S12 NONCLINICAL BIODISTRIBUTION CONSIDERATIONS FOR GENE THERAPY PRODUCTS

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic.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 staff responsible for this guidance as listed on the title page. The draft guidance has been left in the original International Council for Harmonisation format. The final guidance will be reformatted and edited to conform with FDA's good guidance practice regulation and style.

For questions regarding this draft document, contact (CBER) Mercedes Serabian 240-402-8349.

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.

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

NONCLINICAL BIODISTRIBUTION CONSIDERATIONS FOR GENE THERAPY PRODUCTS
S12

Draft version

Endorsed on 3 June 2021

Currently under public consultation

At Step 2 of the ICH Process, a consensus draft text or guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Assembly to the regulatory authorities of the ICH regions for internal and external consultation, according to national or regional procedures.

S12
Document History

Code	History	Date
S12	Endorsement by the Members of the ICH Assembly under *Step 2* and release for public consultation (document dated 22 April 2021).	day/month/year

ICH HARMONISED GUIDELINE

NONCLINICAL BIODISTIBUTION CONSIDERATIONS FOR GENE THERAPY PRODUCTS

ICH S12

ICH Consensus Guideline

TABLE OF CONTENTS

1. INTRODUCTION

1.1. Objectives of the ICH S12 Guideline

The objective of this guideline is to provide harmonised 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 programme and the design of clinical trials are also provided. The recommendations in this guideline endeavour to facilitate the development of GT products while avoiding unnecessary use of animals, in accordance with the 3Rs (reduce/refine/replace) principles.

1.2. Background

An understanding of the BD profile of a GT product following *in vivo* administration is an important element of the nonclinical development programme. 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 guidelines that include recommendations for BD studies have been issued by various regulatory authorities, this document provides a harmonised definition for nonclinical BD and conveys overall considerations for assessing BD for GT products.

1.3. Scope

GT products within the scope of this guideline 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 non-viral methods) are also covered in this guidance. Although not currently considered GT in certain regions, the principles outlined in this guideline are also applicable to oncolytic viruses that are not genetically modified to express a transgene. This guideline does not apply to prophylactic vaccines. Chemically synthesised oligonucleotides or their analogues, which are not produced using a biotechnology-based manufacturing process, are outside the scope of this guideline. The release of a GT product outside the body via excreta (feces), secreta (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 guideline. Assessment of genomic integration and germline integration of GT products are also outside the scope of this guideline.

2. DEFINITION OF NONCLINICAL BD

BD is the *in vivo* distribution, persistence, and clearance of a GT product at the site of administration and in target and non-target tissues, including biofluids (e.g., blood, cerebrospinal fluid, vitreous fluid), in biologically relevant animal species. Nonclinical BD

studies entail 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.

3. TIMING OF NONCLINICAL BD ASSESSMENT

Preliminary BD data obtained at an early stage of a nonclinical development programme can potentially aid in species selection for subsequent pharmacology and toxicology studies (see Section 4.3). In addition, 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 6), thus it is important that nonclinical BD assessment be completed prior to initiation of the clinical trial.

4. DESIGN OF NONCLINICAL BD STUDIES

4.1. General Considerations

BD studies can be conducted as stand-alone BD studies or in conjunction with nonclinical pharmacology and toxicology studies (see Section 5.3). Therefore, in this document the term "BD study" represents either scenario. Nonclinical BD assessment should be performed in a biologically relevant animal species (see Section 4.3) following administration of a GT product that is representative of the intended clinical product (see Section 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 characterisation of the BD profile (see Section 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 accepted; however, when BD evaluation is performed as part of a GLP-compliant toxicology study, it is important that all in-life parameters and sample collection procedures remain in compliance with GLP.

4.2. Test Article

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., titre), and the final clinical formulation (see Section 5.7). In some situations, nonclinical BD data generated with a GT product that consists of the clinical vector containing a different therapeutic transgene or an expression marker gene (e.g., adeno-associated virus vector of the same serotype and promoter with a fluorescent marker protein expression cassette) can be leveraged to support the BD profile (see Section 5.8).

4.3. Animal Species or Model

BD assessment should be conducted in a biologically relevant animal species or model that is permissive for transfer and expression of the genetic material. Selection factors can include species differences in tissue tropism, gene transfer efficiency, and transgene expression in target

and non-target tissues/cells. If working with a replication competent vector, it is important that the animal species or model be permissive to vector replication.

The influence of species, sex, age, physiologic condition (i.e., healthy animal *vs.* 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 5.4). BD data generated from preliminary studies evaluating gene transfer efficiency or assay methodologies can aid justification of an appropriate animal species selected for comprehensive BD assessment in subsequent studies.

4.4. Group Size and Sex of Animals

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 Section 4.6). General recommendations on the number of animals are presented in Note 1. 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 numbers 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.

4.5. Route of Administration and Dose Level Selection

The ROA of the GT product can affect the BD profile, including the cell types that are transduced and the immune response. Therefore, the GT product should be administered using the intended clinical ROA, as feasible (see Note 2).

The selected dose levels of the administered GT product should provide adequate characterisation of the BD profile to aid in interpretation of the pharmacology and toxicology assessments. The highest dose level administered should be the expected maximum dose level in the toxicology studies (usually limited by animal size, ROA/anatomic target, or GT product concentration). However, with appropriate justification, the anticipated maximum clinical dose level can also serve as the highest dose level for BD evaluation.

4.6. Sample Collection

The sample collection procedure for target and non-target tissues and biofluids should be designed to minimise the potential for contamination. It is important to follow a pre-specified process that includes appropriate archiving of the samples obtained from each animal (vehicle control and those administered the GT product), as well as documenting the order of sample collection. Sample collection time points should reflect the anticipated time following GT product administration to reach peak, steady-state (i.e., plateau), and declining (if feasible) GT product levels in target and non-target tissues/biofluids. Additional time points can be included, as applicable, to more comprehensively capture the length of the steady-state period and 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.

The collected samples should include the following core panel of tissues/biofluids: blood, injection site(s), gonads, adrenal gland, brain, spinal cord (cervical, thoracic, and lumbar), liver, kidney, lung, heart, and spleen. This core panel can be expanded depending on additional considerations, such as vector type/tropism, 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, and/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, and existing nonclinical data.

In cases where systemic exposure is not anticipated (e.g., sub-retinal administration) or no leakage from the site of administration can be demonstrated, justification for the selection of a specific panel of tissues/biofluids can be provided.

Collected samples can also be analysed for presence of the expression product. Considerations regarding this assessment are provided in Section 5.2.

5. SPECIFIC CONSIDERATIONS

5.1. Assay Methodologies

Evaluation of the BD profile necessitates quantitating the amount of genetic material (DNA/RNA) of the GT product in tissues/biofluids and, if appropriate, expression products. Currently, real-time quantitative polymerase chain reaction (qPCR) is considered the 'gold standard' for measurement of specific DNA (or, with a reverse transcription step, RNA as well) presence in tissues/biofluids. Quantification of nucleic acid sequences is important for assessing the relative amount of genetic material from a GT product and determining the kinetics of its accumulation or decay. The limit of sensitivity and reproducibility of the quantification method should be established and documented. Spike and recovery experiments, considered part of assay development, should be performed to demonstrate the ability to detect the target sequence in different tissues/biofluids. Other techniques can be used in nonclinical studies to monitor BD of a vector and/or the expression products. These include, but are not limited to: enzyme-linked immunosorbent assay (ELISA); immunohistochemistry (IHC); western blot; *in situ* hybridisation (ISH); digital PCR; 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 of the method.

5.2. Measurement of Expression Products

While quantification of the genetic material of the GT product is the primary BD assessment (see Section 5.1), determination of the level of expression products in different tissues/biofluids can contribute to characterisation 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.

5.3. Nonclinical BD Assessment as a Component of Pharmacology and Toxicology Studies

In addition to stand-alone studies, BD assessment can also be performed as part of nonclinical pharmacology and toxicology studies. In such scenarios, BD assessment should follow the recommendations specified in Section 4. In cases where certain recommendations cannot be met in a single study (e.g., number of animals per group or collection of a pre-determined panel of tissues/biofluids from each animal), BD data should be obtained from several studies (see Section 4.4).

5.4. Immunogenicity

Pre-existing immunity in animals, notably in non-human primates and other non-rodent species, against a GT vector could affect the BD profile. Screening of animals for pre-existing immunity to the vector prior to inclusion in a nonclinical study should be considered. Ideally, selection of animals determined to be negative for pre-existing immunity with appropriate testing is preferred but may not always be feasible. In such situations, it is important that this aspect is factored into the non-biased method used to randomise animals to study groups.

In certain cases, due to the species-specific nature of the expression product, the animal may mount a cell-mediated or humoral immune response to the expression product. Cell-mediated immune response to the vector may also occur after administration of the GT product. This response may result in a BD profile that is not informative. If such a situation is anticipated, sponsors can consider collection and archiving of appropriate 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. Use of a species-specific surrogate transgene can also be considered to circumvent effects of the immune response in some situations.

5.5. *Ex vivo* Genetically Modified Cells

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 haematopoietic 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 should be considered.

5.6. BD Assessment in Gonadal Tissues

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). If the vector or the transferred genetic material signal does not indicate persistence by an appropriate analytical method (see Sections 4.6 and 5.1), further evaluation may not be necessary. Persistent presence of GT product in gonads can lead to additional studies to determine GT product levels in germ cells (e.g., oocytes, sperm) 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. Refer to ICH Considerations document (1) for a more comprehensive discussion on this issue. GT product detection in non-germline cells (e.g., leukocytes, Sertoli cells, Leydig cells) can warrant additional consideration of the function of the affected non-germline cells, particularly if the cell type is important to successful reproduction.

5.7. Triggers for Additional Nonclinical BD Studies

Although nonclinical BD assessment for a GT product is determined prior to a first-in-human clinical trial, various circumstances may elicit the conduct of additional studies for BD assessment. Examples of possible scenarios are provided below:

- A significant change in the clinical development programme, 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, and any other modifications that may result in changes in tropism.
- Changes in the manufacturing process that can affect the final GT product formulation (e.g., addition of excipients that could alter vector tropism) or relevant quality attributes of the GT product (e.g., empty to full capsid ratios, *in vitro* gene transfer activity, product titre). 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).

5.8. Circumstances when Nonclinical BD Studies may not be Needed or are not Feasible

Existing BD data obtained from nonclinical studies conducted with the same GT product in support of a different clinical indication can potentially suffice for a new clinical population. 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 characterised GT product that has the same vector structure and other characteristics that determine its tissue/cell

tropism, but a different transcribed/translated product, 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.

6. APPLICATION OF NONCLINICAL BD STUDIES

Characterisation of the BD profile following administration of a GT product in animals is a critical component of a nonclinical development programme. The nonclinical BD data contribute to the overall interpretation of the study findings to enable a better understanding of the relationship of various findings (desired and undesired) to the administered GT product. Attribution of findings observed in the dosed animals to the genetic material (DNA/RNA) and/or to the expression product 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. In general, it is recommended that a minimum of 5 rodents or 3 non-rodents per sex/group/time point be evaluated; however, inclusion of equivalent numbers for each sex may not be critical. Justification for these decisions should be provided.
2. 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.

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 guideline.

Gene transfer:

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

ROA:

Route of administration.

Transgene:

Transcriptionally or translationally active genetic material intended to be delivered into cells with therapeutic purpose. It does not include vector or chemically synthesised oligonucleotides.

Vectors:

Gene therapy delivery vehicles, or carriers, containing transcriptionally/ translationally active therapeutic genetic material or genetic material to alter the host genome for delivery to cells. They include both genetically modified viruses such as adenovirus or adeno-associated virus, and non-viral 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.

REFERENCE

(1) ICH Considerations: General Principles to Address the Risk of Inadvertent Germline Integration of Gene Therapy Vectors, Oct 2006.