S12 Nonclinical Biodistribution Considerations for Gene Therapy Products

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

May 2023
ICH-Safety

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FOREWORD

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has the mission of achieving greater regulatory harmonization worldwide to ensure that safe, effective, and high-quality medicines are developed, registered, and maintained in the most resource-efficient manner. By harmonizing the regulatory expectations in regions around the world, ICH guidelines have substantially reduced duplicative clinical studies, prevented unnecessary animal studies, standardized safety reporting and marketing application submissions, and contributed to many other improvements in the quality of global drug development and manufacturing and the products available to patients.

ICH is a consensus-driven process that involves technical experts from regulatory authorities and industry parties in detailed technical and science-based harmonization work that results in the development of ICH guidelines. The commitment to consistent adoption of these consensus-based guidelines by regulators around the globe is critical to realizing the benefits of safe, effective, and high-quality medicines for patients as well as for industry. As a Founding Regulatory Member of ICH, the Food and Drug Administration (FDA) plays a major role in the development of each of the ICH guidelines, which FDA then adopts and issues as guidance to industry.

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Contains Nonbinding Recommendations

S12 Nonclinical Biodistribution Considerations for Gene Therapy Products Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA office responsible for this guidance as listed on the title page.

I. INTRODUCTION (1)[2]

A. Objectives of the ICH S12 Guidance (1.1)

The objective of this guidance is to provide harmonized recommendations for the conduct of nonclinical biodistribution (BD) studies in the development of gene therapy (GT) products. This document provides recommendations for the overall design of nonclinical BD assessments. Considerations for interpretation and application of the BD data to support a nonclinical development program and the design of clinical trials are also provided. The recommendations in this guidance endeavor to facilitate the development of GT products while avoiding unnecessary use of animals, in accordance with the 3Rs (reduce/refine/replace) principles.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

B. Background (1.2)

An understanding of the BD profile of a GT product following in vivo administration is an important element of the nonclinical development program. BD data contribute to the interpretation and design of nonclinical pharmacology and toxicology studies conducted to support early-phase clinical trials in the target population. Although guidances that include recommendations for BD studies have been issued by various regulatory authorities, this document provides a harmonized definition for nonclinical BD and conveys overall considerations for assessing BD for GT products. With continued scientific advances in the

[1] This guidance was developed within the Expert Working Group (Safety) of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document has been endorsed by the ICH Assembly at *Step 4* of the ICH process, March 2023. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the ICH regions.

[2] The numbers in parentheses reflect the organizational breakdown of the document endorsed by the ICH Assembly at Step 4 of the ICH process, March 2023.

GT field, incorporation of this topic in early discussions of the nonclinical program for a GT product with the appropriate regulatory authority is also encouraged.

C. Scope (1.3)

GT products within the scope of this guidance include products that mediate their effect by the expression (transcription or translation) of transferred genetic materials. Some examples of GT products can include purified nucleic acid (e.g., plasmids and RNA), microorganisms (e.g., viruses, bacteria, fungi) genetically modified to express transgenes (including products that edit the host genome), and ex vivo genetically modified human cells. Products that are intended to alter the host cell genome in vivo without specific transcription or translation (i.e., delivery of a nuclease and guide RNA by nonviral methods) are also covered in this guidance. Although not currently considered GT in certain regions, the principles outlined in this guidance are also applicable to oncolytic viruses that are not genetically modified to express a transgene.

This guidance does not apply to prophylactic vaccines. Chemically synthesized oligonucleotides or their analogues, which are not produced using a biotechnology-based manufacturing process, are also outside the scope of this guidance.

The release of a GT product outside the body via excreta and secreta (feces, urine, saliva, nasopharyngeal fluids, etc.), or through the skin (pustules, sores, wounds) is termed *shedding*. Evaluation of the nonclinical shedding profile of a GT product is outside the scope of this guidance. Assessment of genomic integration and germline integration of GT products is also outside the scope of this guidance. Considerations for these aspects of nonclinical data can be found in existing International Council for Harmonisation (ICH) consideration documents (1, 2).

II. DEFINITION OF NONCLINICAL BD (2)

BD is the in vivo distribution, persistence, and clearance of a GT product at the site of administration and in target and nontarget tissues, including biofluids (e.g., blood, cerebrospinal fluid, vitreous fluid). Nonclinical BD assessment entails the use of analytical methods to detect the GT product and transferred genetic material in collected samples and can include methods to detect the expression product of the transferred genetic material.

III. TIMING OF NONCLINICAL BD ASSESSMENT (3)

BD data should be available when evaluating and interpreting the nonclinical pharmacology and toxicology findings. Nonclinical BD data can also inform design aspects of a first-in-human clinical trial (see section VI (6)). It is important that nonclinical BD assessments be completed prior to initiation of the clinical trial.

IV. DESIGN OF NONCLINICAL BD STUDIES (4)

A. General Considerations (4.1)

Nonclinical studies for BD assessment can be conducted as stand-alone BD studies or in conjunction with nonclinical pharmacology and toxicology studies. Therefore, in this document the term *BD study* represents either scenario. A nonclinical BD study should be performed in a biologically relevant animal species or model (see section IV.C (4.3)) following administration of a GT product that is representative of the intended clinical product (see section IV.B (4.2) for possible alternate scenarios). It is important that the route of administration (ROA) reflect the intended clinical ROA to the extent possible and that the dose levels studied provide sufficient characterization of the BD profile (see section IV.E (4.5)).

It is important to verify the data quality, integrity, and reliability of the BD evaluation. In principle, nonclinical BD studies that are not conducted in compliance with good laboratory practice (GLP) are acceptable. However, when BD evaluation is performed as part of a GLP-compliant toxicology study, it is important that all in-life evaluations and sample collection procedures remain in compliance with GLP. Sample analysis for BD can be conducted in non-GLP manner.

Considerations specific to ex vivo genetically modified cell products are addressed in section V.E (5.5).

B. Test Article (4.2)

The test article administered in the nonclinical BD studies should be representative of the intended clinical GT product, taking into consideration the manufacturing process, important product characteristics (e.g., titer), and the final clinical formulation. In some situations, nonclinical BD data generated with a GT product consisting of the same vector intended for clinical use and a different therapeutic transgene or an expression marker gene (e.g., adeno-associated virus vector of the same serotype and promoter that directs expression of a fluorescent marker protein transgene) can be leveraged to support the BD profile (see section V.G (5.7)).

C. Animal Species or Model (4.3)

BD assessment should be conducted in a biologically relevant animal species or model that supports transfer and expression of the genetic material (Note 1). Selection factors can include species differences in tissue tropism of the GT product, gene transfer efficiency, and transgene expression in target and nontarget tissues/cells. If working with a replication competent viral vector, it is important that the animal species or model be permissive to vector replication.

The influence of species, sex, age, physiologic condition (e.g., healthy animal versus animal disease model) on the BD profile can also be important. In addition, the potential for the animal species to mount an immune response against the administered vector and/or expression product should be considered (see section V.D (5.4)).

D. Group Size and Sex of Animals (4.4)

An appropriate number of animals per sex (as applicable) should be evaluated at each predetermined sampling time point to generate sufficient data that support comprehensive BD assessment (see Note 2). In keeping with the principles of the 3Rs, the total number of animals can be an aggregate from several studies. Justification should be provided for the number of animals evaluated at each time point, as well as the use of combined data from multiple studies, as applicable. Justification should also be provided when only one sex is evaluated.

E. Route of Administration and Dose Level Selection (4.5)

The ROA of the GT product can affect the BD profile, including the cell types that are transduced and the immune response, and therefore, the GT product should be administered using the intended clinical ROA, as feasible (see Note 3). The selected dose levels of the administered GT product should provide adequate characterization of the BD profile to aid in interpretation of the pharmacology and toxicology assessments. The highest dose level evaluated should be the expected maximum dose level in the toxicology studies (usually limited by animal size, ROA/anatomic target, or GT product concentration). It is important that the dose level for BD evaluation equate to or exceed the anticipated maximum clinical dose level. However, it should not exceed the highest dose level administered in the toxicology study.

F. Sample Collection (4.6)

The sample collection procedure for target and nontarget tissues and biofluids should be designed to minimize the potential for contamination. It is important to follow a prespecified process that includes appropriate retention of the samples obtained from each animal (those that received vehicle control and those administered the GT product), as well as documenting the order of sample collection. Sample collection time points during the nonclinical BD study should be selected to sufficiently characterize the time-related changes in GT product levels over appropriate time points. Additional time points can be included, as applicable, to comprehensively capture the length of the steady-state period or to estimate persistence. Inclusion of time points to permit evaluation of GT product levels after repeat administration should be considered, when applicable.

For replication competent vectors, sample collection time points should also cover the detection of the second peak level due to vector replication and the subsequent clearance phase in relevant sample(s).

The collected samples should include the following panel of tissues/biofluids: injection site(s), gonads, adrenal gland, brain, spinal cord (cervical, thoracic, and lumbar), liver, kidney, lung, heart, spleen, and blood. This panel can be expanded depending on additional considerations, such as vector type/tissue tropism, the expression product, ROA, disease pathophysiology, and animal sex and age. For example, additional tissues/biofluids can include peripheral nerves, dorsal root ganglia, cerebrospinal fluid, vitreous fluid, draining lymph nodes, bone marrow, or eyes and optic nerve. The decision as to the final sample collection panel should be guided by an understanding of the GT product, the target clinical population, the ROA, and existing nonclinical data.

Collected samples can also be analyzed for the presence of the expression product. Considerations regarding this assessment are provided in section V.B (5.2).

V. SPECIFIC CONSIDERATIONS (5)

A. Assay Methodologies (5.1)

Evaluation of the BD profile necessitates quantifying the amount of genetic material (DNA/RNA) of the GT product in tissues/biofluids and, if appropriate, the expression product(s). Currently, quantitation of vector genome and/or transgene DNA/RNA relative to input genomic DNA by established nucleic acid amplification methods (e.g., quantitative polymerase chain reaction (qPCR), digital PCR) is considered the standard for detection of GT products in tissues/biofluids over the course of time. When cellular content varies significantly in a sample (e.g., biofluids), DNA/RNA concentration (e.g., copy number per microliter) can be used. Spike and recovery experiments, considered a part of assay development, should be performed to demonstrate the ability to detect the target nucleic acid sequence in different tissues/biofluids.

Other techniques can be used in nonclinical studies to monitor BD of a vector and/or the expression product(s). These include but are not limited to the following: enzyme-linked immunosorbent assay, immunohistochemistry, western blot, in situ hybridization, flow cytometry, various in vivo and ex vivo imaging techniques, and other evolving technologies.

It is important to provide a comprehensive description of the methodology and the justification for the technique used, including the performance parameters (e.g., sensitivity and reproducibility) of the method.

B. Measurement of Expression Products (5.2)

While quantification of the genetic material of the GT product is the primary BD assessment (see section V.A (5.1)), determination of the level of the expression product in vector genome positive tissues/biofluids can contribute to characterization of the safety and activity profiles following GT product administration. Decisions regarding the conduct of such assessments should be based on the extent of nonclinical BD analyses needed for the GT product, which is determined using a risk-based approach. This approach can include consideration of the GT product levels and persistence in tissues/biofluids; the target clinical population; and potential safety concerns associated with the vector and/or the expression product.

C. Immunological Considerations (5.3)

Preexisting immunity in animals, notably in nonhuman primates and other nonrodent species, against a GT product could affect the BD profile. Screening of animals for preexisting immunity to the vector prior to inclusion in a nonclinical study should be considered. In such situations, it is important that this aspect is factored into the nonbiased method used to randomize animals to study groups.

A cell-mediated or humoral immune response to the GT product can occur after administration of the GT product. These responses may result in a BD profile that is not informative.

Therefore, the sponsor can consider collecting samples for possible immunogenicity analysis to support interpretation of the BD data.

Immunosuppression of animals for the sole purpose of evaluating the BD profile is not recommended. However, if product- or species-specific circumstances warrant immunosuppression, justification should be provided.

In certain cases, due to the species-specific nature of the transgene, the animal may mount a cell-mediated or humoral immune response to the expression product(s). If this occurs, use of a species-specific orthologous transgene can be considered to circumvent the effects of the immune response.

D. Ex vivo Genetically Modified Cells (5.4)

Considerations for BD assessment of GT products that consist of ex vivo genetically modified cells (i.e., cells that are transduced/transfected ex vivo and then administered to the animal/human subject) should include factors such as the cell type, ROA, and the potential for the expression product or gene modification event to affect the expected distribution of the cells within the body (e.g., new or altered expression of cell adhesion molecules). In addition, the occurrence of graft versus host disease in animals can complicate interpretation of BD assessment of genetically modified human T cells. In general, BD assessment of ex vivo genetically modified cells of hematopoietic origin is not critical based on expected widespread distribution following systemic administration. If distribution to a target organ(s)/tissue(s) is expected, BD assessment of select tissues should be considered in appropriate animal species/models.

E. BD Assessment in Gonadal Tissues (5.5)

It is important to conduct BD assessment of the administered GT product in the gonads for both sexes unless the target clinical population is restricted to one sex (e.g., for the treatment of prostate cancer or uterine cancer). If the GT product or its genetic material does not indicate persistence by an appropriate analytical method (see sections IV.F (4.6) and V.A (5.1)), further evaluation may not be necessary.

Persistent presence of the GT product in the gonads can lead to additional studies to determine GT product levels in germ cells (e.g., oocytes, sperm) or nongermline cells in the animals. These data, as well as other factors (vector type, replication capacity, integration potential, dose level, ROA, etc.), can inform the risk of inadvertent germline integration or germline cell genome modification. Refer to the 2006 *ICH Considerations: General Principles to Address the Risk of Inadvertent Germline Integration of Gene Therapy Vectors* document on inadvertent germline integration of GT vectors (2) for a more comprehensive discussion on this issue.

Persistent GT product detection in nongermline cells in gonadal tissues (e.g., leukocytes, Sertoli cells, Leydig cells) can warrant additional consideration of its potential effect on the function of the affected nongermline cells, particularly if the cell type is important to successful reproduction.

F. Triggers for Additional Nonclinical BD Studies (5.6)

During product development, various circumstances can necessitate conduct of additional studies for BD assessment. Examples of possible scenarios are provided below:

- A significant change in the clinical development program, such as a change in the ROA; an increase in the GT product dose level that significantly exceeds the maximum nonclinical dose level tested; changes in the dosing regimen; and inclusion of another clinical indication that includes both sexes instead of the originally proposed single sex. Additional BD assessment can be incorporated into any additional pharmacology and/or toxicology studies that are performed.

- A significant change in the vector structure or serotype, or any other modifications that may result in changes in distribution or transgene expression.

- Changes in the manufacturing process that can affect the final GT product formulation (e.g., addition of excipients that could alter vector tissue tropism) or relevant quality attributes of the GT product (e.g., gene transfer activity, product titer). Other factors to consider about manufacturing changes include vector particle size; aggregation state; antigenicity; and potential interaction with other host components (e.g., serum factors).

G. Considerations for Alternative Approaches (5.7)

Existing BD data obtained from nonclinical studies conducted with the same GT product in support of a different clinical indication can potentially suffice. However, considerations such as the dose level(s), dosing regimen, ROA, and change in promotor will factor into this decision. BD data obtained with a previously characterized GT product that has the same vector structure and other characteristics that determine its tissue tropism, but a different transgene, can also potentially support waiving an additional nonclinical BD study. Justification should be provided for this approach.

In some cases, a biologically relevant animal species that can inform the BD profile in the clinical population does not exist. For example, when the vector binds to the target molecule on human cells but this target is absent on animal cells. In such circumstances, it is important to provide a comprehensive discussion of the issue and justification to support an alternative approach to evaluation of nonclinical BD.

VI. APPLICATION OF NONCLINICAL BD STUDIES (6)

Characterization of the BD profile following administration of a GT product in animals is a critical component of a nonclinical development program. The nonclinical BD data contribute to the overall interpretation of the study findings by enabling a better understanding of the relationship of various findings (desired and undesired) to the administered GT product. Attribution of observed findings in animals to the genetic material (DNA/RNA) and/or to the expression product(s) factor into ascertaining a potential benefit: risk profile of the GT product before administration in humans.

It is important to consider the relevancy of the BD data to the clinical population based on factors such as the ROA, dose level(s), dosing regimen, and animal immune response. These data can also inform elements of a first-in-human trial and subsequent clinical trials, such as the dosing procedure (i.e., dosing intervals between subjects), the monitoring plan, and long-term follow-up assessment.

NOTES

1. For BD assessment, a biologically relevant species or model is one which is anticipated to produce a profile of dose-related GT product tissue distribution and gene product expression similar to that in humans. Supporting data can be derived from prior nonclinical BD studies conducted with GT products composed of the same vector structure, including the same element that determines tissue tropism (e.g., vector capsid) and the transgene promoter of interest, clinical trial data, or peer-reviewed published literature. The supporting data should be from studies where the GT product has been manufactured by a similar process and in which the dose level and ROA are similar to the sponsor's proposed use. For age-specific consideration in experimental animals, Appendix A of the International Council for Harmonisation guidance for industry *S11 Nonclinical Safety Testing in Support of Development of Pediatric Pharmaceuticals* (May 2021) provides comparative information on organ maturation between species (3).[1]

2. In general, it is recommended that a minimum of five rodents or three nonrodents per sex/group/time point be evaluated. Justification for the number of animals, including number per sex should be provided.

3. For each delivery device system used, it is important to provide data that verify the volume and dose level of the administered GT product in animals. This information can affect interpretation of the resulting BD profile. If a novel delivery device system is planned for use in clinical trials, consider collecting BD data in conjunction with the pharmacology and/or toxicology studies conducted with the device system or its equivalent.

[1] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

GLOSSARY

BD:

Biodistribution.

Expression products:

Molecules such as RNA and protein, produced in the cells guided by the transferred genetic materials.

Gene therapy (GT) products:

Therapeutic products that mediate their effect by the expression (transcription/translation) of transferred genetic materials, or by specifically altering the target genome of human cells. This definition is for the purpose of this guidance.

Gene transfer:

Delivery of therapeutic genetic material into the cells using vectors (e.g., transduction for viral vectors and transfection for plasmids).

Persistence:

The continued presence of transferred or modified genetic sequences in the host after acute exposure to a GT product, due to integration of the genetic sequence into the host genome, deletion, insertion, or otherwise modified following genome editing, to latent infection with the viral vector bearing the transgene, or to the transferred genetic material in episomal form.

ROA:

Route of administration.

Tissue tropism:

For GT products, the propensity of a given vector to transduce or transfect a distinct group of tissues (or cells).

Transgene:

Transcriptionally or translationally active genetic material transferred by a vector intended to confer biological activity following expression in cells.

Vectors:

Gene therapy delivery vehicles or carriers, containing transcriptionally/translationally active therapeutic genetic material or genetic material to alter the host genome that is manufactured to transfer the genetic material into the cells. They include both genetically modified viruses, such as adenovirus or adeno-associated virus, and nonviral vectors, such as plasmids and gene

modified microorganisms, and can include targeted nanoparticles, which have the capability to transfer genetic materials or gene editing components to the cells.

REFERENCES

1. International Council for Harmonisation (ICH) Considerations: General Principles to Address Virus and Vector Shedding, June 2009.[1]

2. ICH Considerations: General Principles to Address the Risk of Inadvertent Germline Integration of Gene Therapy Vectors, October 2006.[2]

3. ICH guidance for industry *S11 Nonclinical Safety Testing in Support of Development of Pediatric Pharmaceuticals* (May 2021).[3]

[1] Available at https://admin.ich.org/sites/default/files/2019-04/ICH_Considerations_Viral-Vector_Shedding_.pdf.
[2] Available at https://admin.ich.org/sites/default/files/2019-04/ICH_Considerations_General_Principles_Risk_of_IGI_GT_Vectors.pdf.
[3] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.