14th EBF Open Symposium

Science – Our Universal Language

EBF Feedback on ICH S12

Johannes Stanta, on behalf of the EBF

24-26 November 2021, Barcelona

Guideline on nonclinical biodistribution considerations for gene therapy products

- 3 June 2021 – Document released for public consultation

Objective:

- Provide harmonised recommendations for nonclinical biodistribution (BD) studies of gene therapy (GT) products.
- Facilitate the development of GT products in accordance with the 3Rs.

24 June 2021
EMA/CHMP/ICH/318372/2021
Committee for Medicinal Products for Human Use

ICH guideline S12 on nonclinical biodistribution considerations for gene therapy products

Step 2b

Transmission to CHMP	24 June 2021
Adoption by CHMP	24 June 2021
Release for public consultation	24 June 2021
Deadline for comments	24 October 2021

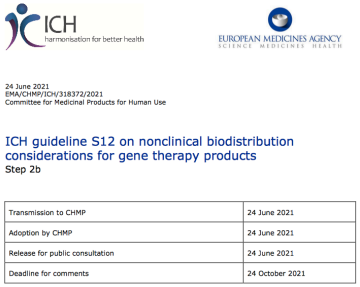
EBF gathering feedback

in addition to giving FB, our goal is also

“stimulating consolidated industry feedback”
in contrast to
“individual company feedback”

in other words…harmonise at the source

Our Feedback journey



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EBF companies to provide individual comments to EBF Expert team

EBF Expert team to consolidate all comments

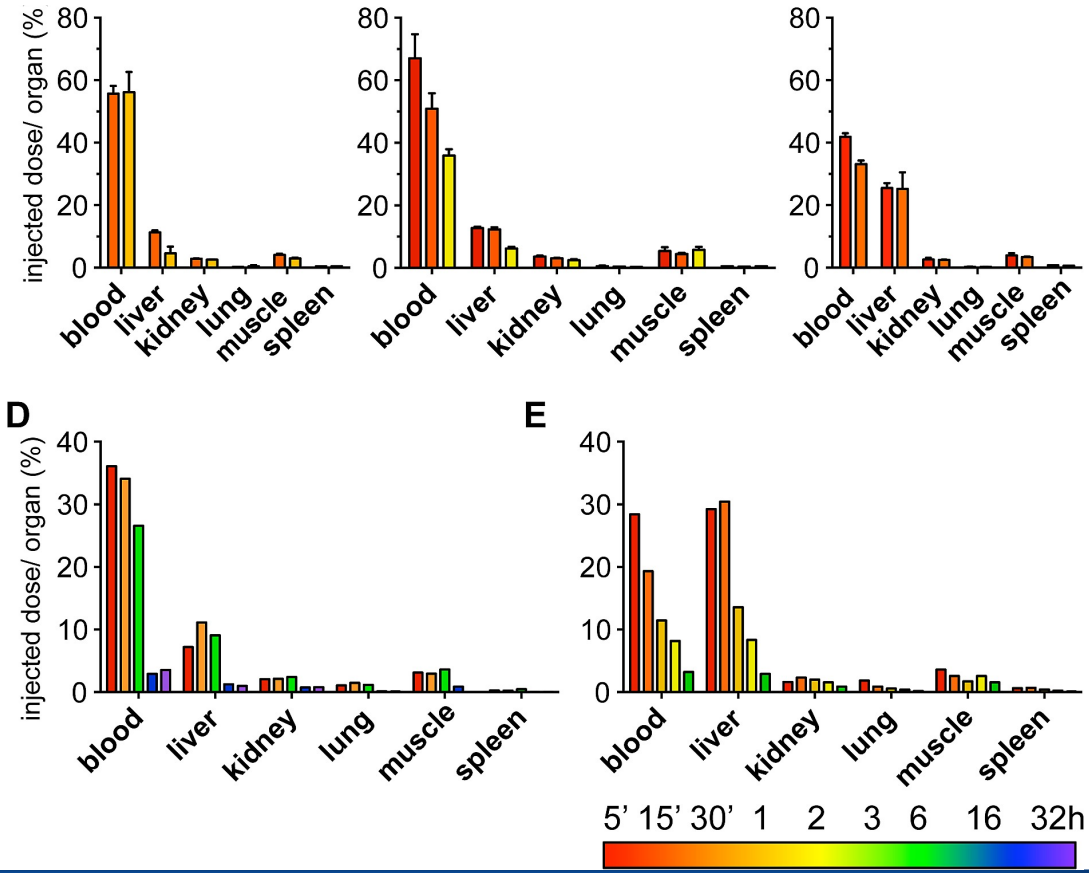
Formal FB sent to EMA on 22OCT2021

Gene Therapy products include:

- Products that mediate their effect by the expression (transcription or translation) of transferred genetic materials:
  - purified nucleic acid (e.g., plasmids and RNA)
  - microorganisms (e.g., viruses, bacteria, fungi) genetically modified to express transgenes
  - ex vivo genetically modified human cells
  - delivery of a nuclease and guide RNA by non-viral methods
  - oncolytic viruses that are not genetically modified to express a transgene
- Outside the scope:
  - Chemically synthesised oligonucleotides
  - Shedding
  - Genomic and germline integration

Nonclinical Biodistribution

- 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).
- Use analytical methods to detect GT product and transferred genetic material
- Can include expression product



4.1 General considerations

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.

- EBF Comment: make it clear that BD endpoints can be taken from studies that are either non-GLP or GLP compliant.

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).

EBF Comment: Clarification needed for representative clinical batch.

- How much change in the full-empty capsid ratio is acceptable?
- Is a CpG content modification acceptable as it does not alter the transgene protein?
- Is it acceptable if different master cell banks are used in genetically modified cell therapies?

Proposed:

…important product characteristics (e.g., titre, copy number, full-empty capsid ratio, CpG content, master cell banks…) and the final clinical formulation (see Section 5.7).

4.6 Sample Collection

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.

- EBF Comment: "Guideline Text: 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.Comment: Please change the word “specific” to “more restricted”"
- Change: Regarding the example of sub-retinal administration as a case where systemic exposure is not anticipated... Some systemic exposure is observed in located RoA of sub-retinal admin. Please consider changing the sentence to read, “where significant systemic exposure of not anticipated”.

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.

- EBF Comment: Narrow and mixed mentioning of technologies. This needs to be broader to stand the test of time.
- Change: Currently, molecular biology techniques, e.g. real-time quantitative polymerase chain reaction (qPCR) or ddPCR is considered the ‘gold standard’ for measurement of specific DNA (or, with a reverse transcription step, RNA as well) presence in tissues/biofluids.

5.1 Assay Methodologies

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.

- EBF Comment: Accumulation most probably isn't the right word, nor is kinetics.

- Change: We suggest replacing with "change in concentrations or exposure over time".

5.1 Assay Methodologies

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.

EBF Comment:

- It is very good that a target sensitivity is not included in the guideline since the analytical range may have different requirements for different GT, depending on the context of use (COU) for each assay. However, difficult and unethical (due to 3R) to characterize assay performance during assay development in all tissues and biofluids. For some matrices a substitute matrix may be considered and the approach of spike/recovery experiments during study conduct in rare tissues/matrices.
- Allow for a scientific mindset on what is needed based on the COU instead of detailing requirements of assay performance such as it is required in the bioanalytical guidelines that are written for the specific purpose of chromatographic and ligand binding assays for detection of drug products in biologic matrices.
- Why are no details of assay validation included only assay development?
- Please note that there is no indication of a specific detection limit that should be achieved (eg 50 copies/microgram of gDNA)"

5.1 Assay Methodologies

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.

- Change to: "It is important to provide a comprehensive description of the methodology and the justification for the technique used. The analytical performance should be characterized, documented and reported to be fit for purpose for each assay for the applicable context of use (COU) of the assay. Assay development and characterization should include main matrices such as most important tissues and biofluids. For rare matrices and biofluids it is acceptable to use substitute matrix during assay development and characterization. "

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.

EBF Comments:

- In this section the use of immune-deficient mice is not mentioned although it is commonly used in the field of CAR T cells.
- When is an animal considered to be negative for pre-existing immunity and based on which selection assay (functional cell-based assay or ligand binding assay)?"

5.4. Immunogenicity

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.

EBF comment:

- Saying that cell-mediated immune response to the vector may occur after administration indicates that a humoral immune response to the vector wont occur. A cell-mediated and humoral immune response to the vector could be considered more likely than an immune response to the expression product.

Notes

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.

EBF comment:

- Consider 3Rs when deciding on number of animals / time points
- If there are unequal numbers of genders, how will you definitively determine distribution to the gonads (which is a critical part of the BD assessment)?

More detail

- You can find all our comments (and the ones from other organisations/companies) on the EMA website

https://www.ema.europa.eu/en/documents/comments/overview-comments-received-ich-guideline-s12-nonclinical-biodistribution-considerations-gene-therapy/chmp/ich/318372/2021_en.pdf

Conclusion

- Welcome attempt to harmonise the conduct of nonclinical BD studies for gene therapy
- Welcome the incorporation of 3R
- Refinement of the text needed
- Mention of specific current methodologies (rt-qPCR) – will it stand the test of time? Keep wording open and generic (molecular biology technologies)?
- Well suited to virus-gene therapy, not always well interpretable for other emerging therapies (cell therapies, synthetic vector constructs, etc.)
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  - Philip Timmerman

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