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4 **Guideline on the quality, non-clinical and clinical aspects**
5 **of gene therapy medicinal products**
6 **Draft**

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8 This guideline replaces the note for guidance on quality, non-clinical and clinical aspects of gene
9 transfer medicinal products (CPMP/BWP/3088/99 draft)

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12 Guideline on the quality, non-clinical and clinical aspects
13 of gene therapy medicinal products

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87 **Executive summary**

88 This guideline defines scientific principles and provides guidance for the development and evaluation of
89 gene therapy medicinal products (GTMP) intended for use in humans and presented for marketing
90 authorisation. Its focus is on the quality, safety and efficacy requirements of GTMP.

91 The quality section addresses mainly the specific requirements for the development and manufacture
92 of a GTMP.

93 The non-clinical section addresses the non-clinical studies required with the aim at maximising the
94 information obtained on dose selection for the clinical trials, to support the route of administration and
95 the application schedule. Non-clinical studies should also allow determining whether the observed
96 effect is attributable to the GTMP.

97 The clinical section addresses the requirements for studying as far as possible the pharmacological
98 properties of the GTMP itself and the transgene. The requirements for efficacy studies emphasises that
99 the same principles apply as for the clinical development of any other medicinal product, especially
100 those of current guidelines relating to specific therapeutic areas. The clinical section further addresses
101 the safety evaluation of the product as well as the principles for follow up and the pharmacovigilance
102 requirements.

103

104 **1. Background**

105 Gene therapy medicinal products generally consist of a vector or delivery formulation/system
106 containing a genetic construct engineered to express a specific therapeutic sequence or protein
107 responsible for the regulation, repair, addition or deletion of a genetic sequence. The active substance
108 is the nucleic acid sequence(s), or genetically modified microorganism(s) or virus(es) or cells. The
109 active substance may be composed of multiple elements. By using such gene therapy constructs *in-*
110 *vivo* genetic regulation or genetic modification of somatic cells can be achieved. Vectors used in GTMP
111 can be engineered to target specific tissues or cells (pseudotyping) or to ensure the safety of the GTMP
112 (deletion of genes associated with virulence, pathogenicity or replication-competence).

113 There are many different types of GTMP vector in development, however the majority fall broadly into
114 one of 3 groups:

- 115 - Viral vectors;
- 116 - DNA vectors e.g. plasmid DNA, Chromosome-based vectors, e.g. iBAC, S/MAR and transposon
117 vectors;
- 118 - Bacterial vectors e.g. modified *Lactococcus* sp, *Listeria* sp and *Streptococcus* sp.

119 Whatever the grouping system, all these active substance(s) are of biological origin.

120 By far the most common vector systems used for gene therapy to date have been viral vectors and
121 plasmid DNA vectors. Viral vectors may be replication defective, replication competent or replication-
122 conditional, each type requiring specific consideration with regard to design and safety. Plasmid DNA
123 vectors may be administered either in a simple salt solution (referred to as "naked" DNA) or may be
124 complexed with a carrier or in a delivery formulation.

125 Historically many gene therapy approaches have been based on expression of a transgene encoding a
126 functional protein. Newer tools include directly acting nucleic acid sequences such as microRNA, RNAi
127 via short hairpin RNAs (shRNA) or molecular scissor approaches and these may effect repair, addition
128 or deletion of a genetic sequence via gene silencing, exon skipping, gene regulation or gene
129 knockdown. The term 'therapeutic sequence' is used in this guideline to reflect the diversity of these
130 approaches and refers to any nucleic acids sequences that may be used in gene therapy.

131 It is recognised that this is an area under constant development and guidance should be applicable to
132 any novel product as appropriate.

133 **2. Scope**

134 This guideline is applicable to gene therapy medicinal products containing recombinant nucleic acid
135 sequences (e.g. DNA vectors) or genetically modified micro-organisms or viruses.

136 This guideline does not specifically consider gene therapy medicinal products containing genetically
137 modified cells (allogeneic or autologous somatic cells modified *ex-vivo* or *in-vitro* with a gene therapy
138 vector prior to administration to the human subject) as they are covered in the guideline on quality,
139 non-clinical and clinical aspects of medicinal products containing genetically modified cells
140 (EMA/CAT/GTWP/671639/2008). However, the principles outlined here apply to the vectors used in the
141 modification of such cells.

142 Although the definition of GTMP does not include chemically synthesised therapeutic sequences, many
143 of the topics regarding design and safety considerations might be relevant to such medicinal products.

144 **3. Legal basis**

145 Advanced therapy medicinal products (ATMPs), as established by Regulation (EC) No 1394/2007,
146 include gene therapy medicinal products, somatic cell therapy or tissue engineering medicinal products
147 for human use.

148 Part IV of the Annex I to Directive 2001/83/EC, as amended by Commission Directive 2009/120/EC
149 includes the definition of a GTMP, the technical requirements for GTMPs and the definitions of starting
150 materials.

151 For Marketing Authorisation Applications (MAAs), the data on quality aspect of GTMPs must be
152 presented in accordance with the standard Common Technical Document (CTD) Module 3 format. The
153 data submitted in this module should be consistent with and complement other parts of the dossier
154 including Module 1.6.2 (GMO Environmental Risk Assessments), Module 2.2 (Risk-based Approach),
155 and 4 (Non-clinical data).

156 This guideline should be read in conjunction with all relevant European guidelines/reflection papers,
157 International Conference of Harmonisation (ICH) guidelines applicable to GTMPs and European
158 Pharmacopeia requirements. References to the relevant guidelines, reflection papers are made within
159 the relevant section of this document and are listed in section 8.

160 Applicants should also consider the environmental impact from the use of GTMPs. If a GTMP is
161 considered as a Genetically Modified Organism (GMO) according to Article 2 of Directive 2001/18/EC,
162 its use needs to comply with Directive 2001/18/EC. Reference is made to Council Directives
163 90/220/EEC or any subsequent amendment and 90/219/EEC (as amended by Council Directive
164 98/81/EC) respectively on the deliberate release and the contained use of genetically modified (micro)-
165 organisms (GMOs).

166

167 **4. Quality**

168 For any GTMP marketing authorisation application, the dossier has to be divided into a drug substance
169 and a drug product section, even though the manufacturing process for GTMPs may not conform to the
170 traditional drug substance/drug product format.

171 Full information on the vector should be provided in the starting material section even if not remaining
172 in the active substance.

173 This guideline follows the CTD headings whenever possible; however the numberings of the CTD are
174 not followed.

175 **4.1 General Information on the GTMP**

176 The name proposed for the drug substance, and whether it is descriptive of the substance should be
177 explained; an INN, if available, should be provided. The trade name proposed for the drug product
178 should be stated.

179 A full description and diagrammatic representation of the GTMP should be given. The clinical indication
180 for the product and the *in vivo* mode of action should be stated: in this context an explanation of the
181 design of the vector should be given along with an outline of the role of individual components and the
182 therapeutic sequence(s). Diagrams should be used to illustrate the description as necessary. The
183 therapeutic sequence(s), junction regions and regulatory elements should be provided.

184 Any component which has been added to ensure delivery, regulation or expression of the GTMP
185 construct should be described.

186 **4.1.1 Vector Design**

187 Whilst the choice of a vector system will depend in part on the proposed clinical indication, mechanism
188 of action and method of administration, consideration should be given to the selectivity of a GTMP for
189 the target cells/tissues, and transduction efficiency of the GTMP in the target cell population or cell
190 type and the functional activity of the therapeutic sequence(s). Barriers to a successful gene therapy
191 include: vector uptake by the target cells, transport and uncoating, vector or sequence persistence,
192 sustained transcription/expression of the transgene, pre-existing or induced immunity to vectors and
193 the protein expressed by the transgene. Consideration should be given to such barriers when designing
194 the GTMP.

195 For products based on viral or bacterial vectors, considerations should be given to:

- 196 i) Pathogenicity and virulence in man and in other animal species of the parental organism and
197 the vector components and, the deletion of virulent determinants where appropriate;
- 198 ii) The minimisation of non-essential accessory vector components or engineering of viral
199 packaging proteins to render, where necessary, the viral vector replication defective;
- 200 iii) The minimisation of vector sequence homology with any human pathogens or endogenous
201 viruses, thus reducing the risk of generating a novel infectious agent or replication
202 competent virus (RCV).
- 203 iv) Tissue tropism;

- 204 v) Transduction efficiency in the target cell population or cell type, e.g. whether the cells are
205 dividing or terminally differentiated or cells expressing the appropriate viral receptor for
206 internalisation;
- 207 vi) The presence and persistence of the viral gene sequence(s) important for anti-viral
208 chemotherapy of the wild type virus;
- 209 vii) The tissue specificity of replication;
- 210 viii) Germline transmission.

211 For integrating vectors, consideration should be given to the risk of insertional mutagenesis. Reference
212 is given to the reflection paper on clinical risks deriving from insertional mutagenesis
213 (EMA/CAT/190186/2012).

214 For replication deficient viral vectors, the strategy taken to render the viral vector replication
215 incompetent should be clearly documented and replication deficiency demonstrated. The drug
216 substance and where appropriate intermediates, as well as any packaging/producer cell lines, should
217 be screened for RCV. The possibility of any recombination events leading to RCV or replication via *trans*
218 regulation should be considered.

219 For replication competent viral vectors or replication-conditional viral vectors, a clear rationale of the
220 construct and the individual genetic elements that control replication should be provided with regard to
221 its safe use for the proposed clinical indications. Consideration should be given to the following factors
222 with regard to the acceptability of using a RCV as a GTMP:

- 223 i) That replication competence is required for the efficacy of the medicinal product;
- 224 ii) That the vector does not contain any element(s) known to induce oncogenicity/tumorigenicity
225 in humans;
- 226 iii) That if the parental viral strain is a known pathogen, the infectivity, virulence and
227 pathogenicity of the RCV should be determined after the desired genetic manipulations and
228 justified for the safety of its use;
- 229 iv) The tissue specificity of replication.

230 For viral vectors which are selected on the basis of their organ/tissue tropism, evidence should be
231 provided on the selective transduction/expression of the inserted gene or an appropriate reporter gene
232 at the desired site. This should form the basis for the design and development of appropriate control
233 methods (See also non-clinical section 5.4.1).

234 **4.1.2 Development genetics**

235 For all vectors, full documentation of the origin where applicable, history and biological characteristics
236 of the parental virus or bacterium should be provided.

237 All the genetic elements of the GTMP should be described including those aimed at therapy, delivery,
238 control and production and the rationale for their inclusion should be given.

239 These include:

- 240 • For plasmid DNA (including plasmids delivered via bacterial vectors): the plasmid backbone,
241 transgene and selection gene and any other regulatory sequences should be described.

- 242 • For viral vectors: these include, but are not limited to, the virus backbone, therapeutic
243 transgene, regulatory sequences and helper-virus.
- 244 • For bacteria: Details of plasmid origin, identification and isolation as well as the nucleotide
245 sequences and functions (including regulative and coding capacity) should be given. For
246 bacteria, their origin and genome should be described.
- 247 For plasmid DNA, full sequence should be provided.
- 248 Additional requirements can be found in the general chapter of the Ph. Eur. 5.14 Gene transfer
249 medicinal products for human use.
- 250 Inclusion in the therapeutic sequence of any intended modification(s) to wild-type sequences, e.g. site-
251 specific mutations, deletions and rearrangements should also be detailed. Where applicable, sequence
252 deviations from the published databases should be highlighted and discussed. For a therapeutic
253 sequence which incorporates transcriptional elements to control the expression of a transgene, e.g. in
254 a temporal or tissue-specific manner, summary evidence should be provided to demonstrate such
255 specificity from a product characterisation and control viewpoint.
- 256 The use of antibiotic resistance genes (or other elements used for selection) in the final GTMP should
257 be avoided if possible and where not possible, justified.
- 258 It is essential to purify and characterise the genetic material as thoroughly as possible before analysis
259 and use. In all cases the likelihood of cross-contamination during construction and recombination with
260 endogenous sequences in the cell substrate used during construction or in production should be
261 evaluated. Contamination of the final GTMP with sequences used in a manufacture process, e.g. read-
262 through from production vectors should be considered. Ideally, steps should be taken in design,
263 construction and production to minimize or eliminate such events.
- 264 Data on the control and stability of the vector and the therapeutic sequence(s) during development
265 and in production should be provided. The degree of fidelity of the replication systems should be
266 ensured as far as possible and described in order to ensure integrity and homogeneity of the amplified
267 nucleic acids. Evidence should be obtained to demonstrate that the correct sequence has been made
268 and that this has been stably maintained during any amplification so that the therapeutic sequence
269 remains unmodified. For example, a gene containing errors in base sequences may encode an
270 abnormal protein which may have undesirable biological and/or immunological activities.
- 271 Cells used in amplification of the genetic material should be fully characterised; the history of the cell
272 line, its identification, characteristics and potential viral contaminants should be described. Special
273 attention should be given to the possibility of contamination with other cells, bacteria, viruses or
274 extraneous genetic sequences. Appropriate process validation studies will contribute to demonstration
275 of genetic stability during production.
- 276 Full details of the construction of any packaging/producer cell line or helper virus should be provided,
277 Details should include the origin, identity and biological characteristics of the packaging cell line or
278 helper virus together with details of the presence or absence of endogenous viral particles or
279 sequences.
- 280 Where, during development, changes to the design of the vector are made to obtain new improved
281 product characteristics, principles outlined in the Reflection paper on changes during development of
282 gene therapy medicinal products (EMA/CAT/GTWP/44236/2009) should be taken into consideration.

283 **4.2 Drug Substance**

284 **4.2.1 Manufacture**

285 Vectors should be produced from well characterised bacterial or virus seeds and/or cell banks, as
286 appropriate, which should be appropriately qualified. Master and working seed/cell banks should be
287 established, thoroughly characterised and subjected to an appropriate quality control strategy (see
288 4.2.2.1). Freedom from contamination with adventitious agents is essential to ensure microbiological
289 safety of the product.

290 Where production involves replication competent viruses it may be necessary to establish working virus
291 seeds before inoculation of the production cell culture. In other cases, DNA plasmids might be used to
292 transfect the production cell culture in addition to or instead of infection with a virus. The number of
293 passages between the working seed/cell lot and vector production should be kept to a minimum and
294 should not exceed that used for production of the vector used in clinical studies, unless otherwise
295 justified and authorised.

296 Different substrates used for production might include primary cells, diploid cells, and/or continuous
297 cell lines. The rationale for the use of a particular substrate should be provided. Where genetically
298 engineered cells are used for production, reference is made to appropriate sections within ICH Q5D
299 Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for
300 Production of Biotechnological/Biological Products.

301 An effective purification process should be in place to eliminate or reduce impurities to acceptable
302 levels. These include host cell proteins, host cell DNA, helper viruses/sequences, packaging viruses or
303 sequences, residues of biological materials introduced during productions such as bovine serum or
304 albumin, antibiotics, leachables from equipment, endotoxins, replication competent vector, and any
305 proteins co-expressed with the transgene. Additional impurities needing consideration may include
306 hybrid viruses in the case of virus vector production, lipids and polysaccharides in the case of
307 production systems which involve bacterial fermentations, and RNA and chromosomal DNA in the case
308 of plasmid purification. Contamination of the final GTMP with manufacture derived sequences, such as
309 read-through from production vector or contamination with helper sequences should always be
310 considered. Ideally steps should be taken in design, construction and production to minimise or
311 eliminate these.

312 In some cases, there may be minimal downstream processing of viral vectors. In such cases, the
313 absence of purification steps to reduce product and process related impurities will need to be robustly
314 justified based on technical considerations and clinical safety and efficacy. The use of purification steps
315 is encouraged for all gene therapy vectors.

316 Substances such as diluents or stabilisers or any other excipients added during preparation of the final
317 vector or final product should be shown not to impair the efficacy and safety of the vector in the
318 concentrations employed.

319 **4.2.1.1 Description of manufacturing process and process controls**

320 A clear definition of Drug substance should be provided. A flow diagram should be provided to illustrate
321 the manufacturing route from the bacterial seed, virus seeds and/or cell banks or sources of nucleic
322 acids up to drug substance. The diagram should include all steps (i.e. unit operations) of the
323 manufacture of the purified drug substance, including inoculation, fermentation/culture, harvesting,
324 clarification, pooling, purification and concentration.

325 Process parameters and control procedures that ensure consistency of production conditions and of the
326 expected product are imperative. Unintended variability, for example in culture conditions or
327 inoculation steps during production may cause alteration to the product, reduce the yield of product
328 and/or result in quantitative and qualitative differences in the quality of the DS or the impurities
329 present.

330 For the process description, information should be included on individual process steps, for example
331 scale, culture media, additives and major equipment. For each stage of the DS manufacturing process,
332 all relevant information (e.g. DNA and virus concentrations, cell densities, cultivation times, holding
333 times, process intermediates and temperatures) should be provided. Critical steps and critical
334 intermediates should be identified and acceptance criteria should be set and justified.

335 For non-replication competent viral vectors and conditionally replicating virus vectors, information
336 should be provided on process parameters, and controls and testing conducted to prevent
337 infection/contamination of the packaging cell line by wild-type, helper or hybrid viruses which might
338 lead to the formation of replication-competent recombinant viruses during production. In process tests
339 with suitably low limits of detection are essential to show that replication-competent viruses are below
340 an acceptable level.

341 The manufacturing process must be set up to minimise the risk of microbiological contamination.

342 Tests performed on harvested vector should as a minimum include identity (desired transgene and
343 vector), purity and yield. Acceptable limits for the purity and yield should be specified and justified.
344 Tests for extraneous agents should be performed on each harvest and should be designed to take into
345 account the need to neutralise the vector where appropriate. Sensitive molecular methods may be
346 used as alternatives to test for the presence of specific extraneous viral sequences.

347 For viral vectors, titre and particle to infectivity ratio should be determined on harvests and minimum
348 acceptable titres should be established. Tests for replication competent viruses may be necessary for
349 certain replication-defective or conditionally replicating viral vectors. For products containing
350 replication-deficient viruses, a test to detect replication competent viruses in supernatant fluids of
351 producing cells and in the viral fraction at appropriate stages of production is essential.

352 A clear definition of a "batch" or "lot" of drug substance should be provided, including details on batch
353 size and scale of production. An explanation of the batch numbering system, including information
354 regarding any pooling of harvests or intermediates should be provided.

355 Where nucleic acid constructs are complexed with polycations, proteins, polymers or are linked to
356 carriers, details of the production process, parameters and controls for all components of the final gene
357 therapy vector should be provided (see 4.2.2.1).

358 **4.2.2 Control of materials**

359 **4.2.2.1 Starting materials**

360 All starting materials¹ used for manufacture of the active substance should be listed and information on
361 the source, quality and control of these materials shall be provided. The establishment of

¹ Annex to directive 2009/120/EC, Part IV, 3.2.: Specific requirements for gene therapy medicinal products

3.2.1.3. In the case of products consisting of viruses or viral vectors, the starting materials shall be the components from which the viral vector is obtained, i.e. the master virus vector seed or the plasmids used to transfect the packaging cells and the master cell bank of the packaging cell line.

362 bacterial/cell/virus seed or bank(s) is expected for starting materials which are bankable. The
363 preparation of a two tiered cell bank and/or bacterial/viral seed system is advisable.

364 The source and history of the cells or bacterial or virus seeds used for generation of the respective
365 banks should be described and genetic stability of the parent material demonstrated.

366 All starting materials, including master and working cell banks and viral seeds should be thoroughly
367 characterised and appropriately monitored (e.g. according to the concepts outlined in ICH Q5D).
368 Evidence of freedom from contamination with adventitious agents is essential. For all starting
369 materials, the absence of microbial/viral and fungal contaminants should be ensured through testing
370 after expansion to the limit of in vitro cultivation used for production (see ICH guidelines Q5A).

371 Where materials of ruminant origin are used in preparation of the master and working seeds or cells,
372 compliance with relevant TSE note for guidance is required. The "EU guideline on the use of bovine
373 serum" (CPMP/BWP/1793/02) should also be consulted, where appropriate.

374 All starting materials should be demonstrated to be genetically stable. For a given product to be
375 prepared in a prokaryotic or eukaryotic cell line, it is necessary to demonstrate that consistent
376 production can be obtained with cells at passage levels at the beginning and the end of production.

377 The following sections provide an indication of the tests expected to be conducted on different types of
378 starting material but do not provide an exhaustive list as the tests required will be essentially product-
379 and production process-specific:

380 i) Virus seed banks

381 Control of virus seed banks should include identity (genetic and immunological), virus concentration
382 and infectious titre, genome integrity, transcription/expression of the therapeutic sequences,
383 phenotypic characteristics, biological activity of therapeutic sequence, sterility (bacterial, and fungal),
384 absence of mycoplasma, absence of adventitious/contaminating virus and replication competent virus
385 (where the product is replication deficient or replication conditional) and inter-vial homogeneity.
386 Complete sequence of the therapeutic and the regulatory elements and, where feasible, the complete
387 sequence of the virus in the seed bank should be confirmed.

388 ii) Mammalian Cell Banks

389 Testing conducted on producer/packaging cell lines (organised in a cell bank system described above)
390 should include identity, purity, cell number, viability, strain characterization, genotyping/phenotyping,
391 verification of the plasmid/transgenic/helper sequence structure (e.g. restriction analysis or
392 sequencing), genetic stability, copy number, identity and integrity of the introduced sequences.

393 Testing of the producer/packaging cell bank for presence of adventitious viruses should be conducted
394 according to ICH Topic Q5A and EP 5.1.7 and should include tests for contaminating and endogenous
395 viruses. The absence of bacterial and fungal contamination, as well as mycoplasma and spiroplasma

3.2.1.4. In the case of products consisting of plasmids, non viral vectors and genetically modified microorganism(s) other than viruses or viral vector, the starting materials shall be the components used to generate the producing cell, i.e. the plasmid, the host bacteria and the master cell bank of recombinant microbial cells.

3.2.1.5. In the case of genetically modified cells, the starting materials shall be the components used to obtain the genetically modified cells, i.e. the starting materials to produce the vector, the vector and the human or animal cells. The principles of good manufacturing practice shall apply from the bank system used to produce the vector onwards.

396 (insect cells), should be determined. Electron microscopy of insect cells should also be carried out,
397 unless otherwise justified.

398 For the packaging cell lines, detailed descriptions of their design, construction, production and the
399 banking system used should be provided, with the same level of detail.

400 iii) RNA or DNA Vectors and plasmids

401 Testing of RNA and DNA vectors, plasmids or artificial chromosome DNA should include tests for
402 genetic identity and integrity including confirmation of the therapeutic sequence and
403 regulatory/controlling sequences, freedom from extraneous agents using a range of tests, sterility and
404 endotoxin levels. The presence/absence of specific features such as CpG sequences should be
405 confirmed by suitable methods.

406 iv) Bacterial cell banks

407 Bacterial cell banks should be tested for phenotypic and genomic identity. The presence/absence of
408 inserted/deleted sequences necessary for the safe use of the GTMP should be confirmed. The
409 immunological identity including the genetically modified components should be determined, for
410 instance by serotyping. Transduction efficiency, absence of contaminating bacteria and bacteriophages,
411 fungal sterility, and inter vial homogeneity of cell bank stocks should be assured. For transduced
412 bacterial cell banks testing should include presence of plasmid or genome sequences containing the
413 therapeutic sequence and associated regulatory/control elements, plasmid copy number and ratio of
414 cells with/without plasmids. The principle described in ICH Q5D guideline on "derivation and
415 characterisation of cell substrates" should also be considered.

416 v) Complexing materials

417 Complexing materials for formulating the drug substance are considered as starting materials and have
418 to be qualified for their intended purpose. The quality and purity of the complexing materials is
419 essential for the later quality of the GTMP, therefore the appropriate characterisation and specification
420 of the complexing material(s) is considered vital as well as the in process controls described above.
421 The level of information to be provided will depend on nature of the complexing material and resulting
422 DS. Use of multiple sources (e. g. animal, plant, synthetic sources) or suppliers for the lipid
423 components would require that information be provided for each, along with additional characterisation
424 and comparability studies to demonstrate equivalence of batches (physico-chemical and purity profile
425 and complexing performances) manufactured with each source or supplier.

426 **4.2.2.2 Raw materials²**

427 A complete description, including source, characteristics and testing of all materials used during
428 manufacture should be provided. Data should be provided to demonstrate that all materials used
429 during production are of suitable quality and consistent between batches and/or between suppliers, in
430 case multiple sourcing is envisaged for some of them. Reference is given to the general chapter of the
431 Ph. Eur. on raw materials used in the manufacture of cell based and gene transfer medicinal products.
432 Information should be provided on the residual level of all raw materials (or components of raw
433 materials such as helper virus/packaging sequences or media) in the final GTMP.

² Regulation defines the raw materials for ATMPs as follows: Materials used during the manufacture of the active substance (e.g. culture media, growth factors) and that are not intended to form part of the active substance shall be considered as raw materials (Dir. 2009/120).

434 For the helper viruses, detailed descriptions of their design, construction, production and the banking
435 system used should be provided, with the same level of detail and amount of confirmatory data, as is
436 required for the starting materials addressed in 4.2.2.1.

437 All raw materials consisting of animal tissue or fluids or containing product of animal origin should
438 comply with the relevant TSE guideline. Penicillin, all other β -lactam antibiotics and streptomycin
439 should neither be used during production nor added to the final product as they are known to provoke
440 sensitivity in certain individuals. This would also apply to other toxic reagents such as ethidium
441 bromide.

442 **4.2.3 Characterisation for the drug substance**

443 Characterisation studies should be conducted throughout the development process, resulting in a
444 comprehensive picture and knowledge of the GTMP, which takes the individual components (including
445 starting materials, intermediates, drug substance and drug product) into full consideration.
446 Characterisation of the vector should include all components, but in particular those present in the final
447 product to be administered. For a complexed nucleic acid vector, the characteristics of the vector, the
448 complexing components and the resulting complexed nucleic acid sequence, should be thoroughly
449 investigated. Characterisation data could encompass data obtained throughout the development
450 and/or manufacturing process. Clear identification of the batches (development, pilot, full scale) used
451 for characterization studies should be made. Batches used for setting specification should be
452 representative of the intended process for marketing (see 4.2.4).

453 An extensive characterisation of the DS should be established in terms of genotypic and phenotypic
454 identity, purity, biological potency/therapeutic sequence activity, infectivity/transduction efficiency and
455 suitability for the intended use, unless otherwise justified.

456 Characterisation studies should use a range of orthogonal state-of-the-art techniques including
457 molecular, biological and immunological tests. The methods used should be described.

458 **4.2.3.1 Elucidation of structure and other characteristics**

459 The complete sequence of the therapeutic and genetic elements required for
460 selectivity/regulation/control of the therapeutic sequence should be provided. Restriction endonuclease
461 mapping data should be provided to complement sequence data and transcription/translation elements
462 and open reading frames analysed. It should be demonstrated that there is no inclusion of known
463 oncogenic/tumorigenic sequences. Tests should be included to show integrity and homogeneity of the
464 recombinant viral genome or plasmid and the genetic stability of the vector and therapeutic sequence.
465 Phenotypic identity and analysis of the therapeutic sequences and selectivity/regulatory elements
466 delivered by the vector should be included.

467 Physicochemical characteristics such as refractive index, particle or molecular size average and
468 distribution, and aggregation levels should be determined in characterization studies.

469 For viral vectors the tissue tropism, infectivity (in a variety of cell cultures), virulence, replication
470 capacity, ratio of infectious to non-infectious particles, and immunological characteristics should be
471 documented. Mean particle size and aggregates should be analysed. For viral vectors, insertion sites
472 should be determined where appropriate and the potential for insertional mutagenesis established and
473 associated risks fully evaluated. Vector shedding and replication-competence and possibility of
474 reactivation of endogeneous viruses or complementarity with endogenous viruses should be discussed
475 in relation to patient safety.

476 For plasmids, the transduction efficiency and copy number should be demonstrated in the relevant cell
477 type(s) and the different plasmid forms should be identified and quantified. The ratio of circular to
478 linear forms, the locations of replication origins, and, if relevant to the design of the product, the
479 presence or absence of CpG sequences should be demonstrated.

480 For complexed nucleic acids, the structure of the complex and the interaction between the vehicle(s)
481 (see 4.2.2) and the negatively charged DNA should be addressed. The properties of the
482 complexing/delivery systems should be adequately characterised include: form, particle size
483 distribution, surface charge, stability under a given condition or in a particular biological environment
484 such as the one expected for the transfection step, and distribution of nucleic acid within the
485 complexing structure. Suitable tests should be included to establish, for example, that complexed
486 nucleic acid has the desired biochemical and biological characteristics required for its intended use.

487 For bacterial vectors, the sequence of the therapeutic and genetic elements required for
488 selectivity/regulation/control of the therapeutic sequence should be provided. Restriction endonuclease
489 mapping data should be provided to complement sequence data and transcription/translation elements
490 and open reading frames analysed. The presence/absence of inserted/deleted sequences necessary for
491 the safe use of the GTMP should be confirmed. It should be demonstrated that there is no inclusion of
492 known oncogenic/tumorigenic sequences. The integrity and homogeneity of the recombinant bacterial
493 genome or plasmid and the genetic stability of the bacterial vector and therapeutic sequence should be
494 investigated. For transduced bacterial vectors testing should include the presence and the sequences
495 of plasmid and associated regulatory/control elements, plasmid copy number and ratio of bacteria
496 with/without plasmids. Phenotypic identity, immunological identity (including the genetically modified
497 bacterial components) and analysis of the therapeutic sequences and selectivity/regulatory elements
498 delivered by the bacterial vector should be included. The absence of contaminating bacteria and
499 bacteriophages, fungal sterility, and inter vial homogeneity of cell bank stocks should be assured.

500 **4.2.3.2 Biological activity**

501 The intended action of regulating, repairing, replacing, adding or deleting a genetic sequence should be
502 demonstrated. The *in vitro* biological activity of all transgene(s) and any other expressed sequences
503 should be determined. The level of transgene expression, associated biological activity, and all factors
504 associated with the proposed mechanism of action of the vector/delivery system including maintenance
505 of the therapeutic sequence in the target cell should be analysed. Any selectivity claimed for the host
506 range and tropism of a viral vector or selectivity of delivery of complexed nucleic acid should be
507 demonstrated, as should selectivity of transgene expression where it is claimed.

508 **4.2.3.3 Impurities**

509 Potential impurities in the drug substance and/or drug product will be influenced by the nature of the
510 expected product and the choice of production/manufacturing process.

511 Product-related impurities, such as vectors with deleted, rearranged, hybrid or mutated sequences
512 should be identified and their levels quantified. The possibilities for co-packaged extraneous DNA
513 sequences being present in the vector should be explored. Reference should be made to potential
514 degradation during the manufacturing process affecting key properties of the vector such as
515 infectivity/non-infectious forms, plasmid forms with reduced transduction efficacy, or degradation of
516 nucleic acid complexes through, for example, oxidation or depolymerisation.

517 Process-related impurities include residues of starting materials (residual DNA and residual host cell
518 protein from each cell bank), raw materials (culture reagents, purification reagents and equipment

519 materials, helper viruses and helper virus nucleic acid used in production), adventitious agents (see
520 section 4.7) and leachables and extractables from the process. In the case of vectors designed to be
521 replication deficient or conditionally replicating, the absence of replication competent vector should be
522 demonstrated and/or conditional replication demonstrated.

523 In the case of complexed nucleic acids, by-products/impurities arising from the complex synthesis and
524 production should be addressed with respect to their impact on safety and performance of the complex
525 for administration to the patients.

526 The characterisation data generated should serve as input into the specification setting for drug
527 substance and drug product; along with data from batch analysis (see 4.2.4). In the case of drug
528 substances which are intended for compounding with ancillary materials acting as carriers or supports
529 the characterisation studies should be repeated for the substance in the complexed state. The nature
530 and strength of the complexation involved should be explored in the studies.

531 **4.2.4 Specifications for the drug substance**

532 The criteria for acceptance or rejection of a production batch must be provided. Drug substance
533 specifications should be justified (see ICH Q6B).

534 A specification table (including parameters, methods and specifications or criteria for acceptance)
535 should be provided. The specifications (at release and at shelf life) for the drug substance should
536 normally encompass tests for identity, purity, content, activity, sterility, endotoxin level and
537 mycoplasma. Tests indicated in relevant sections of Ph. Eur. 5.14. should be considered in the
538 specifications or any departure or omission justified. The analytical methods should be relevant,
539 validated state-of-the-art techniques.

540 The following sections provide an indication of the tests expected to be included in the set of
541 specifications but do not provide an exhaustive list as the tests required will be essentially product-
542 and production process-specific. (Please refer to ICH guideline Q6B, and Ph. Eur. 5.14).

- 543 • Appearance.

544 Qualitative criteria describing the physical form and colour of the drug substance allowing visual
545 inspection of the conformity of the product before it is used for manufacturing the drug product.

- 546 • Identity and integrity

547 The genetic identity and integrity, of the drug substance should be assured using tests that identify
548 both the therapeutic sequence and the vector. Such tests might include DNA sequencing or restriction
549 enzyme mapping and immunological assays.

550 The identity of the drug substance may also be confirmed through infection/transduction assays and
551 detection of expression/activity of the therapeutic sequence(s) (see potency assay section). This
552 identity test is especially important for complexed nucleic acid sequences.

- 553 • Content

554 The quantity of the drug substance should be established. Content might be quantified through tests
555 such as infectious titre, infectious particle concentration, number of particles (infectious/non-
556 infectious), quantity or concentration of DNA or plasmid or a combination of such methods depending
557 of the nature of the active substance. Where relevant, particle to infectivity ratio should be included to
558 define the content of the drug substance.

559 • Potency Assay

560 A suitable measure of the potency or strength of the drug substance should be established.

561 At least one biological potency specification should be established, the attribute(s) reflecting the
562 physiological mode of action and / or the pharmacological effects of the GTMP.

563 The potency assay should normally encompass an evaluation of the efficiency of gene transfer
564 (infectivity/transduction efficiency/ delivery efficiency) and the level and stability of expression of the
565 therapeutic sequence or its direct activity. Where possible the potency assay should include a measure
566 of the functional activity of the therapeutic sequence or the product of it. This functional test may be
567 supplemented with immunochemical methods to determine the integrity and quantity of an expressed
568 protein product if appropriate.

569 In vitro biological potency tests should be developed. If not feasible, biological potency tests in animal
570 tissues maintained *ex vivo* or in whole animals can be considered. Transgenic animals or animals with
571 transplanted human tissues or systems, e.g. a suitable xenograft model, may be suitable for this
572 purpose. In order to reduce the use of animals in accordance with the 3R principles, a validated in vitro
573 method should generally be considered before conducting animal testing (e.g., see Directive
574 2010/63/EU).

575 Whenever possible, suitable ways for expressing potency of vectors should be established and results
576 reported in reference to an appropriately qualified reference material. Specific activity should be
577 determined and a range established.

578 • Product-Related Impurities

579 The presence of product-related impurities such as non-functional forms of the vector, or the presence
580 of co-packaged unwanted genetic sequences should be included in the specification and acceptance
581 limits set to exclude or limit these impurities as appropriate and justified.

582 For viral vectors, empty particle number and aggregates should be controlled. For plasmid DNA Limits
583 for different forms of plasmid should be included. Other impurities' may need to be considered.

584 • Process-Related Impurities

585 Specifications should be set for materials used in vector production, unless process validation data
586 have been provided to demonstrate that such residues are consistently reduced to acceptable levels.

587 For the release specifications, tests should be developed and relevant (upper) limits set to monitor the
588 residual levels of contaminants of cellular origin, e.g. host cell protein or DNA from the bacterial or
589 packaging cell line, as well as raw materials that may have been used during the production process
590 such as benzonase or resins. Other process-related impurities may include: nucleic acids derived from
591 bacteria used for the production of plasmid DNA, extraneous nucleic acids in vector preparations,
592 helper viruses or other impurities such as residual animal serum proteins (e.g. BSA) used in
593 production.

594 If tumorigenic cell lines are used during production the total residual DNA level should be strictly
595 controlled and kept at a minimum unless otherwise justified.

596 • Extraneous agents

597 Tests for extraneous agents should be included to ensure the safety of the vector. For replication-
598 deficient or conditionally-replicating viral vectors, a test for replication competent virus should be
599 included. In the case of vectors which are potentially hazardous to patients' health in their replication-
600 competent forms, such as members of the *Retroviridae*, absence of replication competence should be

601 demonstrated using a validated assay. In other justified cases, it may be acceptable to release vector
602 lots with an upper limit for replication-competent vector. In these cases the justification for the limit
603 should include qualification on the basis of non-clinical and / or clinical data for batches with similar
604 levels.

- 605 • Physicochemical properties.

606 Limits should be applied to measurement of pH and any other relative physicochemical properties such
607 as opalescence, refractive index. Particle number, molecular size average and size distribution should
608 be controlled.

- 609 • Compendial tests

610 Depending on the nature of the drug substance, other compendial tests will apply for release. Inter
611 alia, the sterility test or bioburden limit) should be conformed to the EP standards.

612 **4.3 Finished Medicinal Product**

613 Most of the considerations made for Drug Substance are applicable to the Drug Product (DP) and will
614 not be repeated in this section. However, some specific considerations should be taken into account as
615 regards DP and filing the relevant information in the CTD.

616 **4.3.1 Description of the product and pharmaceutical development**

617 Definition of the DP and its qualitative and quantitative formulation should be provided along with the
618 trade name proposed. The description should take into account the origin, identification, physico-
619 chemical and functional characterisation studies, and the expected function of all components in the
620 final product.

621 **4.3.2 Manufacturing of the Drug product and process controls**

622 A clear description of the DP manufacturing process and the in-process controls, should be provided. A
623 flow diagram should be provided to illustrate the manufacturing route from the purified drug substance
624 up to the final drug product in its primary packaging. The diagram should include all steps (i.e., unit
625 operations) including formulation, filtration, filling and where relevant any further freeze-drying or
626 freezing steps. For each stage of DP manufacturing process, all relevant information, in terms of
627 holding times, temperatures or any parameter relevant for the final quality of the DP should be
628 provided. Process intermediates should be defined. Process parameters and procedures should be
629 defined to ensure consistency of production conditions.

630 The quality controls and critical manufacturing steps should be identified and the control strategy
631 justified.

632 The manufacturing process must be set up to minimise the risk of microbiological contamination.

633 **4.3.3 Excipients**

634 Complexing materials for formulating the drug product are considered as excipients and have to be
635 qualified for their intended purpose. The quality and purity of the complexing materials is essential for
636 the later quality of the GTMP, therefore the appropriate characterisation and specification of the
637 complexing material(s) is considered vital. Functionality-related characteristics as described in the Ph.
638 Eur. monograph 5.15 'Functionality-related characteristics of excipients' should be adequately

639 addressed. The level of information to be provided will depend on nature of the complexing material
640 and resulting final product. The principles of the Guideline on Excipients in the Dossier for Application
641 for Marketing Authorisation of a Medicinal Product (EMA/CHMP/QWP/396951/2006) should be
642 considered unless justified. Use of multiple sources (e. g. animal, plant, synthetic sources) or suppliers
643 for the lipid components would require that information be provided for each, along with additional
644 characterisation and comparability studies to demonstrate equivalence of batches (physico-chemical
645 and purity profile and complexing performances) manufactured with each source or supplier.

646 **4.3.4 Characterisation for the Drug Product**

647 The GTMP can be presented combined with medical devices. If gene therapy products are combined
648 with a medical device at the level of the drug product, characterisation of the drug product is required.
649 This characterisation should take into account the medical device itself and its contribution to the
650 function of the final product. Reference is given to Article 3.4. (specific requirements for advanced
651 therapy medicinal products containing devices) of Directive 2009/120/EC.

652 **4.3.5 Drug Product specification**

653 Quality control tests should be performed at the drug product level, unless appropriate justification can
654 be provided based on release testing at the drug substance level. Tests on attributes which are specific
655 to the formulated product in its final container and quality attributes which may have been impacted
656 by the formulation steps should be included in the release testing.

657 Unless otherwise justified, the release specifications for each batch of drug product are expected to
658 embrace the following:

- 659 • The range of quality attributes listed under “Drug substance” above, including identity, assay and
660 potency. Tests for impurities and process-related impurities from the DS steps could be omitted
661 based on relevant justification and validation data.
- 662 • Infectivity or transduction efficiency: in *vitro* infectivity or transduction efficiency of the drug
663 product in its final formulation should be included.
- 664 • Specification should be applied for appearance and physicochemical properties (e.g. pH and any
665 other relative physicochemical properties such as opalescence, refractive index and osmolality)
666 specific to the drug product.
- 667 • Sterility, endotoxin, particulate matter and other compendial tests such as extractable volume or
668 residual moisture should be included as appropriate.
- 669 • Where appropriate, and subject to a risk-based approach, replication-competent virus acceptance
670 criteria should be applied to ensure the safety of the drug product.
- 671 • Assays for critical excipients, such as albumin or complexing materials used in the formulation (of
672 either DS or DP) should be included, particularly where these ensure the expected bioactivity
673 and/or maintain the stability of the final formulated vector.
- 674 • Specifications should also be set for materials used in the DP formulation and filling unless process
675 validation data have been provided to demonstrate that such residues are consistently reduced to
676 acceptable levels.

677 **4.4 Process development and process validation for drug substance** 678 **and drug product**

679 Changes in the manufacturing process, such as scale-up of culture and/or purification often occur
680 during development as product development progresses to full-scale commercial production. These
681 changes are usually introduced before final validation of the process. This may have consequences for

682 the quality of the product including effects on its biochemical and biological properties, and thus
683 implications for control testing.

684 Approaches to determine the impact of any process change will vary, depending on whether this is at
685 the drug substance or drug product stage and with respect to the specific manufacturing process step
686 concerned. It will also depend on the extent of the manufacturer's knowledge and experience with the
687 process and development data gained. Appropriate, and fully justified comparability studies according
688 to the principles outlined in ICH Topic Q5E for biotechnological/biological products should be conducted
689 in order to demonstrate comparability of the pre- and post-change product. The criteria for
690 determining comparability of GTMP medicinal products after manufacturing changes should be fully
691 justified.

692 For complexed nucleic acids, it is known that small changes to complexed products and the materials
693 used can significantly influence their performance.

694 *In vivo* studies may be necessary to demonstrate that any process changes do not affect the safety
695 and efficacy profile of the product when results from physicochemical and *in vitro* testing indicate a
696 change in the properties of the product.

697 At the end of the process development and when the manufacturing process (for both drug substance
698 and drug product) is deemed finalised, the validation of the entire manufacturing process should be
699 considered to show consistency of the production process using sufficient number of consecutive
700 production runs representative of the commercial scale manufacturing process. The number of batches
701 needed can depend on several factors including but not limited to: (1) the complexity of the process
702 being validated; (2) the level of process variability; and (3) the amount of experimental data and/or
703 process knowledge available on the specific process (further guidance can be found in ICH Q11).
704 Deviations between batches beyond the normal process variability should be noted and investigated.

705 In particular, the ability of the process to remove or inactivate any helper, hybrid or replication
706 competent viruses generated or used during manufacture or components of the production system
707 which may support their formation should be demonstrated where appropriate. If scaled down
708 experiments are used, they should be fully described and justified and such scale-down models should
709 be demonstrated to be representative of the commercial manufacturing scale/site/process.

710 **4.5 Analytical Method, Validation and Reference Standards for drug** 711 **substance and drug product**

712 Full details of all tests used for batch release of drug substance and drug product should be provided,
713 including their analytical performances within their designated use. Individual tests may serve more
714 than one purpose (e.g. identity and potency). All analytical methods used for release of drug substance
715 and drug product batches should be fully validated according to ICH and suitable for their purpose. For
716 assays related to impurities which may affect the safety of the product, such as tests for toxic
717 impurities and tests for replication-competent viruses, it is essential to establish the suitability and the
718 sensitivity of the tests. The limit of detection must be such that the test provides assurance of the
719 safety of the vector product. Also, the appropriateness of the permissive cell type(s) used in the assays
720 for replication-competent virus should be established. Each reference material used in control tests
721 should be described in full and demonstrated to be suitable for its intended purpose. A reference batch
722 of vector of assigned potency should be established and used to calibrate assays. The stability profile
723 and relevant storage conditions of those reference/calibration batches should be established.

724 If the tests proposed for release of commercial batches are not the same as those used throughout
725 development, the differences should be discussed and justified in order to bridge with the data from
726 the clinical trial batches (see 4.2.4).

727 **4.6 Stability for drug substance and drug product**

728 Stability protocols, stability data, justifications for the container-closure system used, and proposed
729 shelf-lives and storage conditions, should be presented for the drug substance, drug product and any
730 intermediate product stored during production (i.e. intermediates for which a holding time is scheduled
731 on the production process scheme). The rules outlined in ICH stability guidelines (and particularly ICH
732 Q5C dedicated to biologics and biotech products) should be followed. Real time stability studies should
733 be undertaken, in particular for the drug substance and drug product intended for marketing. However,
734 it is acknowledged that accelerated stability studies (e.g. at elevated temperatures or under other
735 stress conditions relevant for the product of interest) may provide complementary supporting evidence
736 for the stability of the product and help to establish the stability profile. Forced degradation studies
737 provide important information on degradation products and identify stability indicating tests.

738 In general, the shelf-life specifications should be derived from the release specifications, with
739 additional emphasis on the stability-indicating features of tests used and tests/limits for degradation
740 products. Vector integrity, biological potency (including transduction capacities) and strength are
741 critical product attributes which should always be included in stability studies. In the case of products
742 formulated with carrier or support materials, the stability of the complex formed with the drug
743 substance should be studied. Where relevant, the in-use stability of the drug product (after
744 reconstitution or after thawing) should be properly investigated including its compatibility with any
745 diluents used in reconstitution. The recommended in-use time period should be justified.

746 The transport conditions should be validated. The impact of the transport conditions on the stability of
747 DS or DP with a short term shelf life should be considered.

748 **4.7 Adventitious agent safety evaluation**

749 The risk of contamination of the drug substance or drug product by extraneous viruses should be
750 minimised by rigorous testing of seed and cell banks, intermediates and end products for the presence
751 of adventitious virus. Where appropriate validation studies should be undertaken to establish the
752 reduction factors for elimination or inactivation of adventitious agents, provided by the relevant step(s)
753 of the production processes. In addition, raw materials of biological origin should be thoroughly tested
754 or manufactured by a process validated for the removal of adventitious and endogenous viruses.

755 It should be demonstrated that the production process consistently yields batches which are free from
756 contaminating agents. Depending on the product, the potential contaminating agents to be considered
757 may be of human, animal, arthropod and / or plant origin.

758 The adventitious agent safety information should be presented under respective non-viral and viral
759 headings.

760 **4.7.1 Non-viral adventitious agents**

761 Gene therapy vectors other than bacterial vectors are required to be microbiologically sterile

762 Since it may not be possible to apply direct sterilisation methods such as heat or irradiation, the
763 microbiological sterility of gene therapy vectors should be ensured by the application of a combination
764 of measures including the following:

- 765 • Starting material (including seed and cell banks), reagent and equipment selection and
766 control.
- 767 • Exclusion of ingress of extraneous material during the production process.
- 768 • In-process tests and controls focussing on limiting bioburden levels.
- 769 • The application of bioburden reduction process steps, and sterilisation by filtration.

770 The control of endotoxins should also be addressed in this section.

771 **4.7.2 Viral and non-conventional adventitious agents**

772 The viral safety of each GTMP has to be ensured. Both contaminating extraneous viruses and residues
773 of viruses used during production, such as production viruses and helper viruses need to be excluded.
774 Bacteriophages are relevant contaminating viruses for vectors which are produced on bacterial
775 substrates. The freedom from contamination with TSE agents should also be established any time a
776 biological material from animal species susceptible for TSE is used in the production cess.

777 Since the possibilities for applying virus clearance steps during GTMP production are limited, the viral
778 safety of these products should be ensured by applying a combination of measures including the
779 following:

- 780 • Selection and control of starting materials (including seed and cell banks), raw materials
781 and equipment.
- 782 • application of measures which exclude ingress by extraneous material during production
- 783 • Exclusion of extraneous agent ingress during the production process.
- 784 • Application of vector purification process steps which, where feasible, provide
785 elimination/inactivation capacities vis-a-vis relevant viruses.

786

787 **5. Non-Clinical development**

788 **5.1 Introduction**

789 **5.1.1 General principles**

790 The aim of the non-clinical study programme during the development of Gene Therapy Medicinal
791 Products (GTMPs) has the primary objective of providing sufficient information for a proper benefit-risk
792 assessment for the use of such products in human. This section provides considerations on this
793 programme in order to support clinical trials and marketing authorisations for GTMPs.

794 Features of GTMPs which are specific to this class of medicine and which impact on the requirements
795 for the non-clinical development include the potential in vivo effects of the transgene or other
796 recombinant nucleic acid sequences, the vector backbone (i.e. viral, bacterial or plasmid derived
797 sequences), and of the excipients including any carrier or support medical device employed.

798 Any differences of the non-clinical test article from the clinical material resulting from product
799 development should be highlighted and its potential impact discussed.

800 The non-clinical development should be designed on a risk-based strategy identifying suitable end-
801 points. The non-clinical studies can be carried out as stand-alone or as combined studies. The selection
802 of suitable control groups (e.g. vector with no transgene or with mutated and non-coding transgene)
803 should be considered.

804 Consideration should be given to interim sacrifice groups if it is important to monitor morphological
805 changes at the time of maximum inflammatory response (e.g. to an adenoviral vector) or when gene
806 expression is maximal. Generally, the use of the same animal model in both the toxicology
807 investigations and the pharmacokinetic studies is recommended, in particular in case when vector-
808 related toxicity signals are observed.

809 When a GTMP is combined with a medical device, the medical device should comply with the legislation
810 applicable to medical devices. Depending on previous experience with delivery devices and/or
811 excipients, studies addressing their contribution to GTMP activity may be required.

812 The following guidelines should also be consulted: Guideline on non-clinical studies required before first
813 clinical use of Gene Therapy medicinal products (EMA 125459/2006), Guideline on strategies to
814 identify and mitigate risks for first-in-human clinical trials within investigational medicinal products
815 (EMA/CHMP/WP/28367/07), ICH M3, ICH S6, and ICH S8 and the available product specific
816 guidelines.

817 **5.1.2 Characterisation**

818 The applicant should carefully consider the quality development before progressing with the non-
819 clinical development. Consideration should be given to adequately define the drug product.

820 Products used in non-clinical studies should be sufficiently characterised to provide reassurance that
821 non-clinical studies have been conducted with material that is representative of the product to be
822 administered to humans in clinical studies. The potential impact of any modifications of the
823 manufacturing process and the test article on extrapolation of the animal findings to human during the
824 development programme should be considered. Any modification of the nucleic acid sequence of the

825 GTMP or any other sequence that might impact the characteristics of the final drug product may
826 require additional safety evaluation; reference is also made to the "Reflection paper on design
827 modifications of GTMP during development" (CAT/GTWP/44236/2009). The scientific rationale for the
828 chosen approach should be provided.

829 **5.1.3 Methods of analysis**

830 Methods of analysis used in the non-clinical programme should be technically validated with the test
831 article in the appropriate tissue matrix. Applicants should justify the selection of assays used for these
832 studies and their specificity and sensitivity. The sensitivity limits of the chosen assay should be based
833 on properly validated procedures.

834 When developing a method of analysis to be used in the non-clinical programme, considerations should
835 be given to the procurement of the cells/tissue, and the quality and suitability of the sample
836 preparation for the intended assay.

837 In the case of nucleic acid amplification (NAT), as the specificity of NAT methods depends on the
838 choice and design of the primers and probes, as well as on the reaction conditions and the method of
839 detection, the rationale for the selection of the primer and probe sequences should be carefully
840 justified. Owing to its high sensitivity, NAT assays are prone to cross-contamination and false positive
841 results unless proper precautions are taken. Details of assays used should also be discussed and the
842 negative / positive controls used should be indicated.

843 When performing PCR-based assays to measure copy number of vectors, for integrating vectors and
844 cellular GTMPs, the limits of detection and quantification should be expressed preferably as copy
845 number/genome. For episomal vectors, the limits of detection and quantification should be expressed
846 as copy number/ μ g host cell DNA analysed.

847 Advancing developments in *in-situ* nucleic acid amplification and hybridization techniques may allow
848 localisation of vector DNA / transgene within cells / tissues.

849 **5.2 Animal species/model selection**

850 Due to very specific bioactivity of GTMPs, non-clinical studies should be done with the most appropriate
851 pharmacologically relevant *in vitro* and *in vivo* models available. The rationale for the non-clinical
852 development and the criteria used to choose these models shall be discussed and justified in the non-
853 clinical overview.

854 The choice of animal models and their relevance for the situation in human shall be scrutinised in
855 respect to:

- 856 • The ability of the intended virus/vector to infect/transduce, and to replicate in, the chosen
857 animal species/models. For GTMPs based on a replication-deficient viral vector, the animal
858 model should be sensitive to the viral infection. For GTMP based on replication-competent virus
859 or microorganism, the ability to replicate needs to be taken into consideration when selecting
860 the animal model. For oncolytic viruses which are classified as GTMPs, it may be important to
861 include a tumour-bearing xenograft in immune deficient or immune compromised animals or a
862 syngeneic animal tumour model in order to assess the effects of viral replication in tumour cells
863 in the non-clinical studies.
- 864 • The expression and tissue distribution of cellular receptors for virus/bacteria in the animal
865 model that might affect the efficiency of the uptake by the host and the cellular and tissue
866 sequestration of the vector. Depending on the type of gene therapy vector, tissue tropism may

867 occur or is intended via selective presence of the GTMP in tissues or organs, selective infection
868 of cells/tissues or selective expression of the therapeutic gene(s). When selecting the animal
869 model for such vectors, the comparability of the tissue tropism in the selected animal model
870 and human should be discussed and justified. Specific guidance on tissue tropism is provided in
871 the Reflection paper on quality, non-clinical and clinical issues related to the development of
872 recombinant adeno-associated viral vectors (EMA/CHMP/GTWP/587488/2007 Rev1) and the
873 ICH considerations - oncolytic viruses (EMA/CHMP/ICH/607698/2008).

- 874 • The activity of regulatory elements and their control to drive tissue-specific expression and the
875 expression level of the transgene.
- 876 • The biological response to the transgene product including its target expression, distribution,
877 binding and occupancy, functional consequences, including cell signaling and also regulation of
878 gene(s) associated if relevant.
- 879 • The immune status of the animal, its immune response and potential pre-existing immunity.
880 The immune status and pre-existing immunity in humans should be taken into account when
881 selecting the animal model. The persistence and clearance of administered nucleic acid will
882 largely depend on immune surveillance; therefore the immune status of the animal model
883 should mimic the patient's situation as closely as possible. Effects of pre-existing immunity
884 against the vector vehicle and/or vector gene products in the patient may be mimicked by pre-
885 treatment of the animals with the vector. The animals' immune reaction to the parental virus
886 or bacteria used to derive the GTMP should be taken into consideration, if applicable.
- 887 • Presence of animal genes / gene products homologous to the therapeutic gene / gene product.
888 For example, a vector expressing a human cytokine would best be tested in an animal species
889 in which that cytokine binds to the corresponding cytokine receptor with affinity comparable to
890 that seen with human receptors, and initiates a pharmacologic response comparable to that
891 expected in humans.

892 Transgenic animals are used to model different human diseases: infection, neurodegeneration,
893 apoptosis, atherosclerosis, ageing, cancer, xenografts, etc. Nevertheless the choice of
894 transgenic animal model should be properly discussed. For example, the most common animal
895 models currently used for Alzheimer disease (AD) research are transgenic mice that express a
896 mutant form of human A β precursor protein (APP) and/or some of the enzymes implicated in
897 their metabolic processing. However, these transgenic mice carry their own APP and APP-
898 processing enzymes, which may interfere with the production of different amyloid-beta
899 peptides encoded by the human transgenes.

- 900 • Metabolism and other pharmacokinetic aspects, if needed. Use of large or disease animal
901 models may be needed in order to mimic the clinical condition of biodistribution of the GTMP
902 depending on the nature of the product, its route of administration and, optionally, the delivery
903 system employed (e.g. intra-cerebral administration).
- 904 • Consideration should be given to biological characteristics of the components of the product in
905 the species being used, in relation to the dose administered together with the volume which
906 can be safely administered to the test animals.
- 907 • The active and/or passive distribution of virus/vector in the organism.

908 In case a single animal model might not suffice to address all these aspects, various different animal
909 models should be employed in these studies.

910 The chosen animal model(s) may include wild-type, immuno-compromised, knockout, humanised or
911 transgenic animals.

912 The use of disease models or homologous models (e.g. mouse cells analysed in mice) may be
913 considered (e.g. for immunogenicity and immunotoxicity studies).

914 Small rodent animals including transgenic, knockout, and natural disease models may represent
915 relevant models, but limitations due small size and brief life span should be considered. The number of
916 animals used per dose level tested has a direct bearing on the ability to detect toxicity. A small sample
917 size may lead to failure to observe toxic events due to observed low frequency, regardless of severity.
918 The limitations that are imposed by sample size, as often is the case for non-human primate studies,
919 may be in part compensated by increasing the frequency and duration of monitoring. Both genders
920 should generally be used or justification given for specific omissions. To improve safety issue
921 assessment, special consideration should be given to the size of the control groups especially when
922 historical data is lacking or limited for the chosen animal model/species.

923 **5.3 Pharmacology**

924 **5.3.1 Primary pharmacodynamic**

925 **Proof of concept studies**

926 These studies should generate non-clinical evidence supporting the potential clinical effect or at least
927 provide information on the related biological effect/molecular mechanism of action. This can be shown
928 by *in vivo* studies and/or *in vitro* studies– especially when relevant *in vivo* disease models are not
929 available. When molecular mechanisms of action are investigated *in vivo*, studies need to be performed
930 in a relevant animal model which supports the analysis of the mode of action (e.g. counter-regulatory
931 mechanisms may exist in animals that could impair the function of the GTMP).

932 The use of homologous animal models to explore potential biological effects is encouraged if useful.
933 Expression and, if intended, specific control of expression and production of the “correct” transgene
934 product in the appropriate target organ shall be demonstrated. If synthesis of an aberrant gene
935 product from the GTMP cannot be excluded by quality data, the presence, and if so, the biological
936 consequences of aberrant gene product formation should be investigated.

937 *In vitro* and *in vivo* studies performed to unravel the mechanism of action relating to the proposed
938 therapeutic use (i.e. pharmacodynamic “proof of concept” studies) shall be performed using relevant
939 animal species and models suitable to show that the nucleic acid sequence reaches its intended target
940 (target organ or cells) and provides its intended function (level of expression and functional activity).
941 The duration of the transgene expression and the therapeutic effect associated with the nucleic acid
942 sequence and the proposed dosing regimen in the clinical studies shall be described.

943 When the GTMP is intended to have a selective or target-restricted function, studies to confirm the
944 specificity of this function in target cells and tissues shall be performed.

945 In order to demonstrate the therapeutic effect and evaluate the level of gene expression and functional
946 activity, it is recommended to select and test a relevant choice of markers for the disease and safety.

947 Moreover, it is expected to determine the best effective dose without toxic effects of the product which
948 exerts the desired pharmacological activity in the most suitable animal model. Therefore, it will be
949 useful to determine the safety margin.

950 During insertion into the host chromatin, expression cassettes of integrative vectors (e.g. gamma
951 retrovirus, lentivirus) will be present within a native chromatin environment and thus be subject to
952 host epigenetic regulatory machinery. It has been shown for example that epigenetic modifications
953 such as DNA methylation and histone modifications can negatively impact on the transgene expression

954 profile by reorganizing local chromatin environment that ultimately leads to loss of therapeutic gene
955 expression either via a complete gene silencing or position effect variegation. When designing such
956 vectors, applicants should take into account that epigenetics could interfere with the efficacy and
957 safety of the final GTMP. Therefore applicants are encouraged, where applicable, to investigate these
958 issues further by performing *in vitro* analysis of genomic distribution of integrating vectors which will
959 provide crucial information about 'host-on-vector' influences based on the target cell genetic and
960 epigenetic state during early development.

961 If a replication-competent vector/virus is administered, the detection of viral sequences in non-target
962 sites by NAT techniques should result in quantitative infectivity assays in order to evaluate the
963 infectious potential of the detected nucleic acid. The infectivity assay shall be validated and
964 justifications for the specificity and sensitivity of the assay should be provided.

965 **5.3.2 Safety pharmacology**

966 Safety pharmacology studies are required in order to investigate the potential undesirable
967 pharmacodynamic effects of the GTMP on physiological functions (central nervous system,
968 cardiovascular system respiratory system and any other system based on the biodistribution of the
969 product) in relation to exposure in the therapeutic range and above as recommended in ICH S7A,
970 CPMP/ICH/539/00.

971 The objectives of safety pharmacology studies are the following: 1) to identify undesirable
972 pharmacodynamic properties of the GTMP that may have relevance to its safety in humans based on
973 its biodistribution (e.g. biodistribution of the vector and transgene product) 2) to evaluate adverse
974 pharmacodynamic and/or pathophysiological effects of the GTMP observed in toxicology and/or clinical
975 studies; and 3) to investigate the mechanism of the adverse pharmacodynamic effects observed
976 and/or suspected.

977 The investigational plan to meet these objectives should be clearly identified and delineated. Potential
978 effects of both transgene product and vector should be covered and consequences of preconditioning
979 (e.g. preparatory chemotherapy regimen) taken into account as contributors to possible adverse
980 events.

981 Safety pharmacology studies are generally performed by single dose administration, therefore safety
982 pharmacology study endpoints may be combined with single-dose toxicity and biodistribution studies
983 (e.g. to investigate persistence).

984 However, when pharmacodynamic effects occur only late after treatment, or when results from repeat
985 dose non-clinical studies or results from use in humans give rise to concerns about safety
986 pharmacological effects, the duration of the safety pharmacology studies shall be adjusted accordingly.

987 **5.4 Pharmacokinetics**

988 The standard absorption/distribution/metabolism and excretion studies for conventional medicinal
989 products may not be relevant for GTMPs.

990 Pharmacokinetic studies should focus on the distribution, persistence, clearance and mobilization of the
991 GTMP and should address the risk of germline transmission. Pharmacokinetic studies may be combined
992 with non-clinical safety studies.

993 Pharmacokinetic studies are based on the detection of the administered nucleic acid (vector and/or
994 transgene) and should include all relevant organs and tissues, whether target or not. The

995 pharmacokinetic behavior of the expressed gene product should also be investigated with regard to
996 duration and site of expression and/or release.

997 Investigations of shedding should be performed in accordance with the ICH considerations on general
998 principles to address virus and vector shedding (Concept Paper EMEA/CHMP/ICH449035/2009) and
999 shall be provided with the environmental risk assessment (please refer to the guideline on scientific
1000 requirements for the environmental risk assessment of GTMPs EMEA/CHMP/GTWP/125491/2006,
1001 unless otherwise justified in the application on the basis of the type of product concerned.

1002 For pharmacokinetic studies only validated nucleic acid amplification technology (NAT) assays should
1003 be used to investigate tissue distribution and persistence of the GTMP. Applicants should justify the
1004 selection of assays and their specificity and sensitivity.

1005 **5.4.1 Biodistribution studies**

1006 Biodistribution, persistence, and clearance of administered GTMP

1007 The dosing used for biodistribution studies should mimic the clinical use with appropriate safety
1008 margins, e.g., 10-fold the clinical dose adjusted to the animal model used. The route of administration
1009 and the treatment regimen (frequency and duration) should be representative for the clinical use. In
1010 addition, evaluation of biodistribution of the GTMP after a single administration may add information on
1011 the clearance of the administered GTMP.

1012 Intravenous administration of the GTMP resulting in maximal systemic exposure may be included in the
1013 biodistribution studies as a worst-case-scenario.

1014 The sampling time points and frequency should be chosen in a way that allows determining both the
1015 maximum level of administered GTMP present at target and non-target sites and its clearance over
1016 time. The duration of the study should rely on an observation time until there is no signal detection or
1017 until a long-term signal plateau phase is reached. All relevant organs and tissues should be harvested
1018 and investigated for presence and clearance of the administered GTMP.

1019 If the administered nucleic acid is detected in unintended tissues/organs using a NAT-based assay, it
1020 may be helpful to determine expression of the gene product as well as its duration and level of
1021 expression using RT-NAT, immunological-based assays and/or assays to detect functional protein.

1022 If the administered vector is replication competent, biodistribution studies should be designed to cover
1023 a second viremia as a result of replication of the vector/virus *in vivo*. If the animal model used does
1024 not support *in vivo* replication of the vector/virus, replication could be mimicked by repeated
1025 administration of the GTMP.

1026 Any specific characteristic of the GTMP with potential influence on biodistribution such as latency /
1027 reactivation or vector DNA mobilisation has to be taken into consideration for the design of
1028 biodistribution studies.

1029 Genomic intended- integration

1030 In the cases where the whole vector (e.g. retroviruses or lentiviruses) or part of it (e.g. chimeric
1031 vectors with retroviral/lentiviral portions) is intended for integration in the host genome, this feature of
1032 the vector should be studied by integration studies (*ex vivo* tissue culture or *in vivo*). Integration
1033 studies should focus, at least, on the following issues, unless justified:

- 1034 • Tissues/organs where the integration takes place. It is important to monitor not only the
1035 intended targets, but also to carry out a comprehensive analysis in all tissues where

1036 biodistribution has been observed. The spatial distribution can be studied also locally after
1037 injection into solid tissues.

1038 • Copy number and localisation of the integrated vector copies in the host genome. Information
1039 should be provided regarding the frequency and localization of potential off-target integration
1040 events.

1041 • Structural integrity of the integrated vector (in particular the transgene cassette of interest), to
1042 detect rearrangements/recombination events.

1043 • Stability/persistency of the integrated vector copy/copies.

1044 • Correct targeting, off-target integration events and their probability in case targeted
1045 integration is anticipated.

1046 In the case of plasmid DNA with integrative portions (as in the case of mobile elements), they should
1047 be treated as integrative vectors.

1048 Suitable assay methods for determining vector presence and copy number of vector DNA in the
1049 genome may include nucleic acid amplification technology (NAT) and sequencing assays. The basis for
1050 any integration assay used should be described as well as the limits of sensitivity and the
1051 negative/positive controls used including its potential deficiencies. In addition to investigating the
1052 potential for integration of the nucleic acid into host cell genome, information on the potential for
1053 oncogenesis may also be obtained from *in vitro* studies using a variety of cell lines and primary target
1054 cells, if feasible, to investigate changes in cell morphology, function and behavior due to the
1055 integration events.

1056 When dealing with non-integrating vectors, applicants should investigate if unintended integration is
1057 occurring.

1058 Further guidance on genomic integration of AAV vectors is provided in the Reflection paper on quality,
1059 non-clinical and clinical issues related to the development of recombinant adeno-associated viral
1060 vectors (EMA/CHMP/GTWP/587488/2007 Rev1). Specific guidance on lentiviral vectors is available in
1061 the Guideline on development and manufacture of lentiviral vectors (CHMP/BWP/2458/03). Guidance
1062 on risk mitigation is given in the reflection paper on management of clinical risks deriving from
1063 insertional mutagenesis (EMA/CAT/190186/2012).

1064 For some aspects of GTMP a risk-based approach may be used. The approach taken to address
1065 genomic integration needs to be justified.

1066 Risk of germline transmission

1067 Administration of certain GTMPs to patients/subjects raises the possibility of vertical germline
1068 transmission of vector DNA, which needs to be investigated, unless otherwise justified, e.g. if the
1069 clinical indication and / or patient population indicate that such studies are not warranted.

1070 The risk for germ line transmission should be addressed primarily at the biodistribution level (signal in
1071 gonads, signal in gametes, semen fractionation studies and integration analysis) according to the
1072 Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors
1073 (EMA/273974/2005).

1074 **5.4.2 Shedding**

1075 Shedding is defined as the dissemination of vector/virus through secretions and/or excreta and should
1076 be addressed in animal models. While shedding should not be confused with biodistribution (i.e. spread

1077 within the body from the site of administration), it is advised to integrate shedding studies into the
1078 design of biodistribution studies or other non-clinical studies, when feasible.

1079 The aim of shedding studies is to determine the secretion/excretion profile of the virus / vector.
1080 Information collected from non-clinical shedding studies can then be used to estimate the likelihood
1081 and extent of shedding in humans and to guide the design of clinical shedding studies. It is
1082 recommended to address shedding in non-clinical studies early in development.

1083 More detailed guidance on analytical assays, sampling profiles and schedule as well as the
1084 interpretation of non-clinical shedding is provided in the ICH Considerations on general principles to
1085 address virus and vector shedding (Concept Paper EMEA/CHMP/ICH/449035/2009) and the Guideline
1086 on scientific requirements for the environmental risk assessment of gene therapy medicinal products (
1087 EMEA/CHMP/GTWP/125491/2006).

1088 **5.4.3 Other pharmacokinetic studies**

1089 The pharmacokinetic behavior of any device or structural components of a GTMP should be
1090 investigated. For example, the distribution and clearance of material used to deliver non-viral or viral
1091 vectors (e.g. cationic lipid complexing material, materials for controlled vector release) should be
1092 studied. The impact of these components on temporal and spatial distribution of the vector should be
1093 analysed, if applicable.

1094 **5.5 Toxicology**

1095 The applicant should justify the choice of endpoints and biomarkers predictive of toxicity in the animal
1096 model used.

1097 Toxicity should be assessed for the whole GTMP (virus/vector particle/delivery system, nucleic acid
1098 sequences, etc.) and for the transgene product in order to determine unwanted consequences of the
1099 distribution of the vector, its infection/transduction/transfection, the expression and biological activity
1100 of the therapeutic gene(s) and vector genes, if applicable, as well as immunogenicity or unwanted
1101 pharmacological effects.

1102 For toxicology studies appropriate dose level(s), route and methods of administration should be chosen
1103 to represent clinical use with appropriate safety margins.

1104 Depending on the nature of the GTMP, additional groups may additionally be treated intravenously as
1105 "worst case" scenario representing the effect of widespread dissemination of the GTMP.

1106 Applying a risk-based approach, the applicant should consider to include endpoints in order to address
1107 the safety profile of potential final medicinal product impurity(ies) (e.g. toxicological consequences of
1108 any unforeseen aberrant gene products and of vector-encoded proteins).

1109 **5.5.1 Toxicity study design**

1110 For GTMPs intended for single administration, single dose toxicology studies with an appropriately
1111 extended post-dose observation period shall be performed. Such studies should include endpoints
1112 covered by the Guideline on repeated-dose toxicity studies CPMP/SWP/1042/99 such as necropsy,
1113 histopathological findings and the duration and reversibility of toxicity and should focus on endpoints
1114 relevant to the characteristics of the GTMP involved. Inclusion of interim groups to be sacrificed at
1115 peak levels of biodistribution should be considered.

1116 Single dose toxicity studies for GTMPs should not be designed as acute toxicity studies since the final
1117 endpoint should not be animal death.

1118 The rationale for dose selection and choice of animal model should be justified, as expected for
1119 conventional repeat-dose toxicity. It is recommended to include in the studies a satellite control group,
1120 to improve historical data set regarding the species used, if needed.

1121 Repeated-dose toxicity studies shall be provided when multiple dosing of human subjects is intended.
1122 The mode and schedule of administration shall appropriately reflect the clinical dosing. For those cases
1123 where single dosing may result in prolonged function of the nucleic acid sequence and/or its product in
1124 humans, repeated dose toxicity studies shall be considered. In case replication kinetics of replicating
1125 vectors in animals are not reflecting the situation in humans, repeated dose toxicity studies are
1126 advisable.

1127 The duration of the single dose and repeated dose studies may be longer than standard toxicity studies
1128 for other bio-pharmaceuticals, depending on the persistence of the GTMP, level and site of expression
1129 and the anticipated potential risks. A justification for the duration of the studies shall be provided as
1130 well as the duration of the recovery phase investigations which should rely on the persistence of the
1131 vector and the transgene expression.

1132 The use of one relevant species for the single and repeat dose toxicity studies may be sufficient unless
1133 specific safety concerns require the use of a second animal species.

1134 **5.5.2 Genotoxicity**

1135 Genotoxicity studies might be required depending on the nature of the GTMP. The objectives of such
1136 studies can be addressed by a 3 step approach as follows:

1137 1) To investigate occurrences of genomic modification and detect any subsequent abnormal cell
1138 behavior;

1139 2) To evaluate toxicity issues due to insertional mutagenesis and investigate the mechanism driving
1140 these adverse toxicity effects;

1141 3) To identify/characterise genomic integration sites (IS) and evaluate possible cross-talk between the
1142 transgenic and neighboring sequences.

1143 **5.5.2.1 Overall Safety Considerations**

1144 Genotoxicity issues, including insertional mutagenesis and consequent carcinogenesis shall be
1145 evaluated carefully in relevant *in vitro/in vivo* models. If a positive finding occurs, additional testing
1146 will be needed to ensure the safety of the product before its first administration to humans. The
1147 investigational plan to meet these objectives should be clearly identified in accordance with the type of
1148 the product that will be developed and used.

1149 In these studies, standard genotoxicity assays are generally not appropriate but may be required to
1150 address a concern about a specific impurity or a component of the delivery system, e.g. complexing
1151 material (directive 2009/120/EC; Annex I, Part IV). Particularly, the use of some type of genotoxicity
1152 testing as out-lined in ICH S2 may be necessary to rule out any possible genotoxic effect that might be
1153 attributed to elements present in the formulated final drug product.

1154 Insertional mutagenesis by genomic integration of vector DNA can lead to several scenarios including
1155 altered expression of host genes (activation/inhibition), their inactivation (destruction of the ORF),
1156 activation/repression of neighboring silent/active genes, and generation of a new entity encoding an

1157 active fusion protein. Insertional mutagenesis may have different outcomes. It may not impact cell
1158 growth or it may induce growth advantage or disadvantage.

1159 Insertional mutagenesis could be addressed in *in vitro* and/or *in vivo* studies which shall be designed to
1160 investigate any adverse effects induced by this genetic modification. Performing genotoxicity studies in
1161 established cell lines, primary cells, or animal models shall be considered to be able to estimate the
1162 safety profile of any GTMP.

1163 **5.5.2.2 Vector-Specific Consideration**

1164 The potential for integration of the transgene expression cassette into the host genome should be
1165 investigated and discussed both where it is intended and inherent to the method of expression (e.g.
1166 when retroviral/lentiviral vectors are used), and in cases where integration is not intended (e.g. when
1167 adenoviral or plasmid vectors are used).

1168 Requirement for genotoxicity studies of GTMP with host-DNA integrative capacity will depend on the
1169 way the final product will be delivered (local versus systemic), to which tissue/organ the GTMP will be
1170 targeted and the biological status of the cells to be targeted.

1171 For GTMPs containing an active pharmaceutical ingredient that is not intended for integration, data
1172 from *in vivo* or *in vitro* studies that detect integration may still be required to rule out any possible
1173 safety concern. When expression of a therapeutic gene is lasting over a prolonged period of time, the
1174 persistence of the GTMP and likely the integration of the DNA vector into the genome shall be carefully
1175 investigated. If integration is being confirmed, copy number determination, IS identification, and any
1176 subsequent adverse biological effects and change in cell behavior monitoring shall be performed.
1177 Depending on the nature of the vector used extended *in vitro* and *in vivo* assays addressing insertional
1178 oncogenesis may be warranted before first administration in human.

1179 Genetically modified microorganisms (e.g. Lactobacillus, Salmonella, bacteriophages) can be
1180 considered out of the scope because of the unlikelihood of safety problem raised by DNA transfer and
1181 integration into the host cell genome.

1182 The inability to predict the genotoxic risk of a GTMP simply on the basis of the choice of vector and the
1183 total integration load in the cells arises from the lack of comprehensive understanding of all factors
1184 that determine whether a cell bearing a genotoxic insertion remains established *in vivo*, and whether
1185 its outgrowth eventually progresses to malignancy. Theoretical risks associated with the potential of
1186 vector integration into the human genome should be always taken into account.

1187 Reference is made to the reflection paper on management of clinical risks deriving from insertional
1188 mutagenesis (EMA/CAT/190186/2012).

1189 **5.5.3 Tumorigenicity**

1190 Standard lifetime rodent carcinogenicity studies are usually not required in the non-clinical
1191 development. However, depending on the type of product, the tumourigenic and oncogenic potential
1192 shall be investigated in relevant *in vivo/in vitro* models for neoplasm signals, oncogene activation or
1193 cell proliferation index.

1194 The decision whether the tumorigenic or oncogenic potential of a GMTMP needs to be investigated should
1195 be guided by the Weight of Evidence (WoE) approach according to ICH S6 Carcinogenicity and should
1196 take into consideration the following outcomes:

- 1197 1. Knowledge of intended drug target and pathway pharmacology (e.g. issues with growth factor
1198 transgene).
- 1199 2. Target and pathway related mechanistic/pharmacologic and known secondary pharmacologic
1200 characteristics relevant for the outcome of tumorigenicity studies and the prediction of potential
1201 human oncogenes;
- 1202 3. Potential genetic insertional mutagenesis study results;
- 1203 4. Histopathologic evaluation of repeated dose toxicology studies such as histopathologic findings of
1204 particular interest including cellular hypertrophy, diffuse and/or focal cellular hyperplasia, persistent
1205 tissue injury and/or chronic inflammation, preneoplastic changes and tumors;
- 1206 5. Evidence of hormonal perturbation;
- 1207 6. Immune suppression: a causative factor for tumorigenesis in humans;
- 1208 7. Special studies and endpoints: Data from special staining techniques, new biomarkers, emerging
1209 technologies and alternative test systems can be submitted with scientific rationale to help explain or
1210 predict animal and/or human tumorigenic pathways and mechanisms when they would contribute
1211 meaningfully.

1212 **5.5.4 Other toxicity studies**

1213 *Immunogenicity and immunotoxicity*

1214 Delivery of GTMPs can result in immune responses of the innate (systemic cytokine elevations,
1215 multiorgan inflammation) and adaptive immune system (antibodies against the vector and transgene
1216 product, cytotoxic lymphocytes raised against transfected cells, cytokine-secreting T lymphocytes
1217 specific for the transgene). Many parameters can significantly influence the innate and adaptive
1218 responses towards various GTMPs such as host-factors (prior exposure to virus and/or transgene
1219 product, maturity of the immune system), gene transfer protocols (type of the delivery system, route
1220 of transgene delivery), transgene delivery vehicle (type of viral vector, serotype, and type of transgene
1221 promoter) and the transgene product. These aspects should be considered by the applicant during the
1222 non-clinical development.

1223 Special care should be addressed to complement activation and its consequences. Risk of cross-
1224 reactive or bystander autoimmune responses should be also considered. If repeat-dose administration
1225 can lead to complement activation, markers of the complement activation should be investigated in the
1226 animal and human sera.

1227 **5.5.5 Reproductive and developmental toxicity**

1228 Studies on the effects on fertility and general reproductive function shall be provided according to ICH
1229 S5 (R2). If the risk for germ line transmission cannot be unequivocally determined according to
1230 principles as described in the Guideline on non-clinical testing for inadvertent germline transmission of
1231 gene transfer vectors (EMA/273974/05), then breeding studies should be performed in order to
1232 directly address whether the administered nucleic acid is being transmitted to the offspring. In
1233 addition, the time course of spermatogenesis and oocyte maturation, respectively, will have to be
1234 carefully considered when performing breeding studies.

1235 Embryo-foetal and perinatal toxicity studies and germline transmission studies shall be provided,
1236 unless otherwise duly justified in the application on the basis of the type of product concerned.

1237 Similarly, embryo-foetal and perinatal toxicity studies may be required if women of child-bearing
1238 potential are to be exposed to GTMPs, depending on the clinical use and clinical population in order to
1239 investigate the effect on the foetus such as local cytokine production placenta transfer. Such animal
1240 studies may not be necessary in early development.

1241 **5.5.6 Local tolerance**

1242 Local tolerance studies may be relevant for some GTMPs, depending on their type, route and protocol
1243 of administration (e.g. intra-ocular, intramuscular, intravenous, etc...). If the proposed clinical
1244 formulation and route of administration have been examined in other animal studies, then separate
1245 local tolerance studies are not necessary. If needed, they can be addressed as part of the general
1246 toxicity study and follow the Note for guidance on non-clinical tolerance testing of medicinal products
1247 (CPMP/SWP/2145/00).

1248 **5.6 Drug interactions**

1249 As for any other medicinal products, the effects of co-medications should be investigated on a case by
1250 case basis since it can affect infection efficacy of the vector, therapeutic gene expression, biological
1251 activity of the expressed proteins and tissue distribution of the vector. For instance, if clearance of the
1252 vector/virus may be altered under an immunosuppressive co-treatment and therefore this point has to
1253 be addressed. Moreover, effects of a GTMP including inflammation or cytokine release in liver may
1254 impact liver metabolism of co-administered pharmaceuticals.

1255

1256 **6. Clinical Development**

1257 **6.1 General Considerations**

1258 In general, for GTMP the same principles as for any other medicinal products apply for the clinical
1259 development, especially current guidelines relating to specific therapeutic areas. Of note, GCP
1260 requirements also apply. Any deviation from existing guidelines needs to be justified. For new
1261 therapeutic indications/conditions where limited guidance exists, consultation of national regulatory
1262 authorities and/or EMA for scientific advice on the clinical development plan, including the confirmatory
1263 studies, is recommended.

1264 The choice of the vector should be justified with regards to the tropism of the wild type
1265 virus/bacterium. The indication and the therapeutic concept as well as the target organ/cells will
1266 influence the choice of the vector.

1267 In view of the complexity, the potential benefits and risks of such GTMP approach versus existing
1268 treatment should be discussed in the clinical overview (e.g. factor IX GTMP vs. factor IX).

1269 The design of clinical trials should address the requirements described below.

1270 There may be situations where full compliance to this guideline is not possible. In such cases, proper
1271 justification is expected that includes where feasible alternative approaches for obtaining comparable
1272 information.

1273 All studies should be adequately planned to allow assessment of the feasibility and risks of the gene
1274 therapy approach, carefully balancing the need for retrieving information with respect and protection
1275 for vulnerable patients. The absence of control groups in the clinical design should be justified based
1276 on the disease and the GTMP under investigation. In cases where randomised controlled clinical trials
1277 are not feasible, alternatives (e.g. well documented natural history data or using the patients as their
1278 own control) might be acceptable if appropriately justified and the caveats of using these alternatives
1279 should be discussed. The ICH E10 on choice of control groups in clinical trials (CPMP/ICH/364/96)
1280 should be consulted.

1281 Also, certain conditions targeted for treatment with a GTMP are extremely rare. In such cases, the
1282 guideline on clinical trials in small populations (CHMP/EWP/83561/2005) should be consulted. However
1283 it should be noted that the database on the recruited patients should be as complete as possible; in
1284 order to compensate for the overall shortened/limited clinical development.

1285 Long term monitoring of patients treated with a GTMP is of particular importance, given also the legal
1286 requirement of long term efficacy and safety follow up (according to (EC) Regulation No 1394/2007).
1287 Those studies should be appropriately designed (e.g. sampling plan, sample treatment, analytical
1288 methods, endpoints) in order to maximise information output especially when invasive methods are
1289 used. This is of specific importance when the GTMP is intended to provide life-long persistence of
1290 biological activity and treatment effects (e.g. genetic disease, see Guideline on follow up of patients
1291 administered with GTMPs, EMEA/CHMP/GTWP/60436/2007).

1292 Applicants are advised to develop and validate methods for patient monitoring as early as possible
1293 during clinical development.

1294 Patients screening/eligibility:

1295 In case it is foreseen to apply a live vector, the patients have to be evaluated for immunosuppression
1296 e.g. HIV status, intake of immunosuppressant's.

1297 The pre-existing immunity to the GTMP, i.e. neutralizing antibodies to the vector-derived wild type
1298 virus, and its potential consequences should be addressed prior to clinical administration.

1299 Special populations

1300 Special populations, like children and elderly, should be considered when developing a GTMP. For
1301 example, immunogenicity to a viral vector may vary between children and adults, depending on the
1302 pre-existing exposure to the virus. Yet, as GTMP development is indication and product-specific, no
1303 specific guidance can be given regarding the extent of data to be generated in children and elderly.

1304 The target population might be vulnerable such as pregnant women or children. When the medicinal
1305 products are likely to be of significant clinical value in such populations, robust evidence from the non-
1306 clinical development program should be available to support the safe use in the target population.

1307 In case a GTMP is indicated for use in pregnant women, careful ante-natal monitoring of mother and
1308 foetus should be conducted. In addition, post-partum long term follow-up of the child and the mother
1309 shall be performed.

1310 For children, long-term effects of administration of the GTMP should be specifically considered and
1311 monitored adequately, as defined in (EC) Regulation 1901/2006 (paediatric Regulation) and relevant
1312 paediatric guidelines.

1313 **6.2 Pharmacokinetic studies**

1314 Classical pharmacokinetic studies based on absorption, distribution, metabolism and excretion (ADME)
1315 studies are usually not required for GTMPs but might be relevant in some cases (e.g. oncolytic
1316 viruses). However they are required when the gene product is a protein or another molecule affecting
1317 protein metabolism.

1318 It is expected that the following studies will be carried out:

1319 (a) Usually, shedding studies are required to address the excretion of the GTMPs. Investigations of
1320 shedding and risk of transmission to third parties shall be provided with the environmental risk
1321 assessment, unless otherwise justified in the application on the basis of the type of product concerned.

1322 (b) When possible, dissemination in the body including investigations on persistence, clearance and
1323 mobilisation of the gene therapy vector could be investigated. Biodistribution studies shall additionally
1324 address the risk of germline transmission.

1325 (c) Finally, pharmacokinetic studies of the medicinal product and the gene expression moieties (e.g.
1326 expressed proteins).

1327 For oncolytic viruses specific guidance is provided in ICH considerations on oncolytic viruses.

1328 **6.2.1 Shedding studies**

1329 Shedding studies to address the excretion of the GTMP should be performed. When shedding is
1330 observed, the potential for transmission to third parties might need to be investigated, if relevant (e.g.
1331 with replication competent vectors/oncolytic viruses). The ICH Considerations General Principles to
1332 Address Virus and Vector Shedding (EMA/CHMP/ICH/449035/2009) and the guideline on
1333 environmental risk assessment provide comprehensive recommendations for the design of shedding

1334 studies as well as the interpretation of clinical data in assessing the need for virus / vector
1335 transmission studies. Those data also contribute to appropriate planning of the long term follow up
1336 program.

1337 When there is a risk of shedding through the seminal fluid, at least two means of contraception –
1338 including barrier contraception should be recommended.

1339 **6.2.2 Dissemination studies**

1340 The cell tropism, the route of administration, the target organ/cells, the vector type and the indication
1341 as well as the clinical feasibility and ethical acceptability should be taken into consideration when
1342 designing dissemination studies (e.g. choosing the target and non-target organs/cells/body fluids).

1343 Also special attention should be paid when a GTMP will be applied under conditions in which an
1344 impaired blood brain barrier integrity can be expected.

1345 Invasive techniques (e.g. biopsies, fluid collection) may not always be feasible and ethically
1346 appropriate. Thus the use of other less invasive techniques (e.g. imaging techniques) might prove
1347 useful in some cases to study GTMP dissemination whenever possible.

1348 Special attention should be paid to the use for a replication-competent GTMP. In such cases, the
1349 patients should be monitored for clinical signs of productive infection with replication competent vector
1350 or for signs of unwanted dissemination.

1351 **6.2.3 Pharmacokinetic studies of the medicinal product and of the gene 1352 expression moieties (e.g. expressed proteins or genomic 1353 signatures).**

1354 If appropriate, conventional pharmacokinetic studies, including as a minimum determination of
1355 (plasma) concentration and half-life, should be performed for the therapeutic gene product (i.e.
1356 therapeutic protein); in some cases there might be a need to assess this also for other vector genes
1357 expressed in vivo as shown in non-clinical studies.

1358 For gene expression products such as enzymes or prodrugs, differences in their kinetics and
1359 elimination depending on genetic polymorphism should be taken into consideration.

1360 For the treatment of genetic diseases by gene correction/addition strategies, the therapeutic effects of
1361 the product on different causative gene mutations should be investigated. The potential interference of
1362 residual endogenous proteins with the therapeutic product should be addressed. For example, the
1363 presence of endogenous proteins coded by genes with hypomorphic or dominant negative mutations
1364 may interfere with the half-life and function of the protein product expressed from the delivered gene
1365 and thus respective effects should be carefully considered.

1366 **6.3 Pharmacodynamic studies**

1367 Pharmacodynamic (PD) studies are performed to study the function and/or expression of the
1368 therapeutic nucleic acid sequence. In most cases of GTMP, PD studies address the expression and
1369 function of the gene expression product (e.g. as a protein or enzyme, including conversion of prodrugs
1370 by therapeutic enzymes or induction of immune response) while in other cases the effect of the vector
1371 itself is addressed (e.g. recombinant oncolytic virus).

1372 The selected PD markers should be relevant to demonstrate therapeutic efficacy of the product and in
1373 cases where the PD effects are proposed as surrogate efficacy endpoints this needs to be justified. The
1374 proposed PD marker should be linked to clinical benefit.

1375 **6.4 Dose selection and schedule**

1376 In general, the dose response effect should be evaluated, reference is made to ICH E4 Dose response
1377 information to support drug registration (CPMP/ICH/378/95). When a classical dose finding is not
1378 possible, a minimal effective dose and a maximum tolerable dose may provide useful information on
1379 the relationship between exposure and effect.

1380 **6.5 Immunogenicity**

1381 Prior infection/vaccination with related viruses may affect the safety and efficacy of the GTMP (e.g.
1382 adenoviruses, poxviruses (smallpox vaccine), thus the immune response to the vector should be
1383 evaluated. An immune response to the transgene product might eventually compromise the efficacy of
1384 the product and might have an impact on safety. Thus, evaluation of the immune response to the
1385 transgene product should also be part of the clinical development.

1386 In case repeated administration of the GTMP is foreseen, a comprehensive evaluation of the immune
1387 response to the vector and the transgene product has to be performed. This includes the evaluation of
1388 the cellular and humoral immunity to the vector as well as to the transgene product (e.g. titer and
1389 avidity of antibodies and information on whether the antibodies are neutralising or not). The results
1390 should be documented in relation to the timing of the treatments and correlation of the
1391 immunogenicity results with concurrent safety and efficacy should be provided.

1392 **6.6 Efficacy**

1393 Existing guidelines for the specific therapeutic area should be followed (e.g. cancer, rare diseases)
1394 with regards to study design (e.g. choice of endpoints, choice of comparator, inclusion/exclusion
1395 criteria). Any major deviation(s) from these guidelines should be justified.

1396 The efficacy studies should be designed to demonstrate efficacy in the target population, to support
1397 the proposed posology, and to evaluate the duration of the therapeutic effect of the GTMP.

1398 Clinically meaningful endpoints to demonstrate efficacy are generally required. However in certain
1399 situations (e.g. threshold of FIX or FVIII in case of haemophilia) a validated surrogate parameter as
1400 clinical endpoint might be considered an acceptable alternative, if properly justified. However a clinical
1401 meaningful endpoint has to be investigated in the long term follow up (see guideline on long term
1402 efficacy follow up EMEA/CHMP/GTWP/60436/2007).

1403 Another important aspect is the timing of the efficacy assessment which may be different to
1404 conventional medicinal products and therefore the schedule of clinical evaluation should be planned
1405 accordingly.

1406 If the intended outcome of the treatment is the long-term persistence and functionality of the
1407 transgene expression product (e.g. genetic diseases); this should be reflected with an adequate
1408 duration of follow-up. The design and duration of follow-up has to be specified also considering
1409 potential loss of efficacy and might be completed, post-marketing if justified.

1410 **6.7 Clinical safety**

1411 A safety database should be set up including any adverse events which are linked to the transgene
1412 product and/or to the vector or the transduction mechanism.

1413 Risks of the administration procedure, e.g. invasive procedures to administer the GTMP (e.g. multiple
1414 injection, intra cerebral application), the use of general or regional anesthesia or the use of
1415 immunosuppressive and chemotherapeutic therapy should be addressed.

1416 Special consideration should be taken in the design of the clinical study and risk evaluation when
1417 Medical Devices (MD) are used for the delivery or implant of a combined GTMP. The medical device
1418 effect should be evaluated in the intended use of the combined ATMP. The use of the medical device
1419 with the GTMP should be adequately explained in the Product Information.

1420 In case of an anticipated risk including events with a late onset (e.g. tumourigenicity), measures to
1421 detect the signal and to mitigate this risk should be implemented.

1422 Particular attention should be paid to:

1423 • Infusion-related reactions

1424 Short term tolerability after administration of the GTMP such as infusion-related reactions to the vector
1425 itself or any compound of the product, should be considered.

1426 • Infection and inflammatory responses

1427 Infection related events caused by the presence or appearance of replication competent viruses, the
1428 recombination of the vector with pathogenic strains, the change of tropism.

1429 • Malignancy

1430 Several factors might contribute to tumour development in patients treated with a GTMP. These factors
1431 include product related factors e.g. insertional mutagenesis, altered expression of host genes, the
1432 transgene products itself e.g. growth factors or factors linked to the treatment procedure such as
1433 immunosuppressant therapy or chemotherapy. If malignancy occurs after treatment, a potential link
1434 with the GTMP should be investigated taking into consideration both molecular and biological
1435 characteristics of the GTMP.

1436 • Immune mediated adverse effects

1437 Immune response to the vector itself as well as to the transgene product might lead, in some cases, to
1438 clinical consequences. Applying exogenous transgene product might result in break of tolerance to the
1439 endogenous protein counterpart if present.

1440 • Any unintended transduction of tissues

1441 By nature the vector might have a specific tissue/cell tropism. However unintended transduction of
1442 non-target tissues might occur. Information on the tissue specificity of the virus the vector is derived
1443 of, the focusing on specific target according to the vector type as well as the biodistribution obtained
1444 with the actual GTMP, and the experience with similar GTMPs products should be provided. In case
1445 non-target specific tropism occurs, appropriate monitoring for the clinical consequences of such non-
1446 target tissue transduction should be in place.

1447 • Retention samples

1448 Samples of plasma and tissue/cells of the study subjects should be stored for a sufficient period of time
1449 after the finalisation of the clinical trials in case further investigations are needed to be performed

1450 regarding presence of adventitious agents, autoimmunity and vector integration studies. The duration
1451 of storage is depending on patient population/disease.

1452 **6.8 Pharmacovigilance and Risk Management Plan**

1453 The rules for routine pharmacovigilance (including immediate or periodic reporting) are described
1454 respectively in Volume 10 of the Rules governing medicinal products in the European Union for gene
1455 therapy investigational products, and in the *Guideline on good pharmacovigilance practices (GVP)*.

1456 The EU Risk Management Plan (RMP) requirements are described in the *GVP Module V – Risk*
1457 *management systems* and the template included in the *Guidance on format of the risk-management*
1458 *plan in the European Union* apply.

1459 Careful consideration should be given to efficacy and safety specifications and follow up as GTMPs need
1460 adequately designed long-term studies to monitor specific efficacy and safety issues, including loss of
1461 efficacy. Safety issues, such as infections, immunogenicity/immunosuppression and malignant
1462 transformation, as well as long term efficacy should be addressed in the Risk Management Plan, in the
1463 dedicated chapter RMP module SVII "Identified and potential risks (ATMP version)".

1464 A potentially limited size of the safety database and the need for clinical follow up should also be
1465 addressed. Specific pharmaco-epidemiological studies may be needed. Those requirements are
1466 dependent on the vector type and the biological function of the transgene.

1467 When a GTMP is combined with a medical device, information linked to the safety of the medical device
1468 should be provided (e.g. medication errors, dose delivery, etc...).

1469 **7. DEFINITIONS**

1470 Gene therapy medicinal³ product means a biological medicinal product which has the following
1471 characteristics:

1472 (a) it contains an active substance which contains or consists of a recombinant nucleic acid
1473 used in or administered to human beings with a view to regulating, repairing, adding or
1474 deleting a genetic sequence;

1475 (b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic
1476 acid sequence it contains, or to the product of genetic expression of this sequence.

1477 Gene therapy medicinal products shall not include vaccines against infectious diseases.

1478 Oncogenicity: the cause of producing tumours.

1479 Tumourigenicity: the capacity to induce tumours.

³ Definition in Annex to Directive 2009/120/EC, Part IV, 2.1.

Annex to Directive 2009/120/EC, Part IV, 3.2.: Specific requirements for gene therapy medicinal products:

3.2.1.1. Gene therapy medicinal products containing recombinant nucleic acid sequence(s) or genetically modified microorganism(s) or virus(es) The finished medicinal product shall consist of nucleic acid sequence(s) or genetically modified microorganism(s) or virus(es) formulated in their final immediate container for the intended medical use. The finished medicinal product may be combined with a medical device or active implantable medical device.

The active substance shall consist of nucleic acid sequence(s) or genetically modified microorganism(s) or virus(es).

1480 **8. REFERENCES**

- 1481 Guideline on the risk based approach (EMA/CAT/686637/2011)
- 1482 Guideline on environmental risk assessments for medicinal products consisting of, or containing,
1483 genetically modified organisms (GMOs)" (EMEA/CHMP/BWP/473191/2006-corr)
- 1484 Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically
1485 modified cells" (CHMP/GTWP/671639/2008)
- 1486 Guideline on the use of bovine serum" (CPMP/BWP/1793/02)
- 1487 ICH Q5D guideline on derivation and characterisation of cell substrates
- 1488 Ph. Eur. 5.14
- 1489 Guideline on Excipients in the Dossier for Application for Marketing Authorisation of a Medicinal Product
1490 (EMEA/CHMP/QWP/396951/2006)
- 1491 Guideline on non-clinical studies required before first clinical use of Gene Therapy medicinal products
1492 (EMA 125459/2006)
- 1493 Guideline on strategies to identify and mitigate risks for first-in-human clinical trials within
1494 investigational medicinal products (EMEA/CHMP/WP/28367/07)
- 1495 ICH M3
- 1496 ICH S6
- 1497 ICH S8
- 1498 Guideline on development and manufacture of lentiviral vectors (CHMP/BWP/2458/03)
- 1499 Reflection paper on management of clinical risks deriving from insertional mutagenesis
1500 (EMA/CAT/190186/2012).
- 1501 Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors
1502 (EMEA/273974/2005)
- 1503 ICH Considerations on Oncolytic viruses (EMEA/CHMP/GTWP/607698/2008)
- 1504 ICH considerations: oncolytic viruses" (CHMP/ICH/607698/08)
- 1505 Draft reflection paper on quality, non-clinical and clinical issues relating specifically to recombinant
1506 adeno-associated viral vectors (EMEA/CHMP/GTWP/587488/2007)
- 1507 Guideline on follow-up of patients administered with gene therapy medicinal products"
1508 (EMEA/CHMP/GTWP/60436/2007)
- 1509 Guideline on safety and efficacy follow-up - risk management of advanced therapy medicinal products"
1510 (Doc. Ref. EMEA/149995/2008)
- 1511 ICH E4 Dose response information to support drug registration (CPMP/ICH/378/95)
- 1512 Guideline on good pharmacovigilance practices (GVP)
- 1513 ICH Considerations: General Principles to Address Virus and Vector Shedding (CHMP/ICH/449035/09)
- 1514 Guideline on scientific requirements for the environmental risk assessment of gene therapy medicinal
1515 products (EMEA/CHMP/GTWP/125491/2006).

1516 Guideline on clinical trials in small populations CHMP/EWP/83561/2005

1517 Directive 2001/118/EC on the deliberate release into the environment of genetically modified
1518 organisms and repealing Council Directive 90/220/EEC.