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

ICH HARMONISED GUIDELINE

**NONCLINICAL BIODISTRIBUTION CONSIDERATIONS FOR
GENE THERAPY PRODUCTS
S12**

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S12
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**ICH HARMONISED GUIDELINE
NONCLINICAL BIODISTRIBUTION
CONSIDERATIONS FOR GENE
THERAPY PRODUCTS**

ICH S12

ICH Consensus Guideline

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1 **1. INTRODUCTION**

2 **1.1. Objectives of the ICH S12 Guideline**

3 The objective of this guideline is to provide harmonised recommendations for the conduct of
4 nonclinical biodistribution (BD) studies in the development of gene therapy (GT) products.
5 This document provides recommendations for the overall design of nonclinical BD
6 assessments. Considerations for interpretation and application of the BD data to support a
7 nonclinical development programme and the design of clinical trials are also provided. The
8 recommendations in this guideline endeavour to facilitate the development of GT products
9 while avoiding unnecessary use of animals, in accordance with the 3Rs (reduce/refine/replace)
10 principles.

11 **1.2. Background**

12 An understanding of the BD profile of a GT product following *in vivo* administration is an
13 important element of the nonclinical development programme. BD data contribute to the
14 interpretation and design of nonclinical pharmacology and toxicology studies conducted to
15 support early-phase clinical trials in the target population. Although guidelines that include
16 recommendations for BD studies have been issued by various regulatory authorities, this
17 document provides a harmonised definition for nonclinical BD and conveys overall
18 considerations for assessing BD for GT products.

19 **1.3. Scope**

20 GT products within the scope of this guideline include products that mediate their effect by the
21 expression (transcription or translation) of transferred genetic materials. Some examples of GT
22 products can include purified nucleic acid (e.g., plasmids and RNA), microorganisms (e.g.,
23 viruses, bacteria, fungi) genetically modified to express transgenes (including products that edit
24 the host genome), and *ex vivo* genetically modified human cells. Products that are intended to
25 alter the host cell genome *in vivo* without specific transcription or translation (i.e., delivery of
26 a nuclease and guide RNA by non-viral methods) are also covered in this guidance. Although
27 not currently considered GT in certain regions, the principles outlined in this guideline are also
28 applicable to oncolytic viruses that are not genetically modified to express a transgene. This
29 guideline does not apply to prophylactic vaccines. Chemically synthesised oligonucleotides or
30 their analogues, which are not produced using a biotechnology-based manufacturing process,
31 are outside the scope of this guideline. The release of a GT product outside the body via excreta
32 (feces), secretions (urine, saliva, nasopharyngeal fluids, etc.), or through the skin (pustules, sores,
33 wounds) is termed ‘shedding’. Evaluation of the nonclinical shedding profile of a GT product
34 is outside the scope of this guideline. Assessment of genomic integration and germline
35 integration of GT products are also outside the scope of this guideline.

36 **2. DEFINITION OF NONCLINICAL BD**

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

40 studies entail the use of analytical methods to detect the GT product and transferred genetic
41 material in collected samples and can include methods to detect the expression product of the
42 transferred genetic material.

43 **3. TIMING OF NONCLINICAL BD ASSESSMENT**

44 Preliminary BD data obtained at an early stage of a nonclinical development programme can
45 potentially aid in species selection for subsequent pharmacology and toxicology studies (see
46 Section 4.3). In addition, BD data should be available when evaluating and interpreting the
47 nonclinical pharmacology and toxicology findings. Nonclinical BD data can also inform design
48 aspects of a first-in-human clinical trial (see Section 6), thus it is important that nonclinical BD
49 assessment be completed prior to initiation of the clinical trial.

50 **4. DESIGN OF NONCLINICAL BD STUDIES**

51 **4.1. General Considerations**

52 BD studies can be conducted as stand-alone BD studies or in conjunction with nonclinical
53 pharmacology and toxicology studies (see Section 5.3). Therefore, in this document the term
54 “BD study” represents either scenario. Nonclinical BD assessment should be performed in a
55 biologically relevant animal species (see Section 4.3) following administration of a GT product
56 that is representative of the intended clinical product (see Section 4.2 for possible alternate
57 scenarios). It is important that the route of administration (ROA) reflect the intended clinical
58 ROA to the extent possible and that the dose levels studied provide sufficient characterisation
59 of the BD profile (see Section 4.5).

60 It is important to verify the data quality, integrity, and reliability of the BD evaluation. In
61 principle, nonclinical BD studies that are not conducted in compliance with Good Laboratory
62 Practice (GLP) are accepted; however, when BD evaluation is performed as part of a GLP-
63 compliant toxicology study, it is important that all in-life parameters and sample collection
64 procedures remain in compliance with GLP.

65 **4.2. Test Article**

66 The test article administered in the nonclinical BD studies should be representative of the
67 intended clinical GT product, taking into consideration the manufacturing process, important
68 product characteristics (e.g., titre), and the final clinical formulation (see Section 5.7). In some
69 situations, nonclinical BD data generated with a GT product that consists of the clinical vector
70 containing a different therapeutic transgene or an expression marker gene (e.g., adeno-
71 associated virus vector of the same serotype and promoter with a fluorescent marker protein
72 expression cassette) can be leveraged to support the BD profile (see Section 5.8).

73 **4.3. Animal Species or Model**

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

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

79 The influence of species, sex, age, physiologic condition (i.e., healthy animal vs. animal disease
80 model) on the BD profile can also be important. In addition, the potential for the animal species
81 to mount an immune response against the administered vector and/or expression product should
82 be considered (see Section 5.4). BD data generated from preliminary studies evaluating gene
83 transfer efficiency or assay methodologies can aid justification of an appropriate animal species
84 selected for comprehensive BD assessment in subsequent studies.

85 **4.4. Group Size and Sex of Animals**

86 An appropriate number of animals per sex (as applicable) should be evaluated at each
87 predetermined sampling time point to generate sufficient data that support comprehensive BD
88 assessment (see Section 4.6). General recommendations on the number of animals are presented
89 in Note 1. In keeping with the principles of the 3Rs, the total number of animals can be an
90 aggregate from several studies. Justification should be provided for the numbers of animals
91 evaluated at each time point, as well as the use of combined data from multiple studies, as
92 applicable. Justification should also be provided when only one sex is evaluated.

93 **4.5. Route of Administration and Dose Level Selection**

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

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

103 **4.6. Sample Collection**

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

114 For replication competent vectors, sample collection time points should also cover the detection
115 of the second peak level due to vector replication and the subsequent clearance phase.

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

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

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

130 **5. SPECIFIC CONSIDERATIONS**

131 **5.1. Assay Methodologies**

132 Evaluation of the BD profile necessitates quantitating the amount of genetic material
133 (DNA/RNA) of the GT product in tissues/biofluids and, if appropriate, expression products.
134 Currently, real-time quantitative polymerase chain reaction (qPCR) is considered the ‘gold
135 standard’ for measurement of specific DNA (or, with a reverse transcription step, RNA as well)
136 presence in tissues/biofluids. Quantification of nucleic acid sequences is important for assessing
137 the relative amount of genetic material from a GT product and determining the kinetics of its
138 accumulation or decay. The limit of sensitivity and reproducibility of the quantification method
139 should be established and documented. Spike and recovery experiments, considered part of
140 assay development, should be performed to demonstrate the ability to detect the target sequence
141 in different tissues/biofluids. Other techniques can be used in nonclinical studies to monitor BD
142 of a vector and/or the expression products. These include, but are not limited to: enzyme-linked
143 immunosorbent assay (ELISA); immunohistochemistry (IHC); western blot; *in situ*
144 hybridisation (ISH); digital PCR; flow cytometry; various *in vivo* and *ex vivo* imaging
145 techniques; and other evolving technologies. It is important to provide a comprehensive
146 description of the methodology and the justification for the technique used, including the
147 performance parameters of the method.

148 **5.2. Measurement of Expression Products**

149 While quantification of the genetic material of the GT product is the primary BD assessment
150 (see Section 5.1), determination of the level of expression products in different tissues/biofluids
151 can contribute to characterisation of the safety and activity profiles following GT product
152 administration. Decisions regarding the conduct of such assessments should be based on the

153 extent of nonclinical BD analyses needed for the GT product, which is determined using a risk-
 154 based approach. This approach can include consideration of the GT product levels and
 155 persistence in tissues/biofluids; the target clinical population; and potential safety concerns
 156 associated with the vector and/or the expression product.

157 **5.3. Nonclinical BD Assessment as a Component of Pharmacology and Toxicology Studies**

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

164 **5.4. Immunogenicity**

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

171 In certain cases, due to the species-specific nature of the expression product, the animal may
 172 mount a cell-mediated or humoral immune response to the expression product. Cell-mediated
 173 immune response to the vector may also occur after administration of the GT product. This
 174 response may result in a BD profile that is not informative. If such a situation is anticipated,
 175 sponsors can consider collection and archiving of appropriate samples for possible
 176 immunogenicity analysis to support interpretation of the BD data.

177 Immunosuppression of animals for the sole purpose of evaluating the BD profile is not
 178 recommended. However, if product- or species-specific circumstances warrant
 179 immunosuppression, justification should be provided. Use of a species-specific surrogate
 180 transgene can also be considered to circumvent effects of the immune response in some
 181 situations.

182 **5.5. *Ex vivo* Genetically Modified Cells**

183 Considerations for BD assessment of GT products that consist of *ex vivo* genetically modified
 184 cells (i.e., cells that are transduced/transfected *ex vivo* and then administered to the
 185 animal/human subject) should include factors such as the cell type, ROA, and the potential for
 186 the expression product or gene modification event to affect the expected distribution of the cells
 187 within the body (e.g., new or altered expression of cell adhesion molecules). In addition, the
 188 occurrence of graft versus host disease in animals can complicate interpretation of BD
 189 assessment of genetically modified human T cells. In general, BD assessment of *ex vivo*
 190 genetically modified cells of haematopoietic origin is not critical based on expected widespread

191 distribution following systemic administration. If distribution to a target organ(s)/tissue(s) is
 192 expected, BD assessment should be considered.

193 **5.6. BD Assessment in Gonadal Tissues**

194 It is important to conduct BD assessment of the administered GT product in the gonads for both
 195 sexes unless the target clinical population is restricted to one sex (e.g., for the treatment of
 196 prostate cancer). If the vector or the transferred genetic material signal does not indicate
 197 persistence by an appropriate analytical method (see Sections 4.6 and 5.1), further evaluation
 198 may not be necessary. Persistent presence of GT product in gonads can lead to additional studies
 199 to determine GT product levels in germ cells (e.g., oocytes, sperm) in the animals. These data,
 200 as well as other factors (vector type, replication capacity, integration potential, dose level, ROA,
 201 etc.) can inform the risk of inadvertent germline integration. Refer to ICH Considerations
 202 document (1) for a more comprehensive discussion on this issue. GT product detection in non-
 203 germline cells (e.g., leukocytes, Sertoli cells, Leydig cells) can warrant additional consideration
 204 of the function of the affected non-germline cells, particularly if the cell type is important to
 205 successful reproduction.

206 **5.7. Triggers for Additional Nonclinical BD Studies**

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

- 210 • A significant change in the clinical development programme, such as: a change
 211 in the ROA; an increase in the GT product dose level that significantly exceeds
 212 the maximum nonclinical dose level tested; changes in the dosing regimen; and
 213 inclusion of another clinical indication that includes both sexes instead of the
 214 originally-proposed single sex. Additional BD assessment can be incorporated
 215 into any additional pharmacology and/or toxicology studies that are performed.
- 216 • A significant change in the vector structure or serotype, and any other
 217 modifications that may result in changes in tropism.
- 218 • Changes in the manufacturing process that can affect the final GT product
 219 formulation (e.g., addition of excipients that could alter vector tropism) or
 220 relevant quality attributes of the GT product (e.g., empty to full capsid ratios, *in*
 221 *vitro* gene transfer activity, product titre). Other factors to consider about
 222 manufacturing changes include vector particle size; aggregation state;
 223 antigenicity; and potential interaction with other host components (e.g., serum
 224 factors).

225 **5.8. Circumstances when Nonclinical BD Studies may not be Needed or are not Feasible**

226 Existing BD data obtained from nonclinical studies conducted with the same GT product in
 227 support of a different clinical indication can potentially suffice for a new clinical population.
 228 However, considerations such as the dose level(s), dosing regimen, ROA, and change in
 229 promotor will factor into this decision. BD data obtained with a previously characterised GT
 230 product that has the same vector structure and other characteristics that determine its tissue/cell

231 tropism, but a different transcribed/translated product, can also potentially support waiving an
232 additional nonclinical BD study. Justification should be provided for this approach.

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

238 **6. APPLICATION OF NONCLINICAL BD STUDIES**

239 Characterisation of the BD profile following administration of a GT product in animals is a
240 critical component of a nonclinical development programme. The nonclinical BD data
241 contribute to the overall interpretation of the study findings to enable a better understanding of
242 the relationship of various findings (desired and undesired) to the administered GT product.
243 Attribution of findings observed in the dosed animals to the genetic material (DNA/RNA)
244 and/or to the expression product factor into ascertaining a potential benefit: risk profile of the
245 GT product before administration in humans. It is important to consider the relevancy of the
246 BD data to the clinical population based on factors such as the ROA, dose level(s), dosing
247 regimen, and animal immune response. These data can also inform elements of a first-in-human
248 trial and subsequent clinical trials, such as the dosing procedure (i.e., dosing intervals between
249 subjects), the monitoring plan, and long-term follow-up assessment.

250 **NOTES**

- 251 1. In general, it is recommended that a minimum of 5 rodents or 3 non-rodents per
252 sex/group/time point be evaluated; however, inclusion of equivalent numbers for each
253 sex may not be critical. Justification for these decisions should be provided.
- 254 2. For each delivery device system used, it is important to provide data that verify the
255 volume and dose level of the administered GT product in animals. This information can
256 affect interpretation of the resulting BD profile. If a novel delivery device system is
257 planned for use in clinical trials, consider collecting BD data in conjunction with the
258 pharmacology and/or toxicology studies conducted with the device system or its
259 equivalent.

260 **GLOSSARY**

261 **BD:**

262 Biodistribution.

263

264 **Expression products:**

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

267

268 **Gene therapy (GT) products:**

269 Therapeutic products that mediate their effect by the expression (transcription/transla-
270 tion) of transferred genetic materials, or by specifically altering the target genome of
271 human cells. This definition is for the purpose of this guideline.

272 **Gene transfer:**
273 Delivery of therapeutic genetic material into the cells using vectors (e.g. transduction
274 for viral vectors and transfection for plasmids).

275
276 **ROA:**
277 Route of administration.

278
279 **Transgene:**
280 Transcriptionally or translationally active genetic material intended to be delivered into
281 cells with therapeutic purpose. It does not include vector or chemically synthesised oli-
282 gonucleotides.

283
284 **Vectors:**
285 Gene therapy delivery vehicles, or carriers, containing transcriptionally/ translationally
286 active therapeutic genetic material or genetic material to alter the host genome for de-
287 livery to cells. They include both genetically modified viruses such as adenovirus or
288 adeno-associated virus, and non-viral vectors such as plasmids and gene modified mi-
289 croorganisms, and can include targeted nanoparticles which have the capability to trans-
290 fer genetic materials or gene editing components to the cells.
291

292 **REFERENCE**

293 (1) ICH Considerations: General Principles to Address the Risk of Inadvertent Germline Inte-
294 gration of Gene Therapy Vectors, Oct 2006.