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TOXICITY STUDIES: A CRITICAL STEP IN MOVING FROM DISCOVERY TO DEVELOPMENT

Preparing for toxicity studies is a high-stakes phase that bridges discovery and clinical trials. For a biotech or pharma company, success depends on integrating regulatory compliance, tailored analytical and bioanalytical assays, and manufacturing data that establishes drug product stability and purity. Ensuring that the key building blocks are in place before dosing begins makes for a smooth, reliable study process that delivers the necessary data and documentation from the get-go, supporting decision-making and downstream activity.

Both non-GLP and pivotal GLP safety assessment studies are essential pillars of a strong nonclinical program.

In this issue of *The Altascientist* we examine the strategic role of each study type for small molecules, biologics, and cell and gene therapies, and provide a checklist to help you determine IND/CTA readiness of your program.

Also included is a **case study** demonstrating Altasciences' use of a novel approach to an IND-enabling study in dogs, to counter known pharmacological effects of the test article.

OPERATIONAL PREREQUISITES FOR LEAD CANDIDATE CHARACTERIZATION

Success in downstream pivotal (GLP) studies depends on the thoroughness of pre-IND preparation. Sponsors should align with their drug development partner on the following strategic pillars:

- **Study Protocol:** A clear study plan defining objectives, dosing regimens, and endpoints is necessary to maintain consistency.
- **Bioanalytical Method Development:** Development and qualification of critical bioanalytical assays (e.g., PK and ADA assays) before studies begin, ensuring the drug's PK and ADME (Absorption, Distribution, Metabolism, and Elimination) can be accurately measured.
- **Test Article Characterization:** Although a full Certificate of Analysis (CoA) is only mandated for GLP studies, sponsors still need basic data on the identity, purity, and stability of their drug candidate for dosing accuracy.
- **Chemistry, Manufacturing, and Controls (CMC) Data:** Establishing a rigorous bridge between the nonclinical test article and the intended clinical-grade material is essential for regulatory alignment. While a formal CoA may be pending during early-phase studies, sponsors must provide foundational identity, purity, and stability data to confirm that the drug substance used in safety assessment is representative of the clinical drug product. This characterization is critical to guarantee dosing reproducibility and to validate that toxicological observations accurately reflect the safety profile of the material destined for human administration.
- **Species Selection Rationale Biological and Pharmacological Relevance:** Identifying the most pharmacologically relevant animal species (often one rodent and one non-rodent) to ensure the non-GLP data meaningfully informs the IND.
- **Dose Range Finding (DRF) Strategy:** Defining the Maximum Tolerated Dose (MTD) and Dose-Limiting Toxicity (DLT) to set appropriate levels for subsequent GLP studies.
- **Draft Clinical Synopsis:** Having a preliminary clinical trial protocol ready; the non-GLP studies must be designed to support the specific dose, route, and duration of your proposed Phase I trial.

Exploratory toxicity studies are more than preliminary checkboxes; they are critical for internal go/no-go decisions and defining the therapeutic index.

- **Establishment of NOAEL/HNSTD:** Identifying the no-observed-adverse-effect level (NOAEL) in rodents or the highest non-severely toxic dose (HNSTD) in non-rodents.
- **Exposure-Toxicity Correlation:** Integrating pharmacokinetic (PK) and toxicokinetic (TK) data to establish safety margins based on data rather than mg/kg dosing alone.
- **Target Organ Characterization:** Defining the nature, severity, and reversibility of toxicities to guide clinical laboratory monitoring (e.g., hepatic enzymes, cardiac biomarkers).

Strategic Comparison: Exploratory vs. Pivotal (GLP) Safety Assessment

FEATURE	NON-GLP (EXPLORATORY/DRF)	GLP (PIVOTAL/IND-ENABLING)
Regulatory Objective	Internal De-risking: Lead optimization and DRF	Regulatory Authorization: Formal safety characterization for IND/CTA filing
Protocol Rigor	Iterative/Adaptive: Allows for dose adjustments based on real-time observations	Standardized/Fixed: Protocols are locked; any deviation requires formal impact assessment
Quality Oversight	Sponsor-Defined: Internal standard operating procedures (SOPs)	Independent QA: Mandatory Quality Assurance audits of all phases and raw data
Fiscal Impact	Optimized: Lower cost-to-data ratio; flexible resource allocation	Substantial: Premium pricing due to mandated compliance and documentation overhead
Strategic Value	Decision Support: Informs MTD, DLT, and clinical candidate selection	Clinical Entry: Establishes the legal safety basis for first-in-human (FIH) trials
Data Utility	Internal Roadmap: Supports go/no-go decisions and early investor milestones	Market Asset: Essential for regulatory clearance and late-stage valuation

PRE-IND TOXICITY STUDY READINESS

Before toxicity study planning begins for a specific therapeutic, it is important to review existing data for insights that may influence the study design. For instance, if the therapeutic belongs to an established drug class, data from similar compounds can provide valuable information on previously observed effects, helping to inform the design of the planned safety assessments. Additionally, even compounds from different classes may share common biological pathways, and these similarities can further inform the design of relevant toxicity studies.

If existing data suggest that certain organs or systems may be at risk, toxicity studies can be designed to focus on these areas rather than applying a broad, one-size-fits-all approach. Understanding pharmacokinetics and pharmacodynamics informs the selection of appropriate starting doses for toxicity studies, helping avoid unnecessary harm to animals.

This level of research and planning minimizes risks, promotes more efficient and ethical use of resources, improves safety, and increases the likelihood of successful regulatory approval by anticipating potential issues.

Strategic Framework: Pre-Study Intelligence and Risk Mitigation

INPUT CATEGORY	SCIENTIFIC RATIONALE	IMPACT ON NONCLINICAL STUDY DESIGN
Class-Based Profiling	Leverages historical data from established chemical or pharmacological classes	Identifies off-target liabilities and enables targeted monitoring of known class-effect toxicities
Pathway Mapping	Analyzes conserved biological pathways and molecular targets across different compound classes	Refines end-point selection (e.g., specific biomarkers) based on shared mechanistic risks (e.g., hERG, CYP inhibition)
Organ-System Prioritization	Utilizes *in silico* or *in vitro* signals that suggest tissue-specific vulnerability	Moves away from standardized to tailored histopathology and specialized functional assessments (e.g., telemetry, neurobehavior)
PK/PD Modeling	Integrates ADME data with target engagement	Defines the therapeutic index and ensures dose levels achieve sufficient multiples of clinical exposure without exceeding the MTD
Translational Benchmarking	Compares nonclinical signals with historical human data from similar scaffolds	Informs the starting dose for FIH trials and identifies potential species-specific metabolic differences

OPTIMIZING THE GLP TRANSITION

The timing of the transition from exploratory (non-GLP) to pivotal (GLP) toxicology is a critical determinant of a program's net present value (NPV). While GLP studies are mandatory for an IND application, premature initiation can be detrimental to both budgets and timelines. CMC and nonclinical safety often result in "technical debt," where subsequent changes to the drug substance profile—such as formulation or manufacturing shifts—necessitate costly repeat studies or complex bridging data. To avoid several months of unavoidable regulatory delays, pivotal GLP studies should only commence once the CMC process is sufficiently stable to ensure the test article is representative of the intended clinical material.

Strategic Indicators for Non-GLP Priority

Non-GLP studies serve as the primary de-risking engine when the following conditions exist:

- **CMC Fluidity:** The salt form, polymorph, or formulation is not yet finalized. Pivotal studies must use material representative of the clinical drug product to ensure analytical comparability.
- **PK Uncertainty:** Systemic exposure and dose-proportionality have not been fully characterized in both the rodent and non-rodent species.
- **Capital Preservation:** Pre-Series A or early-seed funding requires high-impact data (e.g., proof of concept safety) before committing to the significantly higher costs of GLP compliance.
- **Dose-Range Optimization:** The MTD and DLTs are not yet defined, which is a prerequisite for selecting the high dose in a GLP protocol.

GLP Readiness Considerations

- **The Material Match Rule:** Do not initiate the GLP in-life phase until the impurity profile of the drug substance is stable. Regulators may reject safety data if the clinical material is significantly different than the toxicology material.
- **The CRO Lead-Time Buffer:** Pivotal GLP slots at top-tier CROs typically require notice for scheduling. Secure your slot based on your CMC-ready date, not your discovery date.
- **The Series B Milestone:** Use robust non-GLP data to anchor a Series A/B raise; use the proceeds of that raise to fund the GLP safety assessment package required for the IND.

Non-GLP Readiness: The Go/No-Go Decision Matrix

To initiate exploratory safety studies, **a "YES" must be confirmed for all four criteria** to ensure the data is actionable and provides a return on investment.

CRITERION	STRATEGIC REQUIREMENT	Check if YES
Scientific Objective	Is the study design powered to define a clear MTD or DLT?	☐
Bioanalytical Readiness	Does a fit-for-purpose assay exist to correlate exposure with observed effects?	☐
Dose Rationale	Is the dose-escalation strategy supported by preliminary PK or *in vitro* potency data?	☐
Strategic Utility	Will the results directly inform the dose levels and species selection for the pivotal GLP study?	☐

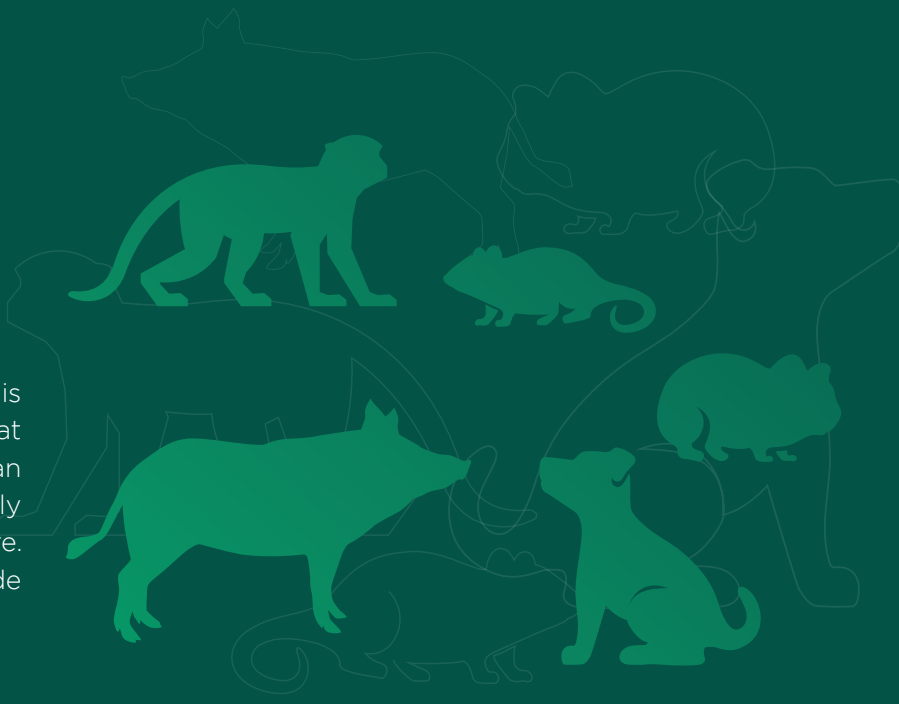
Pivotal GLP Readiness: Strategic Assessment

Before initiating IND-enabling GLP studies, sponsors must confirm the following five technical pillars. **A "NO" in any category indicates a significant risk of regulatory non-compliance or a clinical hold.**

TECHNICAL PILLAR	STRATEGIC REQUIREMENT	Check if NO
Clinical Alignment	Is the Phase I clinical synopsis finalized (route, duration, and dose range) to ensure the GLP package fully supports human entry?	☐
Material Comparability	Is the toxicology test article analytically representative of the intended clinical material (impurity profile, salt form, and potency)?	☐
Formulation Stability	Does stability data confirm the test article maintains its integrity and concentration for the entire in-life duration?	☐
Bioanalytical Validation	Are the PK/TK and ADA assays fully validated to meet GLP regulatory standards?	☐
Translational Safety	Has a preliminary human equivalent dose (HED) been calculated to establish a clear safety margin for the clinical starting dose?	☐

SPECIES SELECTION BY THERAPEUTIC MODALITY

Choosing the appropriate nonclinical model is a regulatory mandate (**ICH M3**, **S6, and S9**) that ensures toxicological data is predictive of human risk. The selection rationale shifts significantly based on the drug's molecular architecture. A scientifically robust program must provide data-driven justification for the chosen models.



FEATURE	SMALL MOLECULES (NMEs)	BIOLOGICS (mAbs/PROTEINS)	CELL AND GENE THERAPY (CGT)
Regulatory Requirement	Two species, typically one rodent (rat) and one non-rodent (dog/minipig)	One relevant species, usually NHP	Case-by-case, driven by pharmacologically relevant or humanized models
Primary Driver	Metabolic profiling, matching human metabolite ratios (>10% exposure)	Target homology: conserved sequence and binding affinity	Vector tropism, species must support viral/cellular distribution
Exposure Profile	Temporary: rapid systemic diffusion and clearance	Extended: target-mediated disposition; longer half-life	Persistent: potential for genomic integration or lifelong expression
Metabolic Fate	Enzymatic metabolism: Phase I/II hepatic processing	Proteolytic degradation: Broken down into endogenous amino acids	Biodistribution: cellular persistence, expansion, or viral shedding
Safety Endpoints	Off-target toxicity: classic organ-system histopathology	Exaggerated pharmacology: On-target effects and immunogenicity (ADA)	Genomic risk: Insertional mutagenesis and long-term vector behavior

ALTASCIENCES CASE STUDY: OVERCOMING KNOWN PHARMACOLOGICAL EFFECTS OF GLUCAGON-LIKE PEPTIDE-1 (GLP-1)

Altasciences conducted an IND-enabling study in dogs involving once-daily administration of an oral tablet containing a GLP-1 receptor agonist. Decreased gastric emptying and increased satiety are common pharmacological effects observed when conducting these types of studies, resulting in decreases in food consumption and body weight loss. This presents a challenge as the test article is being assessed for potential toxicological effects, although it is designed to produce decreased food consumption and body weight loss. These factors must be carefully balanced to maintain animal health and support the successful completion of the 28-day IND-enabling study.

Study Details

- **Drug Development Phase:** IND-enabling
- **Class of Drug:** Small molecule
- **Indication:** Weight loss / type 2 diabetes (T2DM)
- **Animal Model:** Dog
- **# of Animals:** 42 (21 per sex)
- **Dose Route:** Oral administration
- **Dose Regimen:** Once daily for 28 days

Study Design

- Clinical Observations
- Body Condition Scores
- Food Consumption
- Body Weights
- Ophthalmology
- Electrocardiography
- Neurological Assessments
- Toxicokinetics
- Clinical Pathology
- Anatomic Pathology

Study Purpose

To evaluate systemic toxicity and toxicokinetic characteristics of a GLP-1 agonist test article and potential reversibility of any findings.

Methods

During an acclimation period (at least two weeks prior to start of dosing) and throughout the course of the study, the dogs were fed a certified canine dry diet that contained a higher composition of fat/protein. In addition, the animals were provided daily canned and wet food.

These nonstandard diets allowed for the animals to begin the dosing phase at a higher starting body weight in anticipation of the expected weight loss that would result from the pharmacological action of the test article.

Dogs were dosed once daily for 28 days via tablets with a water flush to ensure administration.

Standard toxicological observations and measurements were performed over the course of the study, including detailed clinical observations, body weights, food consumption, ophthalmic examinations, electrocardiograms, clinical pathology, and anatomic pathology.

Animals were euthanized on Day 29. Complete necropsies were conducted, and standard organ weights recorded. A full set of tissues were collected from all animals, processed to slide, stained with hematoxylin and eosin (H&E), and evaluated by Altasciences' board-certified veterinary pathologist.

Group	Test Material	Dose Level (mg/animal)	Dose Concentration (mg/tablet)	Dose Amount (tablet/animal)	Terminal		Recovery	
					M	F	M	F
1	Placebo Tablet 1	0	0	1	3	3	2	2
2	Placebo Tablet 2	0	0	1	3	3	2	2
3	TA Tablet 1	10	10	1	3	3	0	0
4	TA Tablet 2	30	30	1	3	3	0	0
5	TA Tablet 3	100	100	1	3	3	2	2

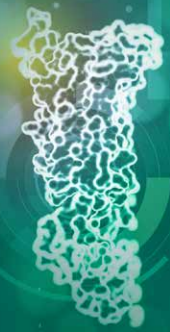
Results

The dogs' food consumption and body weight decreased over the course of the study in a dose-dependent manner with overall body weight loss at 4%, 10%, and 14% decrease when compared to control dogs.

The impact of the nonstandard diet allowed for an extended duration of dosing, and for the potential systemic toxic effects and the toxicokinetic characteristics of the test article to be evaluated without the influence of the pharmacological effect leading to intervention (i.e., dose holiday). In this manner, the objective of the study was achieved.

What Sets Altasciences Apart

We designed a novel approach to feeding the animals, specifically for this type of study. The higher starting weight of the dogs helped to counter the expected pharmacological effect of a GLP-1 test article, which allowed for the expected pharmacological effect to be observed while also allowing for other critical study endpoints to be met and evaluated.



HOW ALTASCIENCES CAN HELP

Whether you are working on preliminary exploratory studies or have a full IND-enabling program planned, Altasciences has the decades of experience and extensive knowledge to get your studies conducted quickly, with your unique needs in mind.

We have board-certified professionals throughout the organization, including:

- DABT study directors
- ACVP veterinary pathologists
- DACVO veterinary ophthalmologists

These experts work with you to determine the most effective approach, develop a strategically relevant program, and refine your plans as new data becomes available.

Our state-of-the-art nonclinical research facilities span over 585,000 square feet in locations throughout North America. We perform over 700 safety studies annually, building our knowledge base, study design database, and regulatory expertise. We offer a full range of *in vivo* GLP and non-GLP safety assessments for your drug substances, including laboratory solutions, ensuring your submissions meet global regulatory requirements.

Leverage our expertise in a broad spectrum of animal models and routes of administration—we ensure meticulous studies for every requirement.

Species

- Rats
- Mice
- Guinea pigs
- Rabbits
- Swine
- Miniature swine
- Dogs
- Nonhuman primates (Cynomolgus, Rhesus, others upon request)

Routes of Administration

- Oral
- Parental
- Infusion
- Ocular
- Dermal
- Implant
- Intravaginal and intrapenile
- Rectal

Tailored Full-Service Nonclinical Solutions

We have an extensive range of customizable nonclinical CRO services to support every aspect of your safety assessment journey. From formulation to bioanalysis and beyond, our full-service offering is designed to meet your unique project needs with precision.

- Formulation
- Analytical chemistry
- Bioanalysis
- PK/TK data analysis
- Immunohistochemistry
- Specialized necropsies
- Anatomic pathology
- Clinical pathology
- SEND (standard for the exchange of nonclinical data)
- *In vivo* study conduct

Whatever your program needs, our experts are here for you.

CONTACT US today to get your preclinical studies started.

ALTASCIENCES' RESOURCES

On-Demand Webinars

Designing IND-Enabling Toxicity Studies for Complex Drug Modalities: Going Beyond the Classical Approach

Preclinical Studies of Gene Therapy Products: Latest Trends

Best Practices to Reduce Animal Use in Toxicology Studies

How Do I Select the Right Species for My Toxicology Program?

Fact Sheets

Preclinical Drug Development

IND-Enabling (Small Molecule)

IND-Enabling (Large Molecule)

Safety Pharmacology

Nonclinical Safety Testing Guide

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.