaller sample sizes, and faster report delivery. It is worth noting that the approaches used by Altasciences lead to streamlined processes and lower administrative burden, delivering quick study initiation, execution, and data review. Ultimately, this accelerated approach allows researchers to make go/no-go decisions more rapidly.

Exploring the Reuse of Non-Naïve Animal Models

Utilizing established animal colonies for discovery studies offers a cost-effective approach to early-stage research while supporting key animal welfare principles. By drawing from existing colonies, researchers can streamline animal selection and reduce the time and resources needed for study initiation. This efficiency minimizes redundant screening and housing costs, contributing to overall project affordability. Importantly, leveraging animals already in use or pre-screened for compatibility allows for smarter study design, reducing the need to source additional animals unnecessarily. This aligns with the "Reduction" principle of the 3Rs (Replacement, Reduction, Refinement), ensuring that animals are only used when scientifically justified and in the most efficient manner possible—ultimately embracing ethical research practices without compromising scientific integrity.

A **survey** published in the International Journal of Toxicology in 2022 discusses the innovative concept of non-naïve NHPs being re-used after non-terminal studies. The IQ Consortium NHP Reuse Working Group (WG) comprised members from 15 pharmaceutical and biotechnology companies, including principal investigators, facility managers, animal welfare officers, and research scientists.

The reuse of protein non-naïve NHPs presents both challenges and a valuable opportunity in early-stage drug discovery. Although concerns such as immunogenicity and variability must be carefully addressed, implementing robust risk assessment and decision-making strategies can enable more efficient and ethical use of NHP resources. With thoughtful planning, including the use of roadmaps and decision trees, research programs can safely expand protein non-naïve NHP reuse. This approach not only supports sustainability and animal welfare goals but also has the potential to accelerate the development of biologics by optimizing resource allocation and reducing study timelines.

The survey results demonstrated that certain pharmaceutical and biotechnology companies have cautiously approached the re-use of protein non-naïve NHPs, and there was strong interest in finding ways to increase such re-use without negatively impacting scientific integrity. The survey showed that of the companies that were re-using protein non-naïve NHPs, 57% reported that reuse was applied only to non-pivotal/non-GLP experiments.

To appropriately increase the re-use of protein non-naïve NHPs, several fundamental concepts must be considered:

- improved accessibility to essential animal history data, both from sponsors and CROs;
- sharing of best practices from industry-wide application of a re-use focus;
- adoption of animal screening strategies to ensure proper test model assignment; and
- collaboration with CROs to support pre-study screening and maintenance of blinded historic treatment data.

Industry-wide alignment is needed to develop a mindset for increased, responsible NHP reuse while maintaining rapid development of safe novel therapies for patients.

DISCOVERY STUDIES SUPPORT SCIENTIFIC EXPLORATION OF NEW DELIVERY METHODS AND APPROACHES

In early-stage research, non-GLP studies are excellent options for exploratory study and proof-of-concept investigations of new modalities and drug delivery technologies. Their flexibility allows researchers to focus on scientific discovery and enables rapid experimental adjustments.

Drug delivery methods are constantly evolving, and discovery studies can provide critical data about whether such advancements are applicable to a new molecule being developed. Here, we explore some current trends in drug delivery methods.

Adeno-associated viruses (AAVs)

Non-GLP discovery studies in NHPs can be used to assess new developments in the field of AAVs for gene therapy (GT) delivery. Screening NHPs for pre-existing AAV antibodies is a common practice to identify animals with low antibody titers which maximizes successful gene transfer in preclinical studies. New research is focusing on developing AAV variants that are less likely to be neutralized by pre-existing antibodies.

Approaches include modifying the AAV capsid to interact with specific cellular receptors, expanding the range of target tissues. Researchers are also actively working to overcome limitations such as the AAV's limited packaging capacity and potential immunogenicity. Strategies include engineering smaller, more compact therapeutic payloads and developing methods to mitigate immune responses.

Non-GLP discovery studies in this space would involve screening for AAVs, POC (e.g., direct injection into the central nervous system), and pCR.

Engineered virus-like particles (VLPs)

Engineered VLPs have several advantages over other delivery methods as a candidate for *in vivo* GT delivery. For example, there is no need to screen animals for antigens like for AAVs. Secondly, there is no known induction of immunological responses on administration and thus, no need for concomitant treatment with immunosuppressants, for example, corticosteroids, a practice common for AAVs and LNPs.

Lipid nano particles (LNPs)

LNPs are a type of nanoscale drug delivery system that carries drugs or genetic material into the body. Commonly used in drug and vaccine delivery, they had a significant role in the mRNA COVID-19 vaccines and gene therapy studies. LNPs offer several advantages, including the ability to protect drugs from degradation, enhance their bioavailability, and potentially target specific tissues or cells.

LNPs do have some challenges in their development, such as optimizing their size and dosage, induction of immune reactions, acute liver enzyme changes, and composition for specific applications. Current and future research is focused on addressing these challenges and expanding their clinical applications.

ALTASCIENCES' CASE STUDIES

AAV-based vectors have emerged as a prominent platform for gene delivery due to their relatively favorable safety profile and versatility in targeting various tissues. However, understanding the biodistribution of these constructs following different routes of administration remains essential, particularly as newer strategies, such as intrathecal or intra-cisterna magna (ICM) delivery, are explored for central nervous system (CNS) targeting.

Here, we present two case studies conducted at Altasciences that exemplify the role of exploratory biodistribution studies in early-stage drug development. The first case study involved the administration of an AAV construct via intra-cisterna magna injection in cynomolgus monkeys, aiming to evaluate its CNS biodistribution. The second study assessed tissue distribution following intravenous administration of a test article in a similar NHP model.

In both studies, streamlined animal selection and in-house capabilities allowed for rapid execution and timely data delivery, demonstrating the advantages of integrated preclinical research environments in accelerating translational science.

CASE STUDY I

An Exploratory Viral Delivery Construct Study in Cynomolgus Monkeys Following Intravenous or Intra-Cisterna Magna Administration

This was an exploratory study to provide samples to assess the biodistribution of viral delivery platform constructs when administered intra-cisterna magna once.

Study Purpose

The purpose of the study was to understand the biodistribution of an AAV construct when administered intra-cisterna magna a single time.

Methods

Fifteen NHP groups (total of 23 females and 3 males) were pre-screened and selected based on screening analysis. To reduce the immune response to the viral construct capsids, the study-assigned animals received an immunosuppression regime of methylprednisolone (20 mg, IM).

Evaluations included mortality/moribundity, food evaluation, cage-side observations, detailed examination, and veterinary physical examination.

Blood and urine samples were collected from selected animals for anti-drug antibody (ADA) analysis and peripheral blood mononuclear cell (PBMC) isolation.

Tissues from 30 different organ systems were collected at necropsy, some fixated and the rest frozen for analysis.

What Set Altasciences Apart

Testing of the viral construct was conducted in a timely manner, allowing subsequent use to deliver gene therapy of interest via the cisterna magna. The selection of animals following screening was seamless since a large colony of animals was already available at our site for screening.

CASE STUDY II

A Biodistribution Study in Cynomolgus Monkeys Following a Single Intravenous Administration

This was an exploratory study to provide samples for the evaluation of the biodistribution of the test article (TA) when administered as a single intravenous (IV) dose to cynomolgus monkeys.

Study Purpose

The purpose of the study was to understand potential tissues where distribution may occur, using a single group of NHPs and one dose of the TA.

Methods

Animals were screened for two subtypes of AAV antibodies, with negative animals assigned to the study from the research facility stock colony. Assigned animals were acclimated to primate chairs prior to Day 1. Animals that seroconverted during the acclimation period were released from the study and replaced with animals seronegative to the two subtypes of AAVs.

Animals were sedated with ketamine (10 mg/kg, IM) for intravenous administration of dose formulations on Day 1, with additional doses of ketamine (5 mg/kg, IM) possible to maintain sedation. The dose formulations were administered over 10 minutes (±1 minute) using a temporary catheter (24G or 26G) inserted into a cephalic vein connected to an infusion line.

Evaluations included mortality/moribundity, food evaluation, and cage-side observations.

Blood samples were taken on the following schedule, and analyzed for NAbs:

GROUP	TIMEPOINT	TARGET VOLUME (ML)
All animals	Day -7	1.2
1	Day 1 – pre-dose	1.2
1	Day 1 – 10 min post end of infusion (EOI)	1.2
1	Day 1 – 24 hours post end of intervention (Day 2)	1.2
1	Pre-necropsy	5-12

Tissues were assessed at necropsy, with special processing for morphology (e.g., muscle tissue) and H&E staining for histopathology.

What Set Altasciences Apart

Study animals were quickly identified and assigned because Altasciences has a large colony of NHPs for screening. The study was initiated and completed on time, enabling the sponsor to obtain results that allowed them to move to the next phase of their program.

HOW ALTASCIENCES CAN HELP

With decades of experience and expertise in biomedical and behavioral research, our 31,000-square-foot preclinical facility in Sacramento, CA, specializes in translational research in NHPs. We have immediate access to a large colony of acclimated animals that are ready to go on study, thus expediting your research.

Sponsors with an ongoing study are welcome to visit the facility, observe study procedures, and discuss data as it is collected. We thrive on working corroboratively with sponsors.

SACRAMENTO SITE HIGHLIGHTS

- Located close to San Francisco International, Oakland International, Buchanan Field Domestic, and Napa County Domestic airports
- Specialized in large molecule NHP studies with expertise in:
  - cell and gene therapy
  - ocular studies
  - CNS research
- Over 140 studies conducted

FACILITY FEATURES

- 18 animal rooms
- Capacity to house ~850 NHPs
- CDC quarantine area

ACCREDITATIONS/CERTIFICATIONS

- AAALAC
- USDA
- CDC
- OLAW

ALTASCIENCES' RESOURCES

Scientific Posters

- The NHP Model of CNS Therapies and Utility of AAV Vectors in Gene Therapy
- Gene Therapy Studies and Germline Integration Assessment in NHPs
- Incidence of Neutralizing AAV Antibody Subtypes in Cynomolgus Monkeys
- CNS-Targeted Therapies Delivery Strategies and Sampling in Non-rodent Preclinical Species
- Historical Review of In-Life Data From Studies Utilizing AAVs for Gene Therapy

Webinars

- Preclinical Studies of Gene Therapy Products: Latest Trends
- Nonclinical Safety Assessment for Gene Therapy Products: Key Considerations
- Gene and Cell Therapy: Enhanced CNS and Ocular Delivery in Nonhuman Primates—Overcoming Technical Challenges
- Key Considerations on Study Design, Laboratory Endpoints and Regulator Guidance for Preclinical Safety Assessment for AAV Gene Therapy Development

eBook

- Key Considerations for Nonclinical AAV Gene Therapy Development

Webpages

- Preclinical Drug Development Solutions
- Gene Therapy Development

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 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. Altasciences helps sponsors get better drugs to the people who need them, faster.