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Nonclinical research guideline for in vivo generated cell therapy products

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INTRODUCTION

The convergence of gene editing technologies and cell-based therapeutics has given rise to a novel class of interventions known as in vivo generated cell therapy products. These agents employ viral or non-viral vectors to introduce genetic instructions directly into a patient's endogenous cells in situ, thereby reprogramming target cells to acquire therapeutic functions (e.g., chimeric antigen receptor (CAR) expression, T cell receptor (TCR) redirection) without ex vivo manipulation or lymphodepletion. Given the rapidly evolving nature of this field, existing regulatory guidelines provide only a fragmented framework. To address this gap, we established a unified nonclinical research standard for in vivo generated cell therapy to provide a scientifically grounded foundation for clinical trial design and risk mitigation.

This guideline establishes four overarching objectives for nonclinical studies of in vivo generated cell therapy products based on our center's experience and international consensus: (1) define mechanistic characterization, including vector design, targeting, immunogenicity reduction, and mechanism of action; (2) quantify delivery efficiency via biodistribution, transduction, expression kinetics, and functional activity; (3) map tissue specificity and pharmacological profiles across key tissues and time points to inform clinical dose and route; and (4) evaluate comprehensive toxicity, covering vector- and cell therapy-related adverse events such as off-target effects, insertional mutagenesis, and long-term organ toxicity.

PRODUCT CHARACTERIZATION AND QUALITY ATTRIBUTES

Formulation and stability

The product formulations shall be characterized in detail, including appearance, pH, osmolarity, buffer composition, and excipients. Stability data must be provided under various storage conditions, including long-term, transport, and in-use scenarios. Packaging materials must be validated for compatibility, safety, and adsorption effects.

Transgene architecture

The transgene cassette, including regulatory elements and coding sequences, must be fully described. For CAR-based products, detailed mapping of extracellular, transmembrane, and intracellular domains is required. Any sequence modifications should be accompanied by structural and safety justifications.

Vector platforms and platform-specific requirements

Vectors are hierarchically categorized by biological nature and integration potential, each requiring a tailored evaluation strategy. [1-3]

Class I: Viral Vectors. (1) Integrating (e.g., lentivirus, gamma-retrovirus, transposons): Key safety considerations include insertional mutagenesis, necessitating comprehensive integration site analysis. (2) Non-integrating (e.g., AAV, helper-dependent adenovirus): Focus on episomal persistence and replication-competent virus risk.

Class II: Non-Viral Vectors. (1) Lipid-based (e.g., lipid nanoparticles [LNPs]): Safety concerns include complement activation, hepatotoxicity, and PEG-related hypersensitivity. (2) Polymer-based (e.g., polyethylenimine [PEI], poly (lactic-co-glycolic acid) [PLGA]): Safety considerations involve cationic polymer cytotoxicity and degradation product accumulation. (3) Biological-derived (e.g., exosomes): Safety focuses on source cell oncogenicity and cargo heterogeneity.

For all targeted vectors, the Target Selectivity Index (TSI) and Tissue Enrichment Factor (TEF) shall be calculated and interpreted alongside absolute transduction levels in target versus non-target tissues. TSI = (Transgene copies per μg DNA in target tissue) / (Transgene copies per μg DNA in non target tissue); a higher TSI indicates greater selectivity. TEF = (Transgene copies per μg DNA in tissue of interest) / (Transgene copies per μg DNA in a reference tissue, e.g., blood); a TEF >1 indicates enrichment. A TSI >10 is considered favorable for selectivity, but if absolute transduction in a high risk non target tissue exceeds 10% of target level, additional studies are warranted regardless of TSI.

Manufacturing and process comparability

Detailed descriptions of raw materials, production systems, purification methods, and critical quality attributes are required. For nonclinical studies that utilize animal-derived components (e.g., promoters, packaging cell lines), comparability to clinical-grade materials must be demonstrated in terms of efficacy, safety, and impurity profiles.

PHARMACODYNAMIC ASSESSMENT

Pharmacodynamic studies evaluate delivery specificity, expression dynamics, and functional activity. The core endpoints recommended for first-in-human enabling studies include transduction efficiency, transgene expression kinetics, cytotoxicity assessment (in vitro), and in vivo measurement of transgene levels in relevant tissues such as blood, spleen, bone marrow, and tumor (as appropriate for the disease model).

In vitro assays should assess CAR/TCR expression kinetics, activation markers (e.g., CD25, CD69, CD107a), and cytotoxicity at varying effector-to-target ratios. In vivo studies using humanized mouse models should measure transgene expression over time across tissues such as peripheral blood, spleen, bone marrow, and tumor.

Humanized mouse model selection should align with study objectives.[4] The peripheral blood mononuclear cell-humanized model supports acute toxicity, short-term transduction (≤ 28 days), and proof-of-concept studies. In contrast, the $CD34^+$ hematopoietic stem cell-humanized model supports long-term persistence (≥ 6 months), memory responses, and integration site analysis. Critical endpoints include bone marrow chimerism, thymic T cell output, and splenic germinal center formation.

Beyond initial transduction efficiency, studies must evaluate therapeutic cell durability through exhaustion marker monitoring and tumor rechallenge protocols. Exhaustion should be assessed by flow cytometric quantification of PD-1, TIM-3, LAG-3, and TIGIT on transgene-positive T cells at weeks 1, 2, 4, 8, and 12 post-administration. For tumor rechallenge, mice achieving an initial complete response should receive secondary tumor inoculation on the contralateral flank at days 60–90. Sponsors may justify alternative thresholds based on product-specific data.

SAFETY EVALUATION

Safety studies must adopt a risk-stratified approach tailored to vector type and mechanism. Viral vector assessments emphasize immunogenicity, insertional mutagenesis, and tissue damage from persistence; lipid-based vectors focus on hepatotoxicity, complement activation, hypersensitivity reactions (e.g., PEG-related), and vascular occlusion; novel vectors require evaluation of degradation product accumulation, immunogenicity, and off-target binding.

Single- and repeat-dose toxicity studies in rodent models should cover neurological, respiratory, and cardiovascular systems, with dose ranges extended to the maximum tolerated dose. Long-term studies (≥ 6 months) are recommended for chronic effects, particularly in tissues with sustained vector retention.

Dynamic monitoring of inflammatory cytokines, complement activation products, and immune cell subsets is essential to assess risks such as cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome. Immunogenicity assessments should differentiate responses attributable to the vector, transgene product, formulation components, and impurities, and must include evaluation of anti-vector immunity (neutralizing and total antibodies against viral capsids, LNP components, or polymer backbones), anti-transgene immunity (antibodies against the transgenic product and their impact on cell persistence), and for vectors with high human seroprevalence (e.g., AAV, PEGylated LNPs), pre-existing immunity studies using serum-screened or pre-immunized models to assess impacts on reprogramming efficiency and safety.

Nonhuman primate studies may provide valuable data on targeted expres-

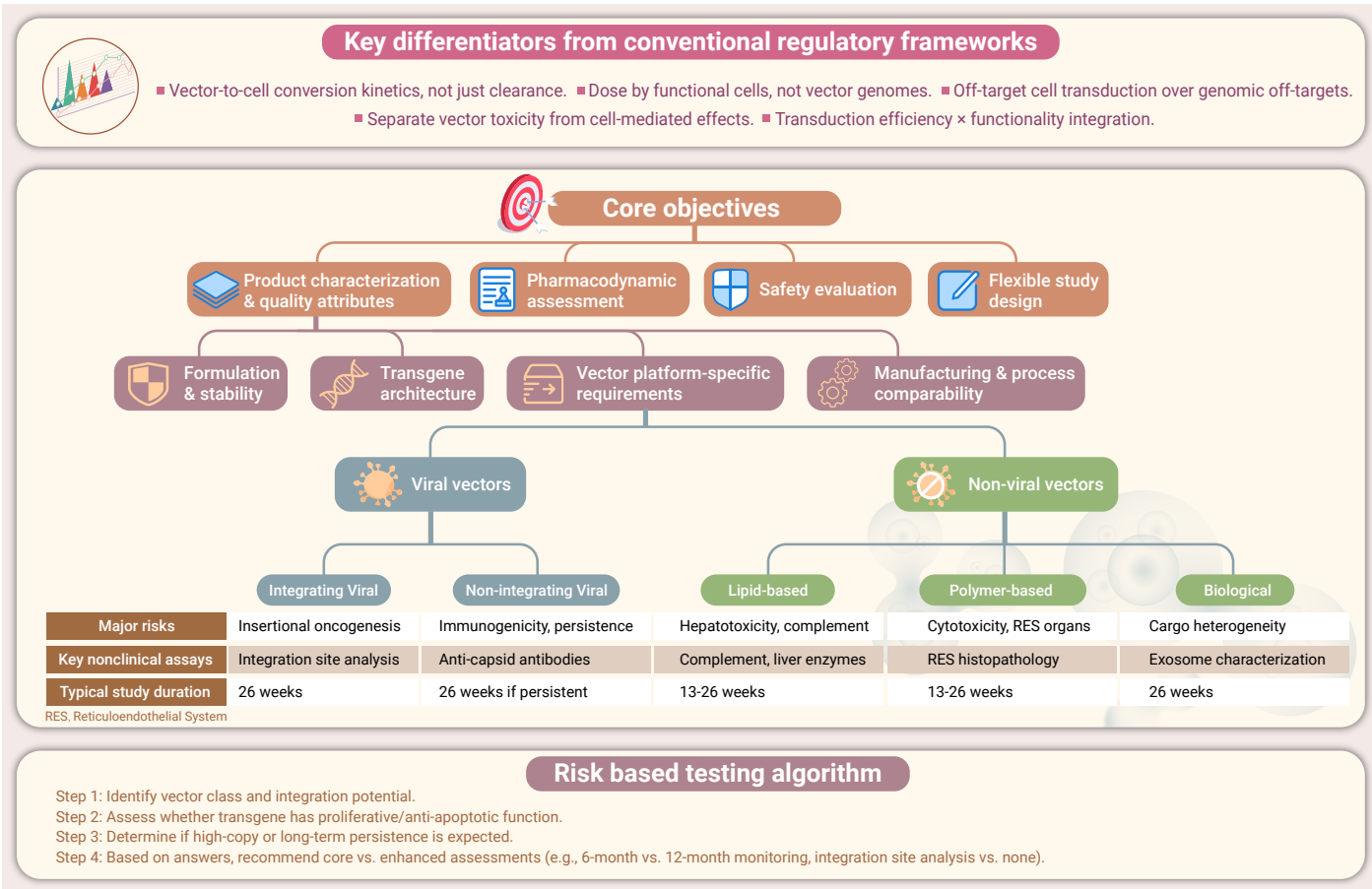

Figure 1. Nonclinical research framework for in vivo generated cell therapy This guideline covers product quality attributes, pharmacodynamic assessment, and risk-stratified safety evaluation to support clinical translation.

sion, T-cell activation kinetics, and immunotoxicity in a system more closely resembling human physiology. Special population studies should follow a risk- and indication-driven approach: geriatric models are recommended when the target population is elderly or mechanisms involve immunosenescence; hepatic or renal impairment models are recommended when clearance is organ-dependent. All general toxicology studies must include both sexes with sex-stratified analyses. Preclinical evaluation of on-target and off-target toxicity should include cross-reactivity studies using human tissue panels and bioinformatic predictions, with single-cell sequencing and in vitro validation encouraged to quantify risks of unintended transduction. These data are intended to inform individualized clinical dosing and monitoring strategies.

Oncogenicity risk assessment should address both integrating and non-integrating vectors.[5] Insertion-related risk is primarily associated with integrating vectors such as lentivirus and transposon systems, for which comprehensive integration site analysis is required, including assessment of proximity to proto-oncogenes or tumor suppressors, and long-term tumor monitoring of at least six months is recommended. Transgene-driven proliferative risk arises when the transgene product has proliferative, anti-apoptotic, or growth factor-like functions, regardless of vector type. Persistence-related risk becomes relevant when non-integrating vectors exhibit high-copy episomal persistence (≥10 copies per cell) for extended periods, or when the vector contains strong enhancer or promoter elements that sustain long-term expression.

FLEXIBILITY AND INNOVATION IN STUDY DESIGN

Given the rapid evolution of in vivo cell therapy technologies, the guideline allows for flexibility in study design, provided that modifications are scientifically justified and risk-based. The use of emerging methodologies—such as noninvasive imaging for real-time biodistribution tracking and single-cell multi-omics for mechanistic insights—is strongly encouraged to enhance data richness and predictive value.

CONCLUSION

This guideline provides a structured framework for the nonclinical evaluation of in vivo generated cell therapy products. By integrating vector-specific requirements with quantitative metrics and considerations for vulnerable populations, it aims to harmonize preclinical development practices and support the safe and efficient translation of these novel therapeutics into clinical application. As the field continues to evolve, this framework is intended to serve as a living document adaptable to emerging scientific evidence and regulatory experience.

The authors declare no competing interests.

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AUTHOR CONTRIBUTIONS

N.L. and S.W. edited and supervised the manuscript. J.D., P.M., and G.Z. wrote the manuscript. All authors contributed to the article and approved the submitted version.

DECLARATION OF INTERESTS

The authors declare no competing interests.