IPRP
International Pharmaceutical Regulators Programme

Expectations for Biodistribution (BD) Assessments for Gene Therapy (GT) Products

Approved by the IPRP Management Committee on 3 June 2018
12 April 2018

IPRP
International Pharmaceutical Regulators Programme

Table of Contents

1. Position Statement 3
2. Executive Summary 3
3. Background 3
4. BD as a component of pharmacokinetics 4
5. BD Considerations for First-in-Human (FIH) Clinical Trials 4
6. Design of BD Studies 4
7. BD assay methods 6
8. BD considerations for modified vector types 6
9. Circumstances that trigger additional BD studies 7
10. General Recommendations 7
11. References 8

1. Position Statement

This reflection paper was prepared by the Gene Therapy Working Group (GTWG) of the International Pharmaceutical Regulators Programme (IPRP). Its purpose is to communicate the current thinking of Experts from various International Regulatory Authorities with respect to the relevance of and need for non-clinical biodistribution (BD) assessments during the development of gene therapy (GT) products. The general principles outlined and discussed in this document are applicable to many types of GT products, such as viral vectors and plasmids, but do not apply to genetically modified cells. Shedding studies and germline transmission studies for gene therapy products are outside the scope of this reflection paper. This reflection paper is not legally binding, does not replace any local guidelines and regulations, and should not be construed to represent the official views of any given Regulatory Authority participating in the IPRP. We encourage discussion of the information contained in this document with the appropriate regional Regulatory Authorities for each regulatory submission.

2. Executive Summary

Understanding the scientific principles that affect the *in vivo* distribution, persistence, and clearance of GT vectors is critical for the development of safer and more effective GT products. BD data for a GT product collected during non-clinical development will contribute to the design of non-clinical safety studies, and will inform dose levels, dosing schedules and monitoring plans for human trials. The BD profile should be determined for a GT product that has not previously been administered to humans and is proposed for a first-in-human (FIH) clinical trial. Under certain circumstances, BD studies may also be conducted during later-phase clinical trials. Considerations for the design of BD studies to support FIH studies and circumstances that may trigger BD studies after FIH use are presented in this document. For BD studies, as well as non-clinical studies in general, incorporation of the principles of the 3Rs (reduce/refine/replace) of animal use are recommended to eliminate the conduct of redundant studies.

3. Background

The Gene Therapy Discussion Group (GTDG) was established within the International Conference on Harmonisation (ICH) framework in 2000. The goals were to share information and explore harmonizing principles which resulted in the drafting of three ICH Considerations documents relevant to the GT field, covering germline integration, vector shedding, and oncolytic viruses (ref 1). In 2009, the GTDG was discontinued due to insufficient resources. However, the field of GT continued to experience significant scientific and clinical innovation worldwide.

In 2012, global regulators proposed an alternative venue to discuss scientific and clinical advances in the GT field. This led to the formation of the Regulators Forum Gene Therapy Discussion Group which was formalized into the IPRF GTWG in 2013 (ref. 2). The objective of the GTWG is to maintain an international networking forum to discuss emerging scientific and regulatory challenges associated with GT products and to share regional updates on guidelines, science and the regulation of GT products. Effective January, 2018, the IPRF was reorganized into the IPRP.

The IPRP GTWG recognizes the importance of conducting BD studies during the development programs of GT products and acknowledges that there may be different requirements and expectations among the various Regulatory Authorities which, in

turn, creates challenges in product development (ref 3). Thus, the IDRP GTWG composed this reflection paper to communicate the current view of Experts from various regions on non-clinical BD data expectations to support clinical trials and marketing authorization applications.

4. BD as a component of pharmacokinetics

Non-clinical pharmacokinetic (PK) studies evaluate the distribution of pharmaceutical agents within the body. Assessment of the PK profile of a GT product is performed by BD determination. BD is defined as the distribution, persistence and clearance of a GT product *in vivo* from the site of administration to target and non-target tissues including biofluids (e.g. blood, lymph node fluid, cerebrospinal fluid). BD studies typically include analytical methods for the collected tissue and biofluid samples to detect the gene therapy vector, but may also include methods to detect the transgene product, as described in more detail in Section 7.

5. BD Considerations for First-in-Human (FIH) Clinical Trials

Prior to initiation of FIH clinical trials, non-clinical pharmacodynamic (PD) studies (also called proof-of-concept or POC studies) are conducted for most GT products. POC studies usually consist of *in vivo* studies in relevant animal species and/or models of disease to better understand the characteristics of the GT product relating to the proposed therapeutic use. Such studies attempt to show that the vector (and when appropriate the transgene product) reaches its intended target tissue/cells, with the subsequent desired activity (i.e., gene expression and function). Therefore, determination of the BD profile of the vector and subsequent expression of the GT transgene product are important for the interpretation of any potential therapeutic effects observed in POC studies.

In addition, BD data, coupled with other non-clinical safety endpoints, such as clinical pathology and histopathology, help determine whether the presence of vector and/or transgene product correlates with any tissue-specific detrimental effects or toxicities. It is important to comprehensively investigate any findings of concern before the initiation of a FIH clinical trial. The nature of these findings and an understanding of their effects will, in turn, help characterize the benefit / risk profile of the GT product and provide a more comprehensive ‘weight-of-evidence’ approach to assure a reasonable expectation of efficacy and safety in clinical trials.

The absence of BD data can lead to an incomplete understanding of risk. In this situation, it may not be possible to establish a starting FIH dose level for the GT product. If the BD data are incomplete or limited (e.g. the study did not incorporate an appropriate route of administration (ROA), dose level, regimen, or relevant animal species), additional BD studies or modifications to a FIH clinical trial may be needed.

6. Design of BD Studies

The objective of non-clinical BD studies is to support the activity and the safety evaluation of the GT product. BD studies should employ a biologically relevant animal species. For example, species that permit vector transduction, and that are biologically responsive to the specific transgene product of interest should be considered.

To generate meaningful data, considerations for the design of BD studies include:

- Use of a GT product in its final clinical formulation is expected, however, it may also be acceptable, if appropriate scientific data and justification are provided to use other formulations when necessary.

- Use of the intended clinical ROA if feasible, or justification of the ROA employed;

- Inclusion of both sexes, or justification of the use of a single sex;

- Use of adequate numbers of animals of each sex per group, per sacrifice time point;

- Evaluation of aspects of the animal test system that might influence or compromise vector distribution and/or persistence, such as the animal's age, physiologic condition, and potential capacity to mount an immune response against the administered vector and/or transgene product;

- Inclusion of a vehicle control group and one group of animals that receives a clinically relevant dose level of the GT product. Additional dose levels, e.g. the maximum feasible dose, might provide dose-dependent information;

- Selection of sampling time points that should reflect the expected time of peak vector detection and clearance of the vector. The study should continue until there is a clear decline in vector signal or until a long-term signal plateau phase is reached. In large animal species, it is not always feasible to obtain tissues at multiple time points due to the use of prohibitive numbers of animals. However, multiple intervals of biofluid sampling over the observation period can be considered, instead;

- Combining BD assessment with another study (i.e., POC or safety study). A combined BD-toxicity study allows for determination of any correlation of vector presence/persistence and safety and activity findings;

- Collection of a standard panel of tissues/biofluids such as, blood; injection site(s); gonads; brain; liver; kidneys; lung; heart; and spleen. Also consider other tissues/biofluids for evaluation (e.g., cerebrospinal fluid, draining lymph nodes, bone marrow, eyes, optic nerve, contralateral sites, etc.), depending on the vector type, transgene product, ROA, disease condition, and other factors. In some cases, where systemic exposure is not expected or no leakage from the site of administration can be demonstrated, a more limited list of tissues/biofluids to collect may be justified;

- Use of a tissue/biofluid collection procedure that minimizes the potential for contamination. It is important to record the collection method;

- Use of a quantitative, sensitive assay to analyze the collected samples. It is important to provide the detailed assay methodology. Refer to Section 7 of this document for a discussion on assay methods;

- For replication competent vectors, the potential exists for product entry into the bloodstream due to vector/virus replication *in vivo*. If the animal species does not support *in vivo* replication of a replication competent vector/virus following

single administration, repeat administration of the GT product should be considered;

7. BD assay methods

Currently, real-time quantitative PCR (qPCR) is considered the 'gold standard' for measurement of vector presence in tissues/biofluids. As developers begin to transition their GT products from research to administration in humans, the limit of sensitivity and reproducibility of this method should be established, and validating the methods should be considered.

qPCR Assays

qPCR is a highly sensitive analytical method to ascertain whether vector DNA sequences are present in tissues. When combined with a reverse transcription step, qPCR reactions can be designed to assess RNA as well.

Quantification of target nucleic acid sequences is important for assessing the relative amount of target material and to determine the kinetics of accumulation or decay. The sensitivity and specificity of the assay are important parameters to consider in the development and qualification of the test method. Spike and recovery experiments can be performed to illustrate the detectability of the target sequence in different tissues/organs, and should be considered as part of assay qualification.

To maximize the detection of specific target sequences, consideration should be given to primer design and qPCR assay conditions. Although lengthening the size of the PCR amplification product may reduce assay sensitivity (i.e. increase the limit of detection), it may be more informative and more likely to yield true positive results. Consideration for enhancing specific detection of a transgene can include incorporating unique differences using codon degeneracy that do not alter the transgene product, but can be distinguished from endogenous molecules.

If the transgene product is designed to be secreted from transduced cells or tissues, additional assays other than qPCR may be considered to assess the transgene product.

Other assays for detection of a transgene product

There are other techniques that can be used in non-clinical studies to monitor the expression of transgene products. These include enzyme-linked immunosorbent assay (ELISA), immunohistochemistry (IHC), or Western blot. The developer should provide the methodology and justification for the method used.

8. BD considerations for modified vector types

The structural components of a vector that interact with host cell-surface receptors usually play a crucial role in determining BD profile. The development of new serotype vectors or engineered viruses with altered tropism has led to improvements in targeting (or de-targeting) viral vector based GT products to certain cell populations or tissues. These vectors, which may be derived from previously characterized viral strains or serotypes, are generally considered new, thus understanding their BD profile is important prior to administration in a FIH clinical trial. Such changes should be carefully assessed for alterations in the distribution of the vector. Therefore, when making any changes to an existing product (or previously studied product), it is important to consider potential effects regarding vector particle

size; aggregation state; antigenicity; decay-rate/half-life; and interaction with other host components (e.g. serum factors) that may also play a role in determining the BD profile of the vector.

9. Circumstances that trigger additional BD studies

Some circumstances may trigger additional BD studies after initiation of FIH studies. Examples of various scenarios are provided below:

- A significant change in the clinical dose level(s), ROA, and/or dosing schedule/dosing frequency of the GT product. The dose level and/or ROA can influence dissemination to various tissue types, as well as the levels of vector and transgene product in those tissues. However, if the maximum feasible dose has been tested in the original BD study, a change in dose might not need an additional BD study. For other proposed changes for clinical trials, the BD information may be collected (during or from) any additional toxicology studies conducted;

- A significant change in the vector structure (refer to Section 8 of this document), and/or serotype, and any other modifications that may result in changes in the tropism;

- Modification of the genetic sequence of an expression cassette. Changes to the expression cassette of a previously characterized vector are not usually expected to alter the BD of the vector and would therefore not trigger additional BD studies. However such changes can impact transgene expression and/or the toxicity of the expressed transgene product expression and/or the toxicity of the expressed transgene. For example, the addition of a new promoter sequence in the transgene cassette may lead to greater transgene expression, which, in turn, may cause toxicities associated with increased expression;

- Incorporation of multiple vectors in a single GT product. This scenario can potentially result in changes in the BD profile of each vector component;

- Changes in the manufacturing process, such as vector formulation and viral titer. The need for any additional BD studies will depend on the type and extent of manufacturing changes; and

- Change in indication or target population (for example if the original BD assessment was performed in animals of one sex, but the clinical population for a second indication will be both sexes).

The goal for a GT product development program is to transition from early-phase to late-phase clinical trials and use by patients in as seamless a manner as possible. Thus, it is important to consider and prepare for the above scenarios as the product development program progresses.

10. General Recommendations

The recommendations in this document constitute the current thinking of the GTWG on this important topic. The information provided in this reflection paper is a result of

a series of discussions among Experts from multiple International Regulatory Authorities. The contents of this document are intended to serve as a starting point and a guide for many developers regarding the conduct of BD studies for GT products within the scope of this reflection paper. Sharing BD study information – both methodology and resulting data – in the public domain (i.e., publications and platform technologies), is encouraged. Such actions will result in non-clinical study designs that incorporate the judicious use of animals and non-clinical testing paradigms that are informative for the overall clinical development program for a GT product.

11. References

1. http://www.ich.org/products/consideration-documents.html
2. https://www.i-p-r-f.org/en/working-groups/gene-therapy-working-group/
3. Biodistribution studies: understanding international expectations, Molecular Therapy Methods and Clinical Development Volume 3, 2016

Disclaimer:

This document reflects the views of subject matter experts participating in the IPRP Gene Therapy Working Group (GTWG) and should not be construed to represent the official views of any given regulatory authority participating in the IPRP.