Ensuring Translational Success: **Preclinical Study Design for CRISPR-based Therapeutics**

White Paper

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I. Introduction

In the drug development process, once a drug candidate is identified, development enters the preclinical study phase. Preclinical studies aim to address two major concerns: the safety and appropriate dosage level of the drug in development.

Preclinical studies differ from earlier discovery-phase studies in that, in order for the results used to make a “best guess” on how clinical studies could be performed in humans, the studies are performed in animal models or, more recently, organoids, which represent the phenotype and genotype of the target disease representative of the intended patient population. Efficacy and essential safety studies, such as proof of concept studies and toxicology studies, are conducted on the chosen model prior to IND submission.

Essential safety studies are required by regulatory agencies prior to IND submission to support the rationale for a first-in-human clinical trial, to make recommendations regarding clinical trial design, such as information on the initial safe starting dose, dose-escalation scheme, dosing schedule, and organ toxicity. In addition, the products investigated should be representative of the intended clinical drug product.

All of the above nonclinical studies must be incorporated into Module 4: the Nonclinical Study Report, one of the key components of the Common Technical Document (CTD) in the IND submission. The adequacy of nonclinical data significantly impacts regulatory approval decisions and the journey of CGT products from clinical trials to market approval. It is recommended that sponsors carefully consider the FDA's nonclinical guidance when developing and compiling information intended for submission.

II. A Strategic Approach to Preclinical Development

Early planning of nonclinical studies is essential, as there are many elements to manage and potential challenges to mitigate. Nonclinical study designs for CGT products require additional assessments because they have unique characteristics relative to other modalities.

At a minimum, preclinical studies of CGT products should obtain the following information prior to clinical trial:

- Proof of concept in relevant models,
- Support for the use of routes of administration, application procedure and application devices,
- Support for the selection of safe and biologically effective starting dose with adequate safety margins for clinical use, and
- Appropriate safety data.

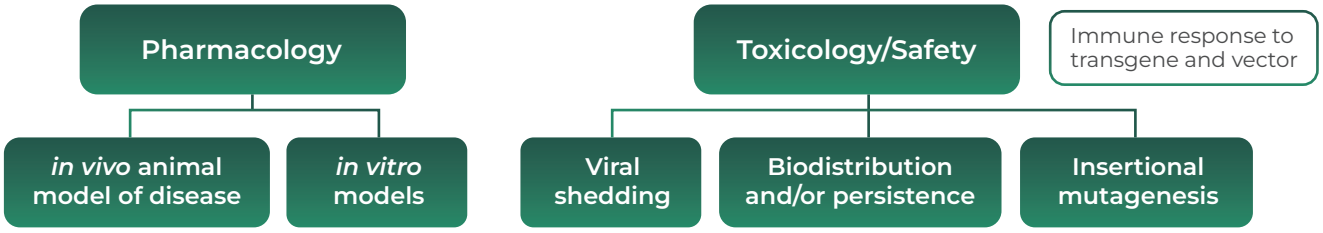

Figure 1. Unique Characteristics of CGT Studies

The preclinical development path for a CGT product generally begins by identifying its intended clinical use and generating a Target Product Profile (TPP). Subsequently, an initial program for preclinical development is designed based on the TPP, available preclinical data, and preliminary *in vitro* screening experiments as a guide. Model selection, pilot studies, and final pivotal nonclinical studies are then conducted.

Due to the specialized and complex functional nature of CGT products, selecting appropriate animal model systems for efficacy and safety testing is critical for preclinical development. If the efficacy of the product requires interaction with human ligands or cells, or if the safety of the product is based on the absence of residual cell populations whose detection requires *in vitro* expansion (e.g., residual proliferating cells in irradiated cellular therapy products), *in vitro* systems may be more appropriate than *in vivo* animal models for generating specific kinds of preclinical data.

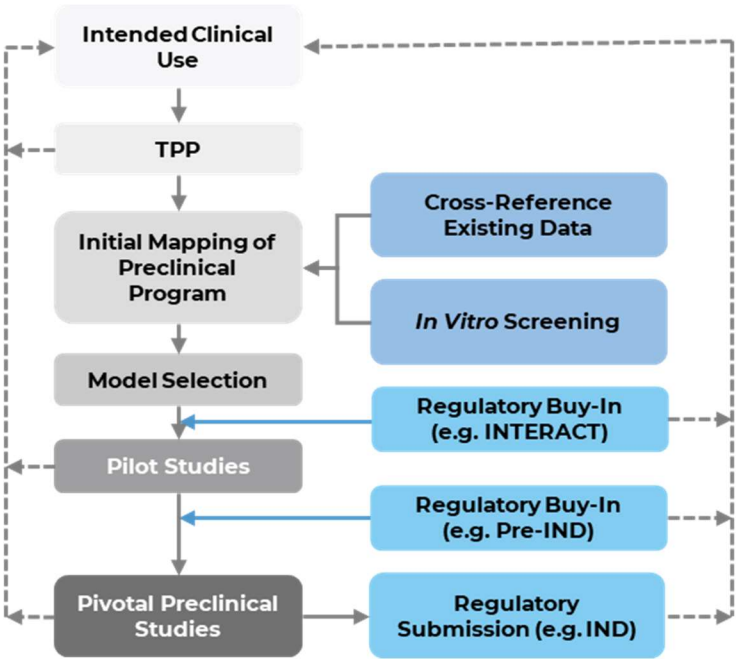

Figure 2: Preclinical Development Path of CGT Products

In general, the selection of an appropriate *in vivo* model for testing needs to be based on the target indication, dosing parameters, and the ability to measure clinically relevant efficacy and safety endpoints to adequately characterize preclinical efficacy or safety. Small and large animal model systems have their own advantages. Small animal models are cost-effective, and immunodeficient strains are more readily available. Large animal models (e.g., NHPs) are closer to humans in terms of pathophysiology, are more clinically appropriate in terms of dosage, and have the potential to provide greater predictability in clinical trials of CGT products.

Once the appropriate preclinical model system has been identified, pilot studies should be conducted to tailor the model to the specific testing needs of the product. Pilot studies may be used to conduct preliminary dose-escalation studies, refine the timing, method, and frequency of product administration, test candidate efficacy and safety endpoints, and provide supporting data for subsequent proof-of-concept (PoC) studies.

A. Using Appropriate Gene Editing Components in Preclinical Studies

For preclinical studies, it is advisable to use batches of guide RNA, Cas protein (or protein-encoding mRNA, etc.), and DNA payloads (if applicable) that are comparable to the intended clinical therapy product in terms of manufacturing process and product attributes, with any relevant differences clearly stated if they exist. Product batches used for pivotal preclinical safety studies need to be manufactured under the clinical manufacturing process (with full adherence to current Good Manufacturing Practices, or cGMP), and each batch should be characterized according to appropriate specifications and consistent with the product development phase. For *ex vivo*-

modified CRISPR-based cellular products, the clinical cell source/type should be used in the final preclinical study. If alternative cell sources/types are used, scientific justification must be provided in the CTD.

However, waiting until pivotal safety studies to switch from research-use-only grade to cGMP grade materials may cause comparability issues, potentially delaying the regulatory review process—particularly if this switch requires a change in vendor. Utilizing a phase-appropriate approach to material selection throughout the development process (i.e., utilizing IND-e or full GMP grade components throughout the preclinical study phase) is recommended to avoid these costly delays.

B. Pharmacology Studies

The activity characteristics of gene therapy products are evaluated through pharmacological studies to investigate the functionality of the corrected or expressed gene product (e.g., protein, RNA), if applicable, to assess the editing efficiency required to achieve the desired biological activity or therapeutic effect, and to observe the specificity and efficiency of editing in target and non-target cells, as well as the persistence of the genome modification and the resulting biological response.

Proof of Concept Studies

For CRISPR-based genome editing (GE) products, before efficacy and safety studies can begin, PoC studies are necessary to support the design of both the preclinical toxicology studies, while helping define reasonable risks for the investigational CGT product in the intended patient population. The in-life duration of PoC studies should be long enough to demonstrate that sustained benefit may translate into improved patient outcomes.

PoC studies investigation scope:

a. The pharmacological minimum effective dose (MED) and optimal biological dose.
b. Optimization of the route of administration (ROA), the timing of product administration relative to onset of disease/injury, and the dosing schedule.
c. Confirmation that the CGT product reaches the target anatomic site/tissue/cell.
d. Characterization of the putative mechanism of action (MOA) or hypothesized biological activities of the investigational CGT product.

In vitro PoC studies are used to identify potential safety concerns and MOAs for investigational CGT products. Preclinical *in vitro* assays employing *in vitro* models that intended to assess aspects of the biological activity of an investigational CGT product (e.g., growth factor secretion, immunological response profile, expression of a neurotransmitter) can provide supporting PoC information. However, the preclinical testing program should incorporate a stepwise, multifactorial approach to achieve an understanding of the biological plausibility for use of the investigational CGT product in the intended patient population, combined with *in vivo* preclinical

testing to anticipate the outcome of physiological and functional integration of the product following *in vivo* administration.

For *in vivo* testing, the use of animal models of disease/injury is recommended, as such studies that allow for the characterization of resulting morphological changes in conjunction with observable functional/behavioral changes. The animal species and/or models should exhibit a biological response to the CGT product. Careful attention to various elements of the preclinical design—such as study endpoints, cohort size, control groups, and in-life duration—is essential, as these parameters affect the interpretability and clinical relevance of the resulting safety data.

Safety Pharmacology

Safety pharmacology data are not routinely needed for CRISPR GE products. However, if the product is expected to have potential effects on major vital physiologic functions (i.e., cardiovascular, central nervous system, or respiratory function) at doses within or above the therapeutic dose range, appropriate safety pharmacology data should be available prior to human exposure. Safety pharmacology studies are usually performed using single-dose administration or, if possible, incorporated in single/repeated dose toxicity testing.

Pharmacokinetics

Pharmacokinetics for CGT products depend on the product type and include biodistribution (distribution and migration), as well as elimination parameters (persistence and clearance).

For CRISPR-based cellular products, the distribution, migration, and persistence of the cells should be understood to determine the risks associated with accidental biodistribution and should also focus on clearance and mobilization for such products. The risk of germline transmission should also be explored prior to clinical trial. The scope of the study depends on the type of cell therapy. For example, gene modified cells with non-integrating vectors or replication incompetent vectors are risk-free, and the scope of the study does not need to be extensive.

For gene therapy products, standard absorption, distribution, metabolism, and excretion (ADME) studies are not applicable in nonclinical studies. Sponsors need to perform biodistribution analyses in relevant animal species or models to determine the distribution and persistence of DNA sequences or expressed proteins in target and non-target tissues after direct administration in animals. Immunogenicity against the sequences or expressed proteins should also be considered. Biodistribution analysis can be performed alone or as part of standard pharmacology and toxicology studies.

In general, biodistribution analysis is performed at the molecular level using highly sensitive bioanalytical methods. The current “gold standard” for biodistribution studies is the use of

quantitative polymerase chain reaction (qPCR) assays to evaluate vectors/genomes in biological fluids and target tissues.

Shedding

The need for shedding studies is assessed according to the characteristics of the CRISPR-based GE products, e.g. replicative gene therapy products require a shedding analysis. Shedding analyses should include testing for the ability of the excreted components to become infected. According to the characteristics of shedding and the risk of infection, take appropriate risk control strategies in clinical trials.

C. Toxicology Studies

Toxicology studies should evaluate CGT products with a comprehensive safety analysis and, if necessary, the safety of transgene expression. Comprehensive safety studies can maximally predict the product's clinical risk. The design of toxicological studies for CGT products should fully consider the characteristics of the product itself, such as the structure and mechanism of action of the transgene, the activity and function of the expression constructs, the nature and biological characteristics of the vector, and the clinical application (e.g., indications, ROA, and therapeutic programs). Toxicological studies for CRISPR-based GE products include not only general toxicology to assess toxicity profiles, reversibility of toxicity, delayed toxicity, dose-toxicity response relationships, but also assessments such as immunogenicity and immunotoxicity, reproductive toxicity, genotoxicity, and carcinogenicity.

Safety studies for CRISPR-based GE products should identify on- and off-target editing events, including type, frequency, and location, and analyze genomic integrity, including chromosomal abnormalities, insertions or deletions, and potential oncogenicity or insertional mutagenesis. The biological consequences associated with on- and off-target editing need to be assessed, including the viability and function of edited cells, as well as the immunogenicity of the GE components and expressed transgenes. In the case of *ex vivo*-modified CRISPR-based cellular products, endpoints should describe the short- and long-term *in vivo* biodistribution using clinically relevant methods of administration and assess systemic and local toxicities associated with the treatment or administration procedure. Thus, the cohort size for safety testing is determined by the expected frequency of potential safety issues, and the *in vivo* duration and sampling for pivotal safety studies are determined by the expected *in vivo* duration and proliferative state of the administered cells. This must be sufficiently long to assess the likelihood of relevant long-term safety issues, such as tumor or ectopic tissue formation.

D. Preparing for Potential Regulatory Hurdles and IND Submission – INTERACT and Pre-IND Meetings

The timing of requesting direction from the FDA is a critical factor for the success of the preclinical phase, especially when it comes to late-stage pivotal studies. The data produced from pivotal preclinical studies plays a critical role in the risk-benefit analysis that determines whether a candidate CGT product is allowed to proceed to clinical trials. Due to this, and the significant impact on program costs, timelines, and animal welfare, it is recommended that sponsors seek regulatory advice on the design and endpoints of preclinical safety studies prior to conducting these studies, commonly through the **IN**itial **T**argeted **E**ngagement for **R**egulatory **A**dvice on **C**BER/CDER Produc**T**s (INTERACT) meeting and Pre-IND meeting.

If sponsors are at the stage of generating preliminary nonclinical data, PoC, and some safety information, but are not ready to conduct definitive pivotal nonclinical safety studies, they should request the right direction and advice. A pre-IND meeting request can be initiated when the sponsor has completed PoC and possibly some preliminary preclinical safety/toxicology studies and desires to move to the definitive toxicology studies.

III. Common Causes of Preclinical Study Failures and Design Challenges

The submission of sufficient information to FDA to assess potential risks to patients can reduce the incidence of preclinical concerns. This effort should include identifying the results of any safety studies, determining their relevance to clinical studies, and providing mitigation and monitoring plans for potential safety issues in clinical protocols. Due to the unique nature of CGT products, a comprehensive and robust preclinical plan to assess potential toxicity, biodistribution, and minimum effective dose should be developed and ideally discussed with the agency prior to IND submission. Otherwise, a clinical hold may result.

A. Common Causes of Preclinical Study Failures

During product development, drug developers need to ensure that all comprehensive nonclinical aspects are fully covered. The following are a few examples of insufficient knowledge and data that can lead to IND deficiencies, delays in product development programs, or even clinical hold:

1. **The CGT product tested in nonclinical studies is significantly different from the version intended for clinical trials.**

2. **Inadequate design of nonclinical studies, such as:**

    - The animal species used to conduct nonclinical safety studies or activity evaluation is not truly permissive to the vector or the final CGT product.
    - Dose levels administered do not adequately bracket the intended clinical dose level, and the rationale or extrapolation for the dose level is missing from the nonclinical program.
    - The safety assessments are not comprehensive enough to fully inform the potential risks to the intended patient population in clinical studies.

3. **The safety concerns, toxicities, or risks identified in nonclinical studies are not sufficiently characterized or justified, for example:**

    - Product-related adverse findings are identified in nonclinical studies, but the animals were not observed long enough to determine whether these adverse findings would resolve or worsen over time.

In addition to these cases, the design of all preclinical studies and the data collected from them must directly support the safety of the intended clinical product.

B. Common Challenges with Preclinical Study Design

The challenges involved in nonclinical studies of CGT products include understanding on-target and off-target activity, immune responses, and other serious adverse events (AEs). Due to the specificity of their properties, the greatest challenges for genome editing therapy products, include controlling activity, mechanism of action, detecting potential off-target mutations, and addressing their inherent immunogenicity. The goal of an efficient gene editing therapy is to achieve perfect specificity for the target sequence without introducing mutations into any other region of the genome. All of this must be carefully monitored, rigorously evaluated, and managed to the highest extent possible. Failing to account for any of the following considerations may result in insufficient nonclinical data and lead to issues with IND approval:

1. Investigational CGT Products Used in Preclinical Studies

Ideally, batches used in preclinical studies should have the same manufacturing and quality control as batches for clinical trials and perform their biological functions in animal models in the expected MOA. If the biological action of a gene therapy product is species-specific, as with

some vector-expressed human transgenes or human-derived cellular therapy products, the representativeness of the product to be used in the clinical trial should be scientifically justified.

As the development of an investigational CGT product moves into late-stage clinical trials, additional preclinical studies should be considered to address any outstanding questions. For example, if major changes occur, like manufacturing/formulation changes, additional *in vitro* and/or *in vivo* preclinical studies may be needed to bridge the pre- and post-change products.

2. Animal Species/Model Selection

The animal species selected should demonstrate a biological response similar to that expected in humans, with physiology and anatomy comparable to those of humans. It should also consider immune tolerance to the CRISPR-based products or the human transgene expressed by the genome editing products.

Depending on the specific characteristics and clinical indications of the products, selection may involve conventional non-human primates (NHPs) that are genetically, physiologically, and immunologically similar to humans, or "non-standard" test species. For CRISPR-based products, genetically modified rodents (i.e., transgenics or knockouts) may be used to visualize the biological response to gene editing.

For proof-of-concept (PoC) studies, the identification of available animal models and the assessment of model limitations are usually performed in conjunction with the genome editing product's characteristics and mechanism of action. If there is no suitable animal model, a new one may need to be constructed, and the basis for its selection needs to be clearly stated. In some cases, more than one animal model may need to be used for investigation.

3. Design of Nonclinical Studies

The design of nonclinical studies for gene therapy products should focus on identifying potential risks, including both safety assessments and proposed clinical trial evaluation elements. The identification and characterization of on- and off-target editing, chromosomal abnormalities, and their biological consequences must be evaluated and analyzed as necessary.

This concern was also highlighted during the Advisory Committee meeting between Vertex Pharmaceuticals, Inc. and FDA prior to the approval of a CRISPR-based product-exa-cel. The complexities involved in assessing off-target effects in genome editing, the importance of methodological rigor, reliance on population-wide data, and the necessity of long-term monitoring to assess safety and efficacy were all key takeaways. Additionally, nonclinical safety

studies should comprehensively address clinical trial elements such as endpoints and dose level ranges to anticipate the biological response in clinical studies.

4. GLP Compliance

Ideally, all safety studies supporting the IND should be conducted in compliance with Good Laboratory Practice (GLP). However, due to the advanced technology and species/model specificity of CGT products, technical limitations may prevent these studies from being conducted at GLP-compliant facilities. In such cases, studies should be conducted according to a prospectively written protocol and in as unbiased a manner as possible. Quality assurance oversight is critical to ensure that studies are conducted following sound procedures and to maintain the integrity of the results and data. Regardless of GLP compliance, vendors performing nonclinical studies should be audited by a contractor, for compliance with GLP regulations or quality assurance strategies necessary to ensure the quality and integrity of safety data.

If nonclinical studies are conducted at non-GLP testing facilities, the reason should be stated in Module 4 of CTD in the IND submission, along with specifications and justifications for any deviations from the proposed written protocol. Inadequate justification for not following GLP may result in failure to successfully proceed to clinical trials.

5. Toxicology Studies

The development of comprehensive toxicology studies is challenging for *ex vivo*-modified CRISPR-based cellular products. A comprehensive nonclinical safety evaluation should focus on cell, gene, and gene expression constructs.

The risks related to cells may arise from the source of the cells, *in vitro* culture and manipulation, cellular properties, the toxicity of non-cellular components, the operations of genetic modifications, or the immune response and recognition. Toxicological studies on cells include evaluation of general toxicity, immunogenicity and immunotoxicity, tumorigenicity and oncogenicity, as well as analysis based on the cellular properties for additional evaluations such as reproductive and developmental toxicity evaluations, local tolerance, histocompatibility, and genotoxicity.

The risks related to genes should focus on genotoxicity resulting from gene modification and operations, as well as evaluating the safety risks caused by gene vectors (e.g., viral vectors or microbial vectors), such as aberrant immune responses, reactivation, or replication, and the reproductive or developmental toxicity and germline transmission risks resulting from gene modification. The safety evaluation shall be based on product characteristics such as the target

gene of gene editing, exogenous sequences, gene integration mode and location, vector design, vector dosage, and target cell population, and its corresponding risks shall be assessed, such as off-target effects, insertion mutations and recombination risk.

The safety evaluation of the gene expression constructs includes the expression of the modified gene (expression level and duration), the distribution and activity of expression constructs, the effect of the expression constructs on the vector characteristics and *in vivo* function, the interaction with endogenous molecules and immunogenicity. Toxicology studies need to allow for the capture of any local or systemic adverse effects caused by the transgene expression constructs.

6. Target Validation and Assessment of Off-Target Effects

A significant part of safety studies of CRISPR-based GE products is the identification and characterization of on- and off-target edits, including type, frequency, and location to identify potential risks associated with administration of the GE products.

The study of off-target editing can be performed using multiple methods (e.g., *in silico*, biochemical, cellular-based assays) including a genome-wide analysis to reduce bias in identification of potential off-target sites. The identified off-target sites are then verified using methods with adequate sensitivity to detect low-frequency events. For *in vitro* genetically modified products, the clinical trial products obtained from multiple donors should be evaluated. It is also important to include appropriate control groups to confirm the quality of the assay and to ensure the interpretability of the results and suitability for the intended use. For *in vivo* GE products, the analysis should also include the major cell types in which editing events are detected.

Finally, the biological consequences associated with on-target and off-target editing need to be evaluated, including, but not limited to, viability and function of edited cells.

7. Delivery and Expression

Gene therapy products require vectors to be delivered into the human body, such as viral vectors, lipid nanoparticles (LNP), or other non-viral vectors. Due to their diverse range of structural properties and mechanisms of action, it is also important to consider the safety of the specific delivery vector used. For example, some genes inserted into integrative vectors may affect subsequent gene expression, causing mutation/carcinogenicity risks.

The mode of gene delivery and vector type can also affect the immune response to gene therapy products. For example, delivery of transgenes that encode various endogenous

enzymes, receptors or structural proteins may elicit antibodies against both the transgene and against the endogenous components expressed in normal cells and tissues, resulting in an adverse response. Therefore, rational nonclinical studies based on different delivery vector types must be performed.

IV. Conclusion

A robust preclinical strategy is critical to the successful development of CGT products. However, the path to an IND submission is fraught with challenges, particularly in the design and execution of nonclinical studies. One of the most significant hurdles is ensuring that preclinical studies sufficiently mirror the product intended for clinical use, as differences in formulation, vector, or transgene between the nonclinical and clinical versions of the CGT product can lead to costly delays or even clinical holds. Thorough planning ensures that key parameters such as dosing, safety margins, and route of administration are addressed early, mitigating challenges that could arise during regulatory review.

Using phase-appropriate materials and relevant animal models or *in vitro* systems further helps generate reliable, translatable data, which can significantly enhance the likelihood of a smooth progression through the drug development pipeline. As it is essential to follow the latest regulatory guidance, having timely discussions with the FDA prior to IND submission through Pre-IND or INTERACT meetings can play a significant role in helping to reduce risks and support more efficient and informed decision-making, ultimately accelerating the path from preclinical development to clinical trials.

Please be advised that the content contained within this document, including any recommendations, insights, opinions, or advice, is solely reflective of GenScript's perspective. The material provided herein is intended for general informational purposes only and should not be construed as official guidance from the FDA.

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