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4 **Guideline on quality, non-clinical and clinical requirements**  
5 **for investigational advanced therapy medicinal products**  
6 **in clinical trials**  
7 **Draft**

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<b>Keywords</b>	<b><i>Investigational ATMPs, Gene therapy medicinal product, Cell therapy medicinal product, tissue engineered product, Exploratory trial, First in human trial, Confirmatory trial</i></b>
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## 55 **Executive summary**

56 The guideline provides guidance on the structure and data requirements for a clinical trial application  
57 for exploratory and confirmatory trials with advanced therapy investigational medicinal products  
58 (ATIMPs).

59 The guideline is multidisciplinary and addresses development, manufacturing and quality control as  
60 well as non-clinical and clinical development of ATIMPs. Considerations on genome editing tools are  
61 included.

62 Throughout the guideline, requirements for exploratory trials (including First in Human studies) and  
63 confirmatory trials are described. The main focus is however on the requirements for exploratory trials.

## 64 1. Introduction (background)

65 Advanced therapy medicinal products (ATMPs<sup>1</sup>) comprise gene therapy, somatic cell therapy medicinal  
66 products and tissue engineered products. Scientific knowledge on gene and cell-based therapy  
67 products is rapidly expanding, and in order to ensure that reliable data are generated on these  
68 complex products, well conducted clinical trials are essential to determine their benefit risk profile.

69 Cell-based medicinal products are heterogeneous with regard to the origin and type of the cells and to  
70 the complexity of the product. Cells can be of human (autologous or allogeneic) or animal origin and  
71 may be self-renewing stem cells, more committed progenitor cells or terminally differentiated cells  
72 exerting a specific defined physiological function. In addition, the cells may also be genetically modified  
73 with newly established genotype/phenotype for the intended therapeutic effect. The cells may be used  
74 alone, associated with biomolecules or other chemical substances or combined with structural  
75 materials that alone might be classified as medical devices (combined advanced therapy medicinal  
76 products).

77 Gene therapy medicinal products generally consist of a vector or delivery formulation/system  
78 containing a genetic construct engineered to express a specific transgene (therapeutic sequence) for  
79 the regulation, repair, replacement, addition or deletion of a genetic sequence. By using such gene  
80 therapy constructs *in vivo*, genetic regulation or genetic modification of somatic cells can be achieved  
81 in situ. The same gene therapy vector can be used *ex vivo* for the manufacture of genetically modified  
82 cells. Quality aspects of vector and cell-based products need to be considered for the development of  
83 products consisting of genetically modified cells.

84 Historically many gene therapy approaches have been based on expression of a transgene encoding a  
85 functional protein (i.e. a transgene product). Newer tools are under development that modify or edit  
86 directly the cellular genome *in vitro* or even *in vivo*. In both cases, the respective tools may be  
87 delivered by a viral vector or by a non-viral approach.

88 In general, the development of an ATMP should follow the same general principles as other medicinal  
89 products. However, it is acknowledged that the distinctive characteristics and features of ATMPs are  
90 expected to have an impact on product development. This guideline will help the developers of ATMPs

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<sup>1</sup> Abbreviations used throughout this guideline:

ATMP – Advanced Therapy Medicinal Product

ATIMP – Advanced Therapy Investigational Medicinal Product

CBIMP – Cell Based Investigational Medicinal Product

ERA – Environmental Risk Assessment

FIH – First in Human

GCP – Good Clinical Practice

GMP – Good Manufacturing Practice

GMO – genetically modified organism

GTIMP – Gene Therapy Investigational Medicinal Product

ICH - International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

IMP – Investigational Medicinal Product

IMPD – Investigational Medicinal Product Dossier

Ph.Eur. – European Pharmacopoeia

91 to design their development programme. The developers are encouraged to seek early advice at the  
92 national or European level to guide product development.

### 93 Risk-based approach

94 In determining the content of the IMPD, a risk-based approach can be applied<sup>2</sup>. The content of the  
95 dossier can be adapted having regard to the identified risks. In particular, the applicant can perform at  
96 the beginning of product development an initial risk analysis based on existing knowledge on the type  
97 of product and its intended use. Aspects to be taken into consideration include the origin of the cells,  
98 the type of vector and/or the method used for the genetic modification, the manufacturing process, the  
99 non-cellular components and the specific therapeutic use as applicable.

100 The risk analysis should be updated by the applicant throughout the product life cycle as new data  
101 become available. Key points relevant to the understanding of the product development approach  
102 chosen, should be summarized in the IMPD.

103 In deciding on the appropriate measures to address the identified risks, the priority should be the  
104 safety of subjects enrolled in the trial. The Guideline on strategies to identify and mitigate risks for  
105 First-in-Human Clinical Trials with Investigational Medicinal Products (Doc. Ref.  
106 EMEA/CHMP/SWP/294648/2007) excludes ATMPs but its principles are nevertheless also useful in the  
107 design of first-in-human (FIH) trials with advanced therapy investigational medicinal products  
108 (ATIMPs). The increasing regulatory expectations along with advancing clinical development are  
109 discussed in section 4.

110 The level of effort and documentation should be commensurate with the level of risk. The application of  
111 a risk-based approach can facilitate compliance but does not obviate the applicant's obligation to  
112 demonstrate the quality and safety of the product to enable the generation of reliable efficacy data. It  
113 likewise does not replace appropriate communications with the authorities.

114 An immature quality development may compromise the use of the study in the context of a marketing  
115 authorisation application (e.g. if the product has not been adequately characterised). A weak quality  
116 system may also compromise the approval of the clinical trial if the safety of trial subjects is at risk.

### 117 Changes during the clinical trial

118 Where changes to the clinical trial dossier become necessary during an ongoing clinical trial, it is the  
119 sponsor's responsibility to evaluate whether the change is substantial or non-substantial. If the change  
120 is deemed substantial, a submission to the competent authorities should be made prior to  
121 implementation thereof.

## 122 **2. Scope**

123 The guideline provides guidance on the structure and data requirements for a clinical trial application  
124 for advanced therapy investigational medicinal products (ATIMPs). The guideline is multidisciplinary  
125 and addresses development, manufacturing and quality control as well as non-clinical and clinical  
126 development of ATIMPs. Considerations on genome editing tools are included.

127 Clinical trial phases in ATMP development are usually not as clear-cut as they might be for other  
128 product types. Therefore distinction is made between exploratory trials and confirmatory trials, where  
129 the latter are performed to obtain pivotal data for a marketing authorisation application (MAA). First-  
130 in-human (FIH) studies constitute a subtype of exploratory trials where a given medicinal product is

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<sup>2</sup> Specific guidance is given in the Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to ATMPs

131 given to human study participants for the first time. The requirements for exploratory trials are the  
132 main focus of this guidance. For confirmatory trials, developers should also take into consideration  
133 existing relevant guidelines outlining marketing authorisation requirements.

134 This guideline does not address environmental aspects of ATIMPs that contain or consist of genetically  
135 modified organisms. Applicants should consult the specific guidelines related to ERA. Information on  
136 national requirements for clinical trials with GMOs can be found on the website of the European  
137 Commission<sup>3</sup>.

138 While extracellular vesicles and cellular fragments originating from human cells or chemically  
139 synthesised therapeutic sequences do not fulfil the definition of ATIMPs, the underlying scientific  
140 principles outlined here may be applicable. For more in depth information on classification, reference is  
141 made to the Reflection Paper on ATMP classification (EMA/CAT/600280/2010 rev 1).

### 142 **3. Legal basis**

143 This guideline should be read in conjunction with the Directive 2001/20/EC and Regulation (EU) No  
144 536/2014 on clinical trials, the ATMP Regulation (EC) No 1394/2007 and the Directive 2009/120/EC  
145 amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code  
146 relating to medicinal products for human use as regards advanced therapy medicinal products.

147 Compliance with GMP requirements as laid down in the Guidelines on Good Manufacturing Practice  
148 specific to Advanced Therapy Medicinal Products (EudraLex Volume 4) is a prerequisite for the conduct  
149 of clinical trials.

150 For those products consisting of, or containing, genetically modified organisms (GMOs) compliance  
151 with the GMO legislation is required.

152 Donation, procurement, and testing of human cell based products need to comply with the  
153 requirements of Directive 2004/23/EC or where applicable Directive 2002/98/EC.

154 In general, for ATIMPs the same principles as for other IMPs apply for the clinical development (e.g.  
155 ICH E8 General considerations for clinical trials), especially current guidelines relating to specific  
156 therapeutic areas. Of note, GCP requirements (ICH E6 Guideline for Good Clinical Practice and the  
157 Guideline on Good Clinical Practice specific to Advanced therapy medicinal products) also apply.

158 The following documents should be consulted from all clinical trials, in addition to the requirements of  
159 the European Pharmacopoeia (Ph.Eur.):

- 160 - Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal  
161 Products [https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-  
162 4/2017\\_11\\_22\\_guidelines\\_gmp\\_for\\_atmps.pdf](https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf)
- 163 - Guideline on strategies to identify and mitigate risks for First-in-Human Clinical Trials with  
164 Investigational Medicinal Products (Doc. Ref. EMEA/CHMP/SWP/294648/2007)
- 165 - Good laboratory practice (GLP) principles in relation to  
166 ATMPs [https://www.ema.europa.eu/documents/other/good-laboratory-practice-glp-principles-  
167 relation-advanced-therapy-medicinal-products-atmps\\_en.pdf](https://www.ema.europa.eu/documents/other/good-laboratory-practice-glp-principles-relation-advanced-therapy-medicinal-products-atmps_en.pdf)

168 In addition, relevant European guidelines and reflection papers provide information on the  
169 requirements at Marketing Authorisation and thus inform on the drug development process, should be

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<sup>3</sup> [https://ec.europa.eu/health/human-use/advanced-therapies\\_en](https://ec.europa.eu/health/human-use/advanced-therapies_en)

170 taken in consideration. They are partially listed below and referred to in the respective sections of this  
171 document and a cumulative listing is provided in section 8:

- 172 - Guideline on human cell-based medicinal products (EMA/CHMP/410869/2006)
- 173 - Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products  
174 (EMA/CAT/80183/2014)
- 175 - Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC  
176 applied to ATMPs (EMA/CAT/CPWP/686637/2011)
- 177 - Guideline on xenogeneic cell-based medicinal products (EMA/CHMP/CPWP/83508/2009)
- 178 - Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy  
179 Agents via Human and Veterinary Medicinal Products (EMA/410/01)
- 180 - Guideline on safety and efficacy follow-up and risk management of Advanced Therapy  
181 Medicinal Products (EMA/149995/2008 rev.1)
- 182 - Guideline on follow-up of patients administered with gene therapy medicinal products  
183 (EMA/CHMP/GTWP/60436/2007).

## 184 4. Quality documentation

185 The data on quality aspects of ATIMP should be presented in a logical structure, ideally according to the  
186 specified structure of a common technical document (CTD) such as that of Module 3. The data  
187 submitted in this module should be consistent with and complement other parts of the clinical trial  
188 submission package.

189 Data requirements evolve as development progresses from exploratory to confirmatory clinical trials:

- 190 - Quality data compiled in the IMPD are expected to reflect increasing knowledge and experience  
191 during product development. At marketing authorisation it needs to be demonstrated that the  
192 medicinal product can be produced consistently and with reproducible quality. For example,  
193 acceptance criteria for tests parameters/in-process controls, even based on limited data should  
194 be set and they should be reviewed at later stages of development.
- 195 - During development, the addition or removal of parameters and modification of analytical  
196 methods may be necessary. In all cases, the suitability of the analytical methods used should  
197 be demonstrated.

198 It is expected to conduct confirmatory clinical trials with a product based on a mature manufacturing  
199 process and specifications that match those for marketing authorisation as closely as possible.

200 Deviations from this principle will lead to comparability issues, a particular challenge for ATMPs, and  
201 may raise questions on the representativeness (validity) of the data obtained.

202 For cell-based investigational ATMPs (CBIMP), the guideline describes activities by manufacturers  
203 following procurement of the cells and tissues or blood. CBIMP often contain, or consist of cell  
204 preparations of limited size and many are intended to be used in a patient-specific manner.

205 The combination of ATMPs with medical devices may give rise to different regulatory scenarios:

- 206 - When a CBIMP incorporates a medical device as an integral part of the active substance, the  
207 medical device will be considered a starting material (see section S.2.3).
- 208 - When an ATMPs necessitates a medical device as part of the final formulation, but the medical  
209 device is not integral part of the active substance (e.g. a medical device added to the active



210 substance shortly before it is administered to the patient which is intended to provide  
211 structural support, to spatially restrict the product or control its release), the medical device  
212 will be considered an excipient (see section P.4 and Annex 3).

213 - When medical device is used as the container closure system (see section P.7) or is intended to  
214 administer an ATMP and the administration device and the ATMP are marketed as a single  
215 integral product and the device is not reusable, the combination will be regulated under the  
216 medicines framework. The latter scenario is not however specifically addressed in this  
217 guideline.

218 The traceability from the recipient of the product to the donor of the cells or tissues should be ensured.  
219 The traceability system should be bidirectional (from donor to recipient and from recipient to donor).  
220 Data should be kept for 30 years after the expiry date of the product, unless a longer time period is  
221 required in the clinical trial authorisation.

222 The requirements for traceability are without prejudice to the provision Regulation (EU) 2016/679 of  
223 the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with  
224 regard to the processing of personal data and on the free movement of such data. Therefore the  
225 system should allow full traceability from the donor to the recipient through a coding system.

## 226 **S Active substance**

227 The IMPD should be divided into a drug substance (DS)<sup>4</sup> and a drug product (DP)<sup>5</sup> section. For certain  
228 ATIMPs, the starting material, the active substance and the finished product can be closely related or  
229 nearly identical. The active substance, any intermediate and the final product should be identified, if  
230 possible. In those cases where the ATIMPs production is a continuous process, it is not necessary to  
231 repeat the information that was already provided in the DS part, into the DP section.

232 The active substance of a CBIMP is composed of the manipulated or non-manipulated cells and/or  
233 tissues. Additional substances (e.g. scaffolds, matrices, devices, biomaterials, biomolecules and/or  
234 other components) when combined as an integral part with the manipulated cells are considered part  
235 of the active substance and are therefore considered as starting materials, even if not of biological  
236 origin. Information on relevant manufacturing and control and viral safety aspect of these additional  
237 substances need to be provided.

238 The active substance of a gene therapy medicinal product based on gene transfer methods *in vivo* is  
239 composed of the recombinant nucleic acid and the viral or non-viral vector used to deliver it. In the  
240 case of *in vivo* genome editing approaches, active substances normally comprise the tools used for the  
241 intended genome edition. This can be as diverse as a recombinant nucleic acid, a recombinant protein,  
242 a synthetic oligonucleotide or RNA, a ribonucleoprotein, etc. and the viral or non-viral vectors used to  
243 deliver them. In the case of gene therapy *ex vivo* (i.e. genetically modified cells), the active substance  
244 is composed of the modified cells. The unmodified cells, the viral or non-viral vectors and any other  
245 nucleic acid and/or protein used in the genetic modification of the cells are considered starting  
246 material. The requirements for the gene/vector component should additionally be taken into  
247 consideration. In this case of *ex vivo* use, viral vectors, plasmids, recombinant proteins and  
248 recombinant mRNA, the components to produce them (e.g. plasmids, cells) are also considered  
249 starting materials. In this case, the principles of GMP, as provided in the General Principles in the  
250 Guidelines for GMP for ATMP, should be applied from the cells bank systems used to produce the  
251 starting materials, when applicable.

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<sup>4</sup> Throughout the guideline, the terminology 'active substance' and 'drug substance' are used interchangeably.

<sup>5</sup> Throughout the guideline, the terminology 'finished product' and 'drug product' are used interchangeably.

252 Of note, if the ATIMP contains additional biological/biotechnological components other than the cells,  
253 reference to an Active Substance Master File or a Certificate of Suitability (CEP) of the European  
254 Directorate for the Quality of Medicines is neither acceptable nor applicable.

## 255 **S.1. General information**

### 256 **S.1.1. Nomenclature**

257 Information concerning the nomenclature of the active substance (e.g. proposed INN-name if  
258 available, pharmacopoeial name, proprietary name, company code, other names or codes, if any)  
259 should be provided. The naming history should be included.

### 260 **S.1.2. Structure**

261 For CBIMP, a description of the active substance should be provided, including information on the cell  
262 composition. Structural components, if they are part of the active substance should be described, e.g.  
263 where cells are grown into sheets or combined with matrices/scaffolds.

264 For gene therapy investigational medicinal products (GTIMP), a description and diagrammatic  
265 representation of the construct should be given. The therapeutic sequence(s), junction regions and  
266 regulatory elements should be provided. Any sequence which has been added for targeting, regulation  
267 or expression of the GTIMP construct should be described.

### 268 **S.1.3. General properties**

269 The proposed mechanism of action should be presented and form the basis for the definition of the  
270 relevant properties of the active substance including biological activity (i.e. the specific ability or  
271 capacity of a product to achieve a defined biological effect).

272 For CBIMPs where the cellular starting materials are obtained through specific technologies (e.g.  
273 reprogramming, genetic modification, activation), the origin and the type of the initial cells,  
274 information on the processing technique together with the target function need to be provided.

275 For GTIMP composed of viral vectors, the following aspects should be described:

#### 276 a. Vector Design

277 A list of physico-chemical and other relevant properties of the GTIMP should be provided.

278 In particular the applicant should set out the rationale for the choice of vector system, in relation to  
279 the proposed clinical indication, mode of administration (*ex vivo* or *in vivo*), transfection/transduction  
280 efficiency on the target cell population, patient and user safety and the functional activity of the  
281 therapeutic sequence(s).

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

- 283 i) Pathogenicity and virulence in man and in other animal species of the parental organism;
- 284 ii) The engineering of viral vectors to render them, where necessary, replication defective;
- 285 iii) Steps taken to minimise the possibility of homologous recombination with any human  
286 pathogens or endogenous viruses;
- 287 iv) Tissue tropism;
- 288 v) Transduction efficiency in the target cell population and whether the cells are dividing or  
289 terminally differentiated;

- 290 vi) The presence and persistence of the viral gene sequence(s) important for anti-viral  
291 chemotherapy of the wild type virus;  
292 vii) The tissue specificity of replication;  
293 viii) Germline transmission.

294 For integrating vectors, the risk of insertional mutagenesis should be addressed. Reference is given to  
295 the *Reflection paper on clinical risks deriving from insertional mutagenesis* (EMA/CAT/190186/2012).

296 For replication deficient viral vectors, the strategy taken to render the viral vector replication  
297 incompetent should be clearly documented and replication deficiency demonstrated. The drug  
298 substance and where appropriate intermediates, as well as any packaging/producer cell lines, should  
299 be screened for Replication Competent Viruses (RCV). The possibility of any recombination events  
300 leading to RCV or replication via *trans* regulation should be considered. In the case of genetically-  
301 modified cells, RCV testing at the Drug Substance or other intermediate levels is not deemed  
302 necessary provided that absence of RCVs has been demonstrated at the level of the virus starting  
303 material and RCV formation during manufacturing of the genetically modified cells can be excluded.

304 For replication competent viral vectors or replication-conditional viral vectors, a clear rationale for the  
305 construct and the individual genetic elements that control replication should be provided regarding to  
306 its safe use for the proposed clinical indications. Consideration should be given to the following factors:

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

#### 314 b. Development Genetics

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

317 All the genetic elements of the GTIMP should be described including those aimed at therapy, delivery,  
318 control and production and the rationale for their inclusion should be given. For helper virus, the same  
319 level of detail should be provided.

320 For plasmid DNA, full sequence should be provided.

321 DNA elements used for selection should be justified. The presence of antibiotic resistance genes in a  
322 GTIMP finished product should be avoided given the burden of bacterial multi-resistance to antibiotics  
323 and the existence of alternatives methods for selection. If unavoidable a risk analysis should be made.

324 Data on the control and stability of the vector and the therapeutic sequence(s) during development  
325 should be provided. The degree of fidelity of the replication systems should be ensured as far as  
326 possible and described. Evidence should be obtained to demonstrate that the therapeutic sequence  
327 remains unmodified and is stably maintained during any amplification.

328 Cells used for the amplification of the genetic material should be characterised.

329 Details of the construction of any packaging/producer cell line or helper virus should be provided,

330 Where, during development, changes to the design of the vector are made to obtain new improved  
331 product characteristics, the clinical impact of the change(s) should be evaluated (consult the Guideline

332 on the quality, preclinical and clinical aspects of gene therapy medicinal products) and comparability  
333 studies should be considered.

334 When GTIMP consists of genetically modified cells, both the required information on the viral vector  
335 plus information on the modified cellular component should be provided following the  
336 recommendations above.

## 337 **S.2. Manufacture**

### 338 **S.2.1. Manufacturer(s)**

339 The name(s) and address(es) and responsibilities of each manufacturer, including contractors, and  
340 each proposed production site or facility involved in manufacture, testing and batch release should be  
341 provided.

### 342 **S.2.2. Description of manufacturing process and process** 343 **controls**

344 The manufacturing process of an ATIMP and process controls should be carefully designed and  
345 described concisely and step-by-step. The suitability of the controls for the intended purpose needs to  
346 be proven.

347 A flow chart of all successive steps of the drug substance manufacturing process should be provided  
348 starting from biological fluid/tissue/organ or from cell banks/viral seeds. Critical steps and intermediate  
349 products should be indicated as well as relevant process parameters, in-process controls (IPCs) and  
350 acceptance criteria. IPC testing (for early phase developments) should focus at the minimum on safety  
351 aspects. Critical steps should already be identified for the manufacture of early clinical trial material  
352 and adequate acceptance criteria for these critical steps established, for other IPCs, monitoring might  
353 be appropriate.

354 During development, as process knowledge is gained, further details of in-process testing should be  
355 provided and acceptance criteria reviewed. As development proceeds, manufacturing consistency  
356 needs to be demonstrated. For a marketing authorisation, the manufacturing process needs to be  
357 validated.

358 For CBIMP the following aspects should be considered as applicable:

- 359 – A clear definition of a production batch from cell sourcing to labelling of final container should  
360 be provided (i.e. size, information on intermediate cell-banking, number of cell passages/cell  
361 population doublings, pooling strategies, batch numbering system). The purpose of the batch  
362 definition is to ensure consistency and traceability.
- 363 – The IMPD should contain information on the volume/number of cells collected and a description  
364 of the manipulation steps after sourcing. This should include a description of any  
365 selection/separation equipment used.
- 366 – The type of manipulation(s) required for cell processing shall be described.
- 367 – Manufacture of combined medicinal products consisting of cells and matrices/devices/scaffolds  
368 require additional consideration regarding cell-matrix/scaffold interactions and associated  
369 quality issues. Attention should be given to biodegradable materials, which may effect  
370 environmental changes (e.g. raising pH) for the cells during the manufacture.

- 371 – Information on procedures used to transport material during the manufacturing process of the  
372 product, including transportation and storage conditions and holding times, should be provided.
- 373 – Microbiological control is a pivotal aspect of process control and quality evaluation of all cell  
374 preparations and should be thoroughly described and justified.

375 For GTIMP the following aspects should be considered as applicable:

- 376 – Batch(es) and scale should be defined, including information on any pooling of harvests or  
377 intermediates.
- 378 – Any reprocessing during manufacture of the active substance (e.g. filter integrity test failure)  
379 should be described and justified.
- 380 – The applicant should establish that the vector sequence remains stable throughout cell culture.  
381 Where sufficient manufacturing experience permits, a maximal passage number for the cells  
382 should be established.
- 383 – The rationale for the use of a particular cell substrate should be provided.
- 384 – A purification process should be in place to reduce impurities. Impurities include hybrid viruses  
385 in the case of virus vector production, host cell-DNA and protein, residual plasmid DNA, lipids  
386 and polysaccharides in the case of production systems which involve bacterial fermentations,  
387 and RNA and chromosomal DNA in the case of plasmid purification. Ideally steps should be  
388 taken over time, in design, construction and production to minimise or eliminate these.
- 389 – For non-replication competent viral vectors and conditionally replicating viral vectors,  
390 information should be provided on process parameters, and controls conducted to prevent  
391 contamination of the packaging cell line by wild-type, helper or hybrid viruses which might lead  
392 to the formation of replication-competent recombinant viruses during production.
- 393 – For conditionally replicating virus vectors, sensitive in process tests with suitably low limits of  
394 detection are essential to show that replication-competent viruses are below an acceptable  
395 level. For non-replication competent viral vectors, the absence of RCV should be controlled with  
396 an assay of appropriate sensitivity.
- 397 – Manufacturers should seek to control unintended variability as far as possible, for example in  
398 culture conditions or inoculation steps during production.
- 399 – The manufacturing process must be set up to minimise the risk of microbiological  
400 contamination.

### 401 **S.2.3. Controls of materials**

#### 402 ***Raw and starting materials***

403 Materials used in the manufacture of the active substance (starting materials and raw materials)  
404 should be listed and their acceptance criteria for production should be provided, identifying where each  
405 material is used in the process. The manufacturing materials and reagents need to be qualified from  
406 the perspective of safety prior to human clinical trials. Reference to quality standards (e.g. compendial  
407 monographs or manufacturer's in-house specifications) should be made where possible. If non-  
408 compendial materials are used, information on the quality and control thereof should be provided.

409 The quality of starting and raw materials is a key factor in the production of ATMPs. Therefore avoiding  
410 contamination, minimising variability of starting and raw materials is vital for the manufacturing

411 process. Where transport conditions impact their quality, the specific conditions of transport should be  
412 described and their suitability verified. Adequate precautions need to be set to ensure proper handling.

413 For viral safety aspects the principles laid down in the general text of the Ph. Eur. 5.1.7. on viral safety  
414 should be followed for every substance of animal and human origin that is used during the production.

415 Measures should be taken to reduce the risk of transmissible spongiform encephalopathy according to  
416 the relevant European legislation and guidelines.

#### 417 **Raw materials**

418 Raw materials are the reagents that are used during the manufacturing process but are not part of the  
419 final product. Examples include foetal bovine serum, trypsin, digestion enzymes (e.g., collagenase,  
420 DNAse), growth factors, cytokines, monoclonal antibodies, antibiotics, resins, cell-separation devices,  
421 and media and media components. Reference to quality standards (e.g. compendial monographs or  
422 manufacturer's in-house specifications) should be made. Information on the quality and control of non-  
423 compendial materials should be provided. Information demonstrating that materials (including  
424 biologically-sourced materials, e.g. media components, monoclonal antibodies, enzymes) are suitable  
425 for their intended use should be provided. While raw materials should be of pharmaceutical grade, it is  
426 acknowledged that, in some cases, only materials of research grade are available. The risks of using  
427 research grade materials should be understood (including the risks to the continuity of supply when  
428 larger amounts of product are manufactured).

429 Considerations for suitability of a given material should focus on its identity, safety and functionality in  
430 respect of the intended use in the manufacturing process. This last point in particular serves to ensure  
431 consistent manufacturing and provides the acceptance criteria in case of supplier changes. Where  
432 possible, the use of animal reagents should be avoided and replaced by non-animal derived reagents of  
433 defined composition. This is due to their potential to introduce adventitious agents and resulting  
434 additional testing requirements.

435 For all raw materials of biological origin, the information on the supplier and the respective stage of the  
436 manufacturing process where the material is used should be indicated and a risk assessment  
437 conducted. Specific guidance is provided in Ph.Eur. (5.2.12) *Raw Materials for the Production of Cell  
438 based and Gene Therapy Medicinal Products*. Summaries of adventitious agents safety information for  
439 biologically-sourced materials should be provided in Appendix A.2.

440 Materials, including cells that function as support for growth and adhesion e.g. feeder cells should be  
441 evaluated and/or validated as to their suitability for the intended use. The same safety principles  
442 should apply to those critical raw materials generated in biological systems that were used for the  
443 manufacture of starting materials such as viral vectors, gene editing products or induced pluripotent  
444 stem cells (iPSC).

445 Raw materials derived from human plasma should be sourced from plasma collected under an EU  
446 approved Plasma Master File (PMF). Otherwise, if the collection and testing has no EU authorisation  
447 and no PMF reference, it should be confirmed that the recommendations provided in Ph. Eur. 5.2.12  
448 and Ph. Eur. 5.1.7 are followed.

449 The relevant characteristics (composition, function, degradation) of any matrices, fibres, beads, or  
450 other materials that are used in manufacture and that are not part of the finished product should be  
451 described.

452 Microbial purity and low endotoxin level of raw materials should be ensured.

453 The manufacturing process of CBIMP usually does not include terminal sterilisation, purification steps,  
454 viral removal and/or inactivation steps. Therefore, stringent sourcing requirements and acceptance

455 criteria for all materials derived from human or animal origin should be adequately defined according  
456 to their intended use. Sterilisation conditions applied to all materials can be found in the *Guideline on*  
457 *the sterilisation of the medicinal product, active substance, excipient and primary container*.

458 In accordance with Article 15 of Regulation 1394/2007, traceability information should also cover raw  
459 materials and all substances coming into contact with the cells or tissues. Details on the  
460 implementation of this obligation have been developed in the Guidelines on Good Manufacturing  
461 Practice specific to Advanced Therapy Medicinal Products.

#### 462 ***Starting materials for CBIMP***

463 This section applies to all materials that will be part of the active substance and is not limited to cells  
464 or tissues.

#### 465 **- Cells**

466 Donated cellular material (cells or tissues) from single or multiple donors, once processed may be:

- 467 • A single primary cell isolate or cell suspensions containing various naturally occurring cell types used  
468 directly for the CBMP;
- 469 • Primary cells cultured for a few passages before being used for the CBMP (cell stocks);
- 470 • Cells based on a well-defined cell bank system consisting of a master cell bank and a working cell  
471 bank.

472 The cell source should be documented, as well as tissue and cell type, and any patient pre-treatment  
473 required prior to donation. The procedure to obtain the cells from their source has to be described  
474 (with respect to the type of enzyme, media, etc.) and the purpose of respective steps explained.

475 Establishment and testing of cell stocks or cell banks should be conducted according to the Guideline  
476 on human cell-based medicinal products.

477 In general, cell pooling should be avoided as it raises questions if the clinical outcome is affected by  
478 the variation of the starting materials from different donors. In case of pooling of similar allogeneic cell  
479 populations the pooling strategies, pool size and measures to ensure traceability shall be described. A  
480 risk analysis should be conducted addressing the possibility of undesired (immunological) responses  
481 and disease transmission due to the pooling. An adequately controlled cell storage system should be  
482 established to allow proper maintenance and retrieval of cells without any alteration of their intended  
483 final characteristics. Storage conditions should be optimised to ensure cell viability, density, purity,  
484 sterility and functionality. The identity of the cells used as starting material should be verified by  
485 relevant genotypic and/or phenotypic markers and the proportion of cells bearing these identity  
486 markers evaluated as an indicator of the intended cell population.

#### 487 ***A. Cells of primary origin***

488 Donation, procurement and testing of human cell based products need to comply with the  
489 requirements of Directive 2004/23/EC or where applicable Directive 2002/98/EC.

490 Procedures and standards employed for the selection of appropriate donors and the exclusion of high-  
491 risk or otherwise unsuitable candidate donors should be clearly delineated and justified. If it is  
492 necessary to pool cells from different donors, the risk analysis should address the possibility that  
493 pooling of allogeneic cell populations may increase the risk of undesired immunological responses in  
494 the recipient and compromise its therapeutic activity. In addition, pooling of cells may increase the risk  
495 of disease transmission. Depending on the nature of the source of the cells and tissues, other risk  
496 factors, e.g. previous radiation exposure, should be also considered and addressed.



497 A specific microbiological screening programme should be in place, adapted to the type of cells, at the  
498 most suitable or relevant step of the manufacturing process, with validated assays capable of detecting  
499 human infectious agents with appropriate sensitivity and taking into consideration the medium  
500 components that might interfere with the assays (e.g. antibiotics). When cells originate from non-  
501 healthy tissues, the product specific acceptance criteria should be defined according to the intended  
502 use.

503 Quality parameters aimed at the definition of acceptance criteria for a given organ or tissues should be  
504 specified, taking into consideration general aspects such as shipment and storage conditions.

505 In the case of autologous donation, the testing regimen of the starting material should be justified,  
506 taking into account the autologous use.

507 Where allogeneic primary cells are collected and expanded for use in multiple patients, the cell stock  
508 should be appropriately characterised. The same characterisation programme shall be applied to each  
509 new cell stock.

### 510 ***B. Banking system for established cell lines***

511 Where cell lines are used, an appropriately characterised Master Cell Bank (MCB) and Working Cell  
512 Bank (WCB) should be established, whenever possible. While a MCB should be established prior to the  
513 initiation of phase I trials, the WCB may not always be established early on. Information on the cell  
514 banking and characterisation and testing of the established cell banks should be provided as well as  
515 available information on cell substrate stability.

516 The MCB and/or WCB (if used) should be characterised and results of tests performed should be  
517 provided. The generation and characterisation of the cell banks should be performed in accordance  
518 with principles of CPMP/ICH guideline Q5D.

519 The history of the cell line derivation and cell banking, including the raw material used during  
520 production, needs to be carefully documented. This is particularly important for human embryonic stem  
521 cells (ESCs). Where ESCs were established before the requirements of Directive 2004/23/EC came  
522 into force, and results from donor testing are not available, extensive viral safety testing of those cell  
523 lines is expected.

524 For the establishment of induced pluripotent stem cell (iPSC) banks, the starting material should be the  
525 primary cells prior to being subject to the dedifferentiation programme. In this regard, the principles of  
526 good manufacturing practice and the recommendations given in this guideline should apply after  
527 procurement of the cells including the generation of iPS cells and the subsequent selection process. It  
528 is understood that in cases where the early steps for the generation of ESC or iPSC banks are  
529 conducted before a clear product concept is present, the initial manufacturing steps might not have  
530 been conducted under full GMP compliance. At the minimum, the GMP principles should be followed in  
531 this exceptional situation, as describe in the GMP for ATMP guidelines section 7.35.

532 Viral and TSE safety of the cells and raw materials should be addressed during cell bank and/or  
533 starting material qualification or early in the production process to minimize the risk of contamination.

534 The origin and procurement of the starting material to isolate the stem cells is considered critical for  
535 the yield and identity/purity of the final cell population. The selection of appropriate markers is  
536 fundamental to the standardisation of isolation conditions and to control cell populations, heterogeneity  
537 and yield.

### 538 ***C. Cell stocks***



539 Primary cells might be organized as cell stocks by expanding them to a given number of cells and  
540 storing them in aliquots which are subsequently used for production of a cell-based ATMP. In contrast  
541 with the two tiered system of master and working cell banks, the number of production runs from a  
542 cell stock is limited by the number of aliquots obtained after expansion and does not cover the entire  
543 life cycle of the product. Cell stock changes (including introduction of cells from new donors) should be  
544 addressed in the clinical trial authorisation and the conditions therein should be complied with. When  
545 cell stocks are used, the handling, storage and manufacturing and testing of cells should be done in  
546 accordance with the principles outlined above for cell banks.

#### 547 **- Structural components**

548 CBIMPs may incorporate structural components as starting materials which may be medical devices or  
549 active implantable medical devices. Those devices should meet the relevant general safety and  
550 performance requirements laid down in under EU legislation on medical devices, and this information  
551 shall be provided in the IMPD. In the case where a Notified Body has evaluated the device part, the  
552 result of this assessment shall be included in the dossier. In cases where the medical device is also in  
553 investigational phase, the specifications set should be adequate to ensure the suitability of the device  
554 to the intended use. In addition, available data on the quality of the device should be provided.

555 CBIMP may also incorporate structural components that are not CE certified or that are certified but  
556 used outside of their intended use. In such cases, the sponsor of the clinical trial should demonstrate  
557 suitability for the intended use (See sections on Characterisation and Development Pharmaceuticals).

#### 558 **Starting materials for GTIMP**

559 Viral vectors are starting materials, also when used to transduce cells and not remaining in the active  
560 substance. Information on the vector should be provided in the starting material section. The same  
561 level of information that is needed for the vector as active substance should be provided in this  
562 situation.

563 Genome editing tools used ex-vivo to generate genetically modified cells are by analogy also  
564 considered as starting materials.

565 Also, for in vitro-transcribed (m)RNAs used as active substances, the linearized template plasmid DNA  
566 should be considered as a starting material.

567 Complexing materials<sup>6</sup> for formulating the drug substance are considered as starting materials and  
568 have to be qualified for their intended purpose. The level of information to be provided will depend on  
569 nature of the complexing material and resulting DS.

570 For further requirements refer to S.3.1.

#### 571 **- Source, history and generation**

572 A summarised description of the source and generation (flow chart of the successive steps) of the cell  
573 substrate/ viral seed should be provided.

574 Where cells or tissues of human origin are used, the procurement and testing should comply with  
575 conditions provided for primary cells above in the section on starting materials for CBIMP.

576 For genome editing approaches, the starting materials shall be, as appropriate, the vector (viral or  
577 non-viral vector) carrying the nucleic acid sequences encoding the modifying enzyme, the mRNA

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<sup>6</sup> A substance used to form a complex with DNA which facilitates transfer of that DNA into a cell (for example: calcium phosphate, lipids or proteins.)

578 expressing the modifying enzyme, the modifying enzyme itself, the genetic sequence for modification  
579 of the cell genome (e.g. a regulatory guide RNA) or a ribonucleoprotein (e.g. Cas9 protein pre-  
580 complexed with gRNA), the template (e.g. linear DNA fragment or a plasmid), and the components to  
581 produce them. When mRNA or proteins are used to generate genetically modified cells, the principles  
582 of good manufacturing practice shall apply from the bank system used to produce these materials  
583 onwards.

584 For medicinal products based on induced pluripotent stem (iPS) cells generated by genetic  
585 modification, the principles of good manufacturing practice and the scientific recommendations given in  
586 this guideline should apply after procurement of the cells including the generation of iPS cells and the  
587 subsequent selection process. It is acknowledged that at the early steps in iPS cells generation, cell  
588 material may be limited and availability of samples may impact on the extent of testing and process  
589 qualification. The Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal  
590 Products should be considered.

591 For the manufacture of active substances consisting of genetically modified cells derived from  
592 genetically modified animals, good manufacturing practice shall apply after their procurement and  
593 testing according to the *Guideline on xenogeneic cell-based medicinal products*.

#### 594 - **Banking system, characterisation and testing**

595 The establishment of bacterial/cell/virus seed or bank(s) is expected for starting materials which are  
596 bankable. Where possible, a Master Cell/Seed Bank (MCB/MSB) should be established prior to the  
597 initiation of exploratory trials. It is acknowledged that a Working Cell/Seed Bank (WCB/WSB) may not  
598 always be established.

599 The MCB/MSB and/or WCB/WSB should be characterised and results of tests performed should be  
600 provided. Banks should be characterised for relevant phenotypic and genotypic markers so that the  
601 identity, viability, and purity of cells used for the production are ensured.

602 The safety assessment for adventitious agents and qualification of the cell banks used for the  
603 production of the active substance should be provided in A.2, if needed.

604 Applicants should consult the requirements for banking as described in the Guideline on the quality,  
605 non-clinical and clinical aspects of gene therapy medicinal products.

#### 606 **A. Virus seed banks**

607 Control of virus seed banks should include identity (genetic and immunological), virus concentration  
608 and infectious titre, genome integrity, transcription/expression of the therapeutic sequences,  
609 phenotypic characteristics, biological activity of therapeutic sequence, sterility (bacterial, and fungal),  
610 absence of mycoplasma, absence of adventitious/contaminating virus and replication competent virus  
611 (where the product is replication deficient or replication conditional). The sequence of key elements  
612 such as the therapeutic and the regulatory elements should be confirmed.

#### 613 **B. RNA or DNA Vectors and plasmids**

614 Testing of RNA and DNA vectors, plasmids or artificial chromosome DNA should include tests for  
615 genetic identity and integrity including confirmation of the therapeutic sequence and  
616 regulatory/controlling sequences, freedom from extraneous agents using a range of tests, sterility and  
617 endotoxin levels. The presence/absence of other genetic features such as immunomodulatory CpG  
618 sequences should be determined, unless otherwise justified.

#### 619 **C. Mammalian Cell Banks**

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

624 Testing of the producer/packaging cell bank for presence of adventitious viruses should be conducted  
625 according to the principles of ICH guideline Q5A. Ph.Eur. 5.2.3 and 5.1.7 should be followed as  
626 indicated in Ph.Eur 5.14 and should include tests for contaminating and endogenous viruses. The  
627 absence of bacterial and fungal contamination, as well as mycoplasma and spiroplasma (insect cells),  
628 should be determined. Electron microscopy of insect cells should also be carried out, unless otherwise  
629 justified.

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

#### 632 **D. Bacterial cell banks**

633 Bacterial cell banks should be tested for phenotypic and genomic identity. The presence/absence of  
634 inserted/deleted sequences necessary for the safe use of the GTIMP should be confirmed. The  
635 immunological identity including the genetically modified components should be determined, for  
636 instance by serotyping. Transduction efficiency, absence of contaminating bacteria and bacteriophages,  
637 fungal sterility, and inter vial homogeneity of cell bank stocks should be assured. For transduced  
638 bacterial cell banks testing should include presence of plasmid or genome sequences containing the  
639 therapeutic sequence and associated regulatory/control elements, plasmid copy number and ratio of  
640 cells with/without plasmids. The principle described in ICH Q5D guideline on derivation and  
641 characterisation of cell substrates should also be considered.

#### 642 **S.2.4. Control of critical steps and intermediates**

643 Critical steps in the manufacturing process should be identified as appropriate for the stage of  
644 development and all available data and acceptance criteria should be provided. It is acknowledged that  
645 due to limited data at an early stage of development complete information may not be available.  
646 Where applicable, hold times and storage conditions for process intermediates should be justified and  
647 supported by data, as appropriate. Intermediate cell products are products that can be isolated during  
648 the process; specifications of these products should be established in order to assure the  
649 reproducibility of the process and the consistency of the final product. Tests and acceptance criteria  
650 should be described. Any storage periods during production need to be controlled (e.g. time,  
651 temperature).

652 Monitoring of *in vitro* cell culturing at selected stages of the production should be performed where  
653 feasible and the *in vitro* cell age (population doublings) should be controlled. The culture should be  
654 examined for any microbial contamination.

#### 655 **S.2.5. Process evaluation / validation**

656 Process validation is the documented evidence that the manufacturing process can consistently  
657 produce a result within specific parameters. The manufacturing process for ATIMPs is not expected to  
658 be validated for early clinical trials but appropriate monitoring and control measures should be  
659 implemented to ensure compliance with the requirements in the clinical trial authorisation. It is noted  
660 that for the confirmatory clinical trial to be used in support of a marketing authorisation process  
661 validation is required to demonstrate that the manufacturing process of the ATIMP ensures consistent  
662 production.

663 Process characterisation/evaluation data should be collected throughout the development. It is  
664 acknowledged that some degree of variability of the active substance due to the characteristics of the  
665 starting materials is intrinsic to ATMPs. In this regard, it is recommended that critical process  
666 parameters, critical quality attributes and the associated acceptance criteria should be set based on the  
667 development data and current knowledge. This is achieved through implementation of appropriate  
668 monitoring and control measures. Summaries of the process characterisation and verification studies  
669 need to be provided, but the reports themselves are not required to be submitted as part of the IMPD.  
670 Reference is made to the *Guideline on process validation for the manufacture of biotechnology-derived*  
671 *active substances and data to be provided in the regulatory submissions*  
672 (EMA/CHMP/BWP/187338/2014) and to the GMP for ATMP Guidelines.

673 In addition the process characterisation/ evaluation summaries, validation of the aseptic process and  
674 the viral removal/inactivation steps are expected to be validated prior to the FIH clinical trials. Details  
675 on manufacturing steps intended to remove or inactivate viral contaminants should be provided in the  
676 section A2, Adventitious agents safety evaluation.

677 - CBIMPs:

678 Characterisation/evaluation with surrogate materials: The limited availability of the cells/tissues e.g.  
679 autologous ATMPs, allogeneic cell stocks where there is no expansion of cells to MCB, requires the  
680 development of pragmatic approaches for characterization/evaluation of the manufacturing process or  
681 subsequent changes, taking into account the quantities of tissue/cells available. The goal needs to be  
682 to gain maximum experience from each batch processed.

683 The representativeness of surrogate starting material should be evaluated, considering -for example-  
684 donor age, donor health status, anatomical source (e.g. femur vs iliac crest) or other characteristics  
685 (e.g. use of representative cell-types or use of cells at a higher passage number than that foreseen in  
686 the product specifications). Where possible, consideration should be given to complementing the use of  
687 surrogate materials with samples from the actual starting materials for key aspects of the  
688 manufacturing process. For instance, in the case of an ATMP based on genetically modified cells, use of  
689 patient material may be limited to process characterization of the genetic modification. Other aspects  
690 could be qualified/evaluated using a representative surrogate cell type. For further information, consult  
691 the GMP Guide on ATMPs.

692 - GTIMPs:

693 Where replication defective viral vectors are used, measures should be in place to prevent the  
694 introduction of wild-type viruses, which may lead to the formation of replication competent  
695 recombinant virus. Absence of formation of replication competent virus should demonstrated at the  
696 level of the viral production system.

## 697 **S.2.6. Manufacturing process development**

### 698 ***Process improvement***

699 Manufacturing processes and their control strategies are continuously being improved and optimised,  
700 especially during early phases of clinical trials and development. These changes need to be adequately  
701 documented and evaluated for the need to submit a substantial amendment. In general, these  
702 improvements and optimisations are considered as normal development work, and should be  
703 appropriately described in subsequently submitted dossiers. Changes to the manufacturing process  
704 and controls should be summarized and the rationale for changes should be presented. This description  
705 should allow a clear identification of the process versions used to produce each batch used in non-  
706 clinical and clinical studies, in order to establish an appropriate link between pre-change and post-

707 change batches. Comparative flow charts and/or list of process changes may be used to present the  
708 process evolution. Process modifications may require adaptation of in-process and release tests, and  
709 thus these tests and corresponding acceptance criteria should be reconsidered when changes are  
710 introduced.

711 While changes to the manufacturing process commonly occur during development, the complex and  
712 dynamic nature of AMTPs presents a challenge for the evaluation of pre-versus post-change product.  
713 Orthogonal methods need to be applied in this evaluation and the potential impact on the entire  
714 product needs to be taken into consideration rather than on a single parameter.

715 GTIMPs:

716 It is recognised that in particular for GTIMPs, only a limited number of batches may be produced prior  
717 to MAA. Therefore, it is particularly important to gather sufficiently detailed manufacturing process and  
718 batch analytical data throughout the development process as these can be used as supportive  
719 information during a licence application.

## 720 **Comparability**

721 Depending on the consequences of the change introduced and the stage of development, a  
722 comparability exercise may be necessary to ensure that the change does not have an adverse impact  
723 on impact on the quality of the product and therefore on the safety and clinical efficacy of the product.  
724 The main purpose of this exercise is to provide assurance that the post-change product is suitable for  
725 the forthcoming clinical trials and that it does not raise any concern for the safety of the patients  
726 included in the clinical trial. The extent of the comparability exercise needed depends on the nature of  
727 the change introduced and the stage of development.

728 This comparability exercise should normally follow a stepwise approach, including comparison of  
729 quality attributes of the active substance and relevant intermediates, using suitable analytical  
730 methods. Analytical methods usually include routine tests, and should be supplemented by additional  
731 characterisation tests (including orthogonal methods), as appropriate. Developing a panel of suitable  
732 assays for comparability is highly recommended from the first steps of development. As such,  
733 biological characterisation and the potency assay(s) are the most important parameters to perform  
734 comparability on quality grounds.

735 The analytical tools for comparability need to be chosen based on critical parameters identified  
736 throughout development.

737 During early phases of non-clinical and clinical studies, comparability testing is generally not as  
738 extensive as for an approved product.

739 When only non-clinical data has been generated, normally at an early stage of development, and prior  
740 to clinical exposure, analytical results should support safety data filiation, i.e. demonstrating  
741 representativeness of the non-clinical safety profile of the batches studied to those to be used in  
742 exploratory clinical trials. In the case of exploratory clinical trials, it is recommended to use  
743 investigational product representative of the material used in non-clinical studies (see *Guideline on  
744 Strategies to Identify and Mitigate Risks for First-In-Human Clinical Trials with Investigational Medicinal  
745 Products* (EMA/CHMP/SWP/28367/07)). More stringent equivalence is required when toxicity and dose  
746 finding studies have been conducted.

747 When exploratory trials already took place, data filiation program should expand to a full comparability  
748 exercise where a higher degree of sameness is expected and a more comprehensive analytical package  
749 should be in place. For confirmatory trials, the principles as can be found in ICH Q5E Comparability of  
750 Biotechnological/Biological Products should be applied. During the confirmatory clinical studies

751 introducing changes to the manufacturing process and the final product should be avoided, because  
752 comparability issues may impact the acceptability of the data.

753 Where the relevant information is not sufficient to assess the consequences introduced by the change  
754 and if a potential risk to the patients cannot be excluded, a comparability exercise based only on  
755 quality considerations most likely will not be sufficient and further non-clinical data will be required.

756 It is particularly important that all stages of development are fully evaluated, justified and tracked  
757 within the evolving dossier.

758 In case of complex CBIMP with a tri-dimensional architecture, the extended characterisation for  
759 comparability should consider possible structural changes as well as functional changes.

### 760 **S.3. Characterisation**

#### 761 **S.3.1. Elucidation of structure and other characteristics**

762 Characterisation studies should be conducted throughout the development process, resulting in a  
763 comprehensive picture and knowledge of the ATIMP to allow appropriate control of quality parameters  
764 related to efficacy and safety. Reference to the literature data alone is not acceptable. Sufficient  
765 characterisation to define the product profile should be performed in the development phase prior to  
766 FIH clinical trials and, where necessary, following significant process changes.

767 Characterisation data could encompass data obtained throughout the development and/or  
768 manufacturing process and should reflect the most complete knowledge of the product.  
769 Characterisation is also the basis for comparability and stability studies. Ultimately, characterisation  
770 allows setting the routine controls that will be applied for release of the active substance.  
771 Characterisation data are likely to be necessary for single components as well as for the final product.

772 Biological characterisation of the product is essential part of the documentation. The strategy to  
773 demonstrate biological activity should be justified. It is recognised that the extent of characterisation  
774 data will increase in later phases.

775 Generally the biological activity measurement will become the potency test for DS and DP.

776 From the characterisation and evaluation of the biological activities, the quality attribute(s) relevant for  
777 the potency should be identified. Potency is the quantitative measure of biological activity, which is  
778 linked to the relevant biological properties and the claimed mechanism of action. The potency assay  
779 should be developed based on the biological activity (i.e. the specific ability or capacity of a product to  
780 achieve a defined biological effect).

781 It is strongly recommended that the development of a suitable potency assay be started as soon as  
782 possible. Preferably, a suitable potency assay should already be in place when material for the FIH  
783 clinical trial is produced and it should be validated prior to confirmatory clinical trials unless otherwise  
784 justified. Surrogate potency markers can be considered for release tests, but appropriate justification  
785 on their relevance in the context of the intended action of the ATIMP is needed.

#### 786 **1. Characterisation studies of CBIMP**

787 The characterisation should encompass all the components present in the active substance.  
788 Characterisation may prove particularly challenging for where cells are combined with matrices,  
789 scaffolds and innovative devices. At minimum characterisation of the cellular component should be  
790 established in terms of identity, purity, impurities (see also S.3.2), viability, quantity (cell number) and  
791 potency.

792 It is noted that in a combined product the characteristics of both the cellular and the non-cellular  
793 components may be altered by the process of integration.

#### 794 **- Cellular Component**

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795 The identity of the cellular components, depending on the cell population and origin, should be  
796 characterised in terms of phenotypic and/or genotypic profiles.

797 When addressing the phenotype of the cells, relevant identity markers could be used. These markers  
798 may be based on gene or surface marker expression, the capacity to present antigen, biochemical or  
799 immunological activities, response to exogenous stimuli, capability to produce biologically active or  
800 otherwise measurable molecules, etc. They should be specific for the intended cell population(s) and  
801 should be based on an understanding of the biological or molecular mechanism of the proposed  
802 therapy. For adherent cells, morphological analysis may be a useful tool in conjunction with other tests  
803 whereas for stem cells, markers of pluripotency, lineage commitment or differentiation state might be  
804 appropriate.

805 Tumourigenicity/genetic stability should be evaluated for stem cell preparations that undergo extensive  
806 in vitro manipulation such as prolonged cell culture.

807 The cellular population could contain other cells that are of different lineages and/or differentiation  
808 stage or that may be unrelated to the intended population. Where a specific cell type is required for  
809 the indication, other cell populations should be defined and their amount in the final product should be  
810 controlled by appropriate specifications, i.e. acceptance criteria for the amounts of contaminating cells  
811 should be set. In cases, where the desired biological activity and efficacy of the product requires a  
812 complex mixture of cells, the cell mixture needs to be characterized and its composition controlled by  
813 appropriate in-process controls and release testing.

#### 814 - **Non-cellular Components of the active substance**

815 Non-cellular components are starting materials that should be characterised on their own in the  
816 context of their required function. This includes biomaterials, proteins or chemical entities which may  
817 supply structural support, a suitable environment for growth, biological signalling or other functions.

818 These components should be identified and characterised with respect to their composition, structural  
819 characteristics and mechanical properties. The general principles that are applied to the biological  
820 evaluation of medical devices can also be applied to the evaluation of biomaterials intended for use in  
821 CBIMP. Such an evaluation involves a programme of characterisation, testing and review of existing  
822 data to assess the potential for an adverse biological reaction to occur as a result of exposure to the  
823 biomaterial. These principles are set out in international standard ISO 10993 Part 133. Other parts of  
824 the ISO 10993 series of standards specify methods that may be relevant to the assessment of material  
825 characteristics, biological safety and degradation of biomaterials used in CBIMP. For example, ISO  
826 10933 Parts 1826 and 1927 refers to chemical / physical characterization such as porosity, density,  
827 microscopic structure and particular size. The summary of performed analysis and studies should be  
828 submitted.

829 If the device has been CE marked for the same intended use, the 'Instructions for Use' should be  
830 provided. Additional studies (e.g. cell adhesion studies, growth studies) may be necessary to  
831 demonstrate aspects of biocompatibility specific to the cell-based product.

832 In addition, effects of potential impurities that can be present in non-cellular components should be  
833 taken into consideration. Also it should be ensured that the non-cellular component is of consistent  
834 quality.

835 Since the identity of both the cellular and the non-cellular components may be altered by the process  
836 of combination a distinctive way to define identity should be established for the components in the  
837 combination, unless justified. Special consideration should also be given to their degradation profile  
838 and impact on the combination.

## 839 **2. Characterisation studies of GTIMP**

840 Characterisation of a gene therapy active substance (which includes the determination of physico-  
841 chemical, biological and functional properties, purity and impurities) by appropriate techniques is  
842 necessary to allow relevant specifications to be established. Tests should be included to show integrity

843 and homogeneity of the recombinant viral genome or plasmid and the genetic stability of the vector  
844 and therapeutic sequence.

- 845 • Tests performed on harvested vector should as a minimum include identity (desired transgene  
846 and vector), purity and yield. For viral vectors, titre and particle to infectivity ratio should  
847 normally be determined.
- 848 • For complexed nucleic acids, the structure of the complex and the interaction between the  
849 vehicle(s) and the negatively charged nucleic acids should be addressed. Suitable tests should  
850 be included to establish, for example, that the complexed nucleic acid has the desired  
851 biochemical and biological characteristics required for its intended use.
- 852 • For bacterial vectors, the presence/absence of inserted/deleted sequences necessary for the  
853 safe use of the GTMP should be confirmed. It should be demonstrated that there is no inclusion  
854 of known oncogenic/tumorigenic sequences. Phenotypic identity, immunological identity  
855 (including the genetically modified bacterial components) and analysis of the therapeutic  
856 sequences and selectivity/regulatory elements delivered by the bacterial vector should be  
857 included. The absence of contaminating bacteria and bacteriophages, fungal sterility, and inter  
858 vial homogeneity of cell bank stocks should be assured.
- 859 • For genetically modified cells, *in vitro* assays for transduction efficiency and transgene copy  
860 number per transduced cell should be conducted. For GM cells derived using genome editing  
861 tools, *in vitro* assays for editing efficiency and off-target editing should be conducted.
- 862 • The intended action of regulating, repairing, replacing, adding or deleting a genetic sequence  
863 should be demonstrated. The potency assay should normally encompass an evaluation of the  
864 efficiency of gene modification (infectivity/transduction efficiency/delivery efficiency) and the  
865 level and stability of expression of the therapeutic sequence or its direct activity or deletion.  
866 Where possible the potency assay should include a measure of the functional activity of the  
867 therapeutic sequence or the product of it.

868 The rationale for selection of the analytical methods used for characterisation should be provided and  
869 their suitability should be justified.

### 870 **S.3.2. Impurities**

871 During the production of an ATIMP, variable amounts of impurities, product- and process-related, may  
872 be introduced into the active substance. Any reagents known to have clinical impact in humans should  
873 be analysed in the active substance (or in individual components if otherwise not possible) and  
874 acceptance criteria should be set. The specification limits should be justified by levels detected in  
875 batches used for toxicological and/or clinical studies.

876 The aim should be to maximise the active components and minimise features which do not contribute,  
877 or may negatively impact on therapeutic activity/safety. The setting of purity specifications should be  
878 based on characterisation studies conducted as part of product development. Purity does not  
879 necessarily imply homogeneity, however, product consistency needs to be demonstrated.

880 Any material capable of introducing degradation products during the production, e.g. biodegradable  
881 materials, should be thoroughly characterised in this respect and the impact on the cellular  
882 component(s) should be addressed.

883 Analytical procedures should be demonstrated to be suitable to detect, identify, and quantify  
884 biologically significant levels of impurities.



885 Process related impurities (e.g. media residues, growth factors, host cell proteins, host cell DNA,  
886 column leachables) and product related impurities (e.g. cell types not linked to the therapeutic effect,  
887 cell fragments or non-viable cells, precursors, degradation products, aggregates) should be kept to the  
888 minimum or a risk assessment provided. Based on the risks identified, consideration should be given to  
889 the maximum amount for the highest clinical dose and an estimation of the clearance should be  
890 provided. In case only qualitative data are provided for certain impurities, this should be justified.

891 Product-related impurities, such as or unrelated or non-viable cells, as well as vectors with deleted,  
892 rearranged, hybrid or mutated sequences or co-packaged nucleic acids should be considered, with a  
893 particular focus initially on safety. In the case of vectors designed to be replication deficient or  
894 conditionally replicating, the absence of replication-competent virus should be demonstrated and/or  
895 conditional replication demonstrated. The absence of any helper or hybrid viruses generated or used  
896 during manufacture or components of the production system should be demonstrated. If genetically  
897 modified cells are used in the product, any additional proteins expressed from the vector, e.g.  
898 antibiotic resistance factors or other selection markers should be analysed and their presence in the  
899 product should be justified.

900 Where only a selected population of cells in a mixture is responsible for the therapeutic effect, other  
901 cell populations should be defined and their amount controlled by appropriate specifications.

902 Irrespective of the cell type, the cell population can contain with non-viable cells. Since cell viability is  
903 an important parameter for product integrity and directly correlated to the biologic activity, the ratio  
904 between non-viable and viable cells should be determined and specifications should be set.

#### 905 **S.4. Control of the active substance**

906 During the clinical trial phases, where process validation data are incomplete, the quality attributes to  
907 control the active substance are important to demonstrate pharmaceutical quality, product consistency  
908 and comparability after process changes. Therefore the quality attributes controlled throughout the  
909 development process should be more comprehensive than the tests included in the specification for  
910 which preliminary acceptance criteria have been set.

911 For quality control the active substance should be subjected to release testing, whenever possible. If  
912 justified, it can be acceptable to have reduced testing at one level provided an exhaustive control is  
913 performed at another.

##### 914 **S.4.1. Specification**

915 The specifications for the batch(es) of the active substance to be used in the clinical trial should be  
916 defined. The acceptance criteria together with the tests used should ensure sufficient control of the  
917 quality of the active substance.

918 The release specification of the active substance should be selected on the basis of parameters defined  
919 during the characterisation studies. The selection of tests is product-specific and needs to be defined  
920 and justified by the applicant.

921 During early phases of clinical development specification can include wider acceptance criteria based  
922 on the current knowledge of the risks. As the acceptance criteria are normally based on a limited  
923 number of development batches and batches used in non-clinical and clinical studies, they are by their  
924 nature preliminary and need to be subject to review during development.

925 Product characteristics that are not completely defined at a certain stage of development or for which  
926 the available data is too limited to establish relevant acceptance criteria, should also be recorded. As a

927 consequence, such product characteristics could be included in the specification, without pre-defined  
928 acceptance limits. The results should be reported in the Batch Analyses section (S.4.4). It is  
929 nevertheless stressed that these parameters cannot replace existing and sufficient specification.

930 If certain release tests cannot be performed on the active substance or finished product, but only on  
931 key intermediates and/or as in-process tests, this needs to be justified.

932 Specifications should be meaningful and quantitative and a limit of 'record' or 'report results' should be  
933 avoided whenever possible. For test parameters relevant to safety, the absence of defined limits is not  
934 acceptable. Tests and defined acceptance criteria are expected for quantity, identity, purity,  
935 microbiological assays and biological activity. For a FIH trial the absence of quantitative limits for  
936 potency / biological activity would have to be justified by the applicant. Upper limits, taking safety  
937 considerations into account, should be set for impurities. Microbiological safety testing of the active  
938 substance should be specified.

939 When development and validation was performed using cells from healthy volunteers, acceptance  
940 criteria should be revised when sufficient data with patient material is available.

941 In case of GTIMP, the genetic identity and integrity of the drug substance should be assured. Test  
942 should identify both the therapeutic sequence, the vector and, if applicable and possible, the  
943 complexed nucleic acid sequences. In addition to sequencing data, the identity of the drug substance  
944 may also be confirmed through infection/transduction assays and detection of expression/activity of  
945 the therapeutic sequence(s) (see potency assay section).

#### 946 ***Additional information for confirmatory clinical trials***

947 As knowledge and experience increase, the addition or removal of parameters and modification of  
948 analytical methods may be necessary. Parameters, analytical methods and acceptance criteria set for  
949 previous trials should be reviewed and, where appropriate, adjusted to the current stage of  
950 development.

951 For confirmatory trials, the active substance specifications should be in place to allow sufficient and  
952 accurate evaluation of quality that is linked to the clinical outcome.

### 953 **S.4.2. Analytical procedures**

954 The analytical methods used for the active substance should be listed for all tests included in the  
955 specification (e.g. phenotypic characterisation, biological assay, chromatographic methods, biological  
956 assay etc.) including those tests reported without acceptance limits. A brief description for all non-  
957 compendial analytical procedures, i.e. the way of performing the analysis, should be provided  
958 highlighting controls used in the analysis. For methods, which comply with a monograph of the Ph.Eur.,  
959 the pharmacopoeia of an EU Member State, USP or JP, reference to the relevant monograph is  
960 acceptable.

961 Stability methods should be demonstrated as suitable to monitor product degradation.

### 962 **S.4.3. Validation of analytical procedures**

963 Validation of analytical procedures during clinical development is an evolving process. An appropriate  
964 degree of method qualification should be applied at each stage to demonstrate the methods are  
965 suitable for their intended use at that time.

966 Analytical procedures, which are either described in Ph.Eur., the pharmacopoeia of a Member State,  
967 USP or JP general chapter, or are linked to a product specific monograph, are normally considered as

968 validated. Proposed modifications, or alternatives, to compendial methods when duly justified must be  
969 qualified / validated.

970 For exploratory clinical trials, the suitability of the analytical methods used should be confirmed and  
971 preliminary acceptance limits defined (e.g. acceptance limits for the determination of the content of  
972 impurities). The parameters for performing qualification of the analytical methods (specificity, linearity,  
973 range, accuracy, precision, quantitation and limit of detection, as appropriate) should be presented in  
974 tabulated form. It is not necessary to provide full interim validation report. If validation studies have  
975 been undertaken for early phase trials, a tabulated summary of the results of analytical method  
976 validation studies could be provided for further assurance.

977 Irrespective of the clinical trial phase, the suitability of the analytical methods used for viral testing,  
978 either as a qualitative or a quantitative method, should be substantiated. ICH Q5A Chapter 3.2  
979 "Recommended Viral Detection and Identification Assays" is applicable. Validations of sterility and  
980 microbial assays, as well as RCR testing are required whatever the clinical trial phase.

981 When routine release testing is limited or not possible, characterisation / evaluation of process  
982 robustness becomes more important in lieu of batch testing.

983 For GTIMP or genetically modified cells transduced using retro/lentiviral vectors, each viral batch  
984 should be tested for the presence of replication competent virus with a validated method. When using  
985 assays determining residual replication competent virus (RCV) the limit of detection must be such that  
986 the test provides assurance of the safety of the vector product. Also, the appropriateness of the  
987 permissive cell type(s) used in the assays for replication-competent virus should be established.

#### 988 ***Information for confirmatory clinical trials***

989 For confirmatory clinical trials, the guidelines applicable to Marketing Authorisation Applications do  
990 apply. Validation of analytical methods for batch release and stability testing is expected. It is not  
991 necessary to provide full validation reports. A tabulated summary of the results of the validation  
992 carried out should be provided.

#### 993 **S.4.4. Batch analyses**

994 The focus of this section is to demonstrate quality of the batches (conformance to established  
995 preliminary specification) to be used in the given clinical trial. The manufacturing history is important  
996 for this purpose. As acceptance criteria may be initially wide, actual batch data are important for  
997 quality assessment. For quantitative parameters, actual numerical values should be presented. These  
998 values serve to evaluate process variability/manufacturing consistency.

999 Batch number, batch size, manufacturing site, manufacturing date, control methods, acceptance  
1000 criteria and the test results should be listed together with the use of the batches. The manufacturing  
1001 process version used for each batch should be identified.

1002 For exploratory clinical trials, which are often characterised by a limited number of batches, results for  
1003 relevant non-clinical and test batches should be provided, including the results of batches to be used in  
1004 the given clinical trial, when available. In case of genetically modified cells, the batch data on the  
1005 vector used to produce the active substance should be provided.

1006 In confirmatory trials, data from all batches produced should normally be provided, although,  
1007 depending on the nature of the product and the production history, it could be acceptable to provide  
1008 results from a justified number of representative batches. In the autologous setting, each  
1009 manufactured product should be viewed as a batch.

#### 1010 **S.4.5. Justification of specification**

1011 A justification for the quality attributes included the specification and the acceptance criteria for purity,  
1012 impurities, biological activity and any other quality attributes which may be relevant to the  
1013 performance of the medicinal product is required already for an exploratory clinical study. Early  
1014 selection of a potency assay and its proposed acceptance limits is recommended.

1015 The justification of specifications should be based on sound scientific knowledge supported by the  
1016 available development data, the batches used in non-clinical and/or clinical studies and data from  
1017 stability studies, taking into account the methods used for their control. The justification should  
1018 address how the respective quality attributes and acceptance criteria are relevant for the performance  
1019 of the medicinal product.

1020 It is acknowledged that during early clinical development when there is only limited experience, the  
1021 acceptance criteria may be wide. However, for those quality attributes that may impact patient safety,  
1022 the limits should be carefully considered taking into account available knowledge (e.g. impurities).

1023 Further refinement is expected as knowledge increases and data become available. Changes to a  
1024 previously applied specification (e.g. addition or removal of parameters, widening of acceptance  
1025 criteria) should be indicated and justified.

#### 1026 **S.5. Reference standards or materials**

1027 For medicinal products reference materials are normally utilised to ensure consistency between  
1028 different batches but also to ensure the comparability of the product to be marketed with that used in  
1029 clinical studies and to provide a link between process development and commercial manufacturing. For  
1030 ATIMPs it is recommended to establish a reference batch as soon as possible.

1031 Information regarding the manufacturing process used to establish the reference material should be  
1032 provided. If more than one reference standard has been used during the clinical development, a  
1033 qualification history should be provided describing how the relationship between the different  
1034 standards was maintained.

1035 For CBIMPs identification of suitable product reference standard may be challenging, especially in cases  
1036 where the manufacturing process does not foresee a freezing step and stored (frozen) reference  
1037 material might differ from the actual product.

1038 For GTIMPs, once a potency assay is established, a reference batch of vector of assigned potency  
1039 should be used to calibrate assays. The stability profile and relevant storage conditions of those  
1040 reference/calibration batches should be established.

1041 If other reference materials are available and used, they should be characterised with reliable state-of-  
1042 the-art analytical methods, to be sufficiently described.

#### 1043 **S.6. Container closure system**

1044 The immediate packaging material used for the active substance should be stated. A description of the  
1045 container closure system should also be provided. It should be indicated if the container closure *per se*  
1046 has a CE marking for the intended use under the EU legislation on medical devices. Information on the  
1047 sterilisation procedures of the container and the closure should be provided. A possible interaction  
1048 between the immediate packaging and the active substance should be considered (see stability).

1049 **S.7. Stability**

1050 ***Stability summary and conclusions (protocol / material and method)***

1051 A stability protocol covering the proposed storage period and storage conditions of the active  
1052 substance should be provided, including specification, analytical methods and test intervals. Unless  
1053 justified, the testing interval should follow ICH Q5C. The re-test period (as defined in ICH Q1A  
1054 guideline) is, however, not applicable to ATMPs.

1055 The quality of the batches of the active substance placed into the stability program should be  
1056 representative of the quality of the material to be used in the planned clinical trial.

1057 The stability samples of active substance entered into the stability program should be stored in  
1058 containers that use the same materials and container closure system as the active substance used to  
1059 manufacture the clinical trial batch. Containers of reduced size are usually acceptable for the active  
1060 substance stability testing.

1061 Studies should evaluate the active substance stability under the proposed storage conditions.  
1062 Accelerated and stress condition studies may help understanding the degradation profile of the product  
1063 and support extension of shelf-life and comparability studies.

1064 Stability-indicating methods should be included in this stability protocol to provide assurance that  
1065 changes in the purity / impurity profile and potency of the active substance would be detected. A  
1066 potency assay should be included in the stability protocol, unless otherwise justified.

1067 CBIMP:

1068 For CBIMPs, particularly in the autologous setting, stability studies can pose a challenge, due to ethical  
1069 considerations of using patient material. In these cases, it is acceptable to base early stability  
1070 evaluations on results with cells from healthy donors. The representativeness of this approach for  
1071 patient material, however, needs to be justified and investigated as development proceeds.

1072 GTIMP:

1073 For GTIMP, vector integrity, biological activity (including transduction capacity) and strength are critical  
1074 product attributes which should always be included in stability studies. It is appreciated, however, that  
1075 during early development the potency assay may not be fully developed. Where feasible forced  
1076 degradation studies may also provide important information on degradation products and identify  
1077 stability indicating parameters to be tested.

1078 In the case of products formulated with carrier or support materials, the stability of the complex  
1079 formed with the drug substance should be studied.

1080 ***Stability data / results***

1081 Stability data should be presented for at least one batch representative of the manufacturing process  
1082 of the clinical trial material. In addition, stability data of relevant development batches or batches  
1083 manufactured using previous manufacturing processes could be provided. Such batch data may be  
1084 used in the assignment of shelf life for the active substance provided appropriate justification of  
1085 representative quality for the clinical trial material is given.

1086 The relevant stability data available should be summarised in tabular format, specifying the batches  
1087 tested, date of manufacture, process version, composition, storage conditions, time-points, test  
1088 methods, acceptance criteria and results.

1089 For quantitative parameters, actual numerical values should be presented. Any observed data trends  
1090 should be discussed.

1091 The increase of available data and improved knowledge about the stability of the active substance will  
1092 need to be demonstrated during the different phases of clinical development. For confirmatory clinical  
1093 trials the applicant should have a comprehensive understanding of the stability profile of the active  
1094 substance.

#### 1095 ***Shelf-life determination***

1096 The claimed shelf-life of the active substance under the proposed storage conditions should be  
1097 provided and accompanied by an evaluation of the available data. Any observed trends should be  
1098 discussed.

1099 The foreseen storage period should be based on long term, real time and real temperature stability  
1100 studies, as described in ICH Q5C. Extension of the shelf-life beyond the period covered by real-time  
1101 stability data may be acceptable, if supported by relevant data, including accelerated stability studies  
1102 and/or relevant stability data generated with representative material.

1103 The maximum shelf-life after the extension should not be more than double, or twelve months longer,  
1104 whichever is the longest, than the period covered by real time stability data obtained with  
1105 representative batch(es).

1106 Where extensions of the shelf-life are planned, the applicant should commit to perform the proposed  
1107 stability program according to the presented protocol, and, in the event of unexpected issues, to  
1108 inform Competent Authorities of the situation, and propose corrective actions.

#### 1109 ***P Investigational medicinal product***

1110 Most of the ATIMP specific considerations made for Drug Substance are also applicable to the Drug  
1111 Product (DP) and will therefore not be repeated in this section. However, some specific considerations  
1112 as regards DP are outlined.

#### 1113 ***P.1. Description and composition of the investigational medicinal product***

1114 The qualitative and quantitative composition of the ATIMP should be provided including:

- 1115 • a short statement or a tabulated composition of the dosage form;
- 1116 • description of the product composition, i.e. list of all components (active substances, excipients  
1117 and any other structural components) of the product and their amount on a per-unit basis  
1118 (including overages, if any), the function of each component, and a reference to their quality  
1119 standards (e.g. compendial monographs or manufacturer's specifications);
- 1120 • description of accompanying components (e.g. medical devices to administer the product)  
1121 and/or accompanying diluent(s);
- 1122 • a brief description of the type of container and closure used for the product and accompanying  
1123 components or diluents, if applicable.

#### 1124 ***P.2. Pharmaceutical development***

1125 For early development there may be only limited information to include in this section.

1126 A short description of formulation development, including justification of any new pharmaceutical form  
1127 or excipient, should be provided. The usage of cryopreservation agent and its concentration should be  
1128 justified.

1129 For products requiring additional preparation of the medicinal product (e.g. reconstitution), the  
1130 compatibility with the used materials (e.g. solvents, diluents, matrix) should be demonstrated and the  
1131 method of preparation including the equipment used should be summarised (reference may be made  
1132 to a full description in the clinical protocol or in a separate document). Through appropriate studies it  
1133 should be demonstrated that the specified reconstitution process is sufficiently robust and consistent to  
1134 ensure that the product fulfils the specifications and can be administered without negative impact on  
1135 quality/safety/clinical properties of the ATMP.

1136 It should be documented that the combination of intended formulation and packaging material does  
1137 not impair correct dosing, ensuring for example that the product is not adsorbed to the wall of the  
1138 container or infusion system. This is particularly relevant for low dose and highly diluted presentations.  
1139 Where applicable, the reliable administration of very small doses in exploratory studies should be  
1140 addressed as laid down in the Guideline on strategies to identify and mitigate risks for first-in-human  
1141 and early clinical trials with investigational medicinal products (EMA/CHMP/SWP/28367/07 Rev. 1).

#### 1142 **Manufacturing process development**

1143 Any changes in the product during the clinical phases should be documented and justified with respect  
1144 to their impact on quality, safety, clinical properties, dosing and stability of the medicinal product.

1145 The relevance of the structural and functional characteristics of the non-cellular components in a  
1146 combination product should be discussed. Interaction of the cellular component and any additional  
1147 non-cellular components with the device should be evaluated and the development and characteristics  
1148 of the combined product as a whole should be presented.

#### 1149 **Comparability**

1150 Development of an ATIMP may encompass changes in the manufacturing process that might have an  
1151 impact on the final product. Changes in the manufacturing process including changes in formulation  
1152 and dosage form compared to previous clinical trials should be described. An appropriate comparability  
1153 exercise should support significant changes, e.g. formulation changes, considering their impact on  
1154 quality, safety, clinical properties, dosing and stability. In this regard, expectations are similar to those  
1155 described in S.2.6. This data should be sufficiently detailed to allow an appropriate understanding of  
1156 the changes and assessment of possible consequences to the safety of the patient. The same principles  
1157 to demonstrate comparability throughout development that apply to the active substance also apply to  
1158 the finished product.

### 1159 **P.3. Manufacture**

#### 1160 **P.3.1. Manufacturer(s)**

1161 The name(s), address(es) and responsibilities of all manufacturer(s) for each proposed production site  
1162 involved in manufacture, testing and batch release should be provided. In case multiple manufacturers  
1163 contribute to the manufacture of the ATIMP, their respective responsibilities need to be clearly stated.



1164 **P.3.2. Batch formula**

1165 The batch composition / formula for the batch(es) to be used for the clinical trial should be presented.  
1166 This should include a list of all components to be used. The batch sizes or range of batch sizes should  
1167 be given.

1168 **P.3.3. Description of manufacturing process and process**  
1169 **controls**

1170 A flow chart of all successive steps including in-process-testing should be given. The results of in-  
1171 process testing may be recorded as action limits or reported as preliminary acceptance criteria. During  
1172 development, as process knowledge is gained, further detail of in-process testing and the criteria  
1173 should be provided and acceptance criteria reviewed.

1174 **P.3.4. Control of critical steps and intermediates**

1175 Tests and acceptance criteria for the control of critical steps in the manufacturing process should be  
1176 provided. It is acknowledged that due to limited data at an early stage of development complete  
1177 information may not be available.

1178 The critical manufacturing steps required to ensure a given stage of cellular differentiation necessary  
1179 for the intended use should be controlled with relevant markers. Considerations on the manufacturing  
1180 process should also take into account the product-associated risk profile.

1181 If holding times are foreseen for process intermediates, periods and storage conditions should be  
1182 provided and justified by data in terms of physicochemical, biological and microbiological properties.

1183 For sterilisation by filtration the maximum acceptable bioburden prior to the filtration must be provided  
1184 in the application.

1185 Reprocessing may be acceptable for particular manufacturing steps only if the steps are adequately  
1186 described and appropriately justified.

1187 **P.3.5. Process validation and/or evaluation**

1188 Process characterisation / evaluation data should be collected throughout the development preparing  
1189 for Marketing Authorisation Application. At that stage the entire manufacturing process, storage etc.  
1190 should be validated. Refer to S.2.5 for further details on the extent of evaluation / validation data  
1191 required throughout development.

1192 The manufacturing process for CBIMP includes cell harvesting, cell manipulation, combination with  
1193 other components of the product, filling and packaging. Characterisation/evaluation of the production  
1194 process of a combined product should encompass all steps from separate components up to the final  
1195 combination to ensure consistent production.

1196 Aseptic processes (and, where applicable, sterilising processes) should be validated.

1197 Reconstitution of product:

1198 Reconstitution activities can be performed at the administration site. This covers activities required  
1199 after batch release and prior to the administration of the ATMP to the patient, and which cannot be  
1200 considered as manufacturing steps, e.g. thawing or mixing with other substances added for the  
1201 purposes of administration (including matrices). Grinding and shaping are part of surgical procedures



1202 and therefore are neither manufacturing, nor reconstitution activities. No activity that entails  
1203 substantial manipulation can, however, be considered reconstitution (e.g. cultivation).

1204 The reconstitution process has to be qualified and needs to be described. The description of the  
1205 reconstitution process should include all components that come into contact with the cells as part of  
1206 the clinical application (e.g. membranes for local containment, fibrin glues). For confirmatory clinical  
1207 trials the defined reconstitution process is expected to be validated.

#### 1208 **P.4. Control of excipients**

1209 Information on the choice of excipients, their properties, their characteristics and the design and  
1210 testing of a final scaffold/matrix should be provided in the dossier as part of the development  
1211 pharmaceuticals. Information on the vendor and source should also be provided. Matrices, scaffolds,  
1212 devices, biomaterials, or biomolecules or complexing materials which are not an integral part of the  
1213 active substance are considered as excipients of the finished product. The general principles that are  
1214 applied to the biological evaluation of medical devices can also be applied to the evaluation of  
1215 biomaterials intended as excipients.

1216 Established (non-novel) excipients should preferably be of pharmaceutical grade. When non-  
1217 pharmaceutical grade materials are used, the developers will have to invest more effort in in-house  
1218 characterisation and testing.

1219 - CBIMPs:

1220 Excipients should be qualified with respect to their combination with cells.

1221 The stability of the non-cellular components should also be assessed in the presence and absence of  
1222 cellular components. The effect of the cellular component or of the surrounding tissues on the  
1223 degradation (rate and, if appropriate, products) or stability of the structural component should be  
1224 investigated.

1225 - GTIMPs:

1226 Diluents or stabilisers or any other excipients added during preparation of the final vector or final  
1227 product should be shown not to impair the properties of the vector in the concentrations employed.

1228 Complexing materials for formulating the GTIMP drug product are considered as excipients and have to  
1229 be qualified for their intended purpose. The quality and purity of the complexing materials is essential  
1230 for the later quality of the GTIMP, therefore the appropriate characterisation and specification of the  
1231 complexing material(s) and qualification for their intended purpose are considered vital.

#### 1232 **P.4.1. Specification**

1233 References to the Ph.Eur., the pharmacopoeia of an EU Member State, USP or JP may be applied. For  
1234 excipients not covered by any of the aforementioned standards, an in-house specification should be  
1235 provided. Acceptance criteria should be presented preferably as quantitative limits, ranges, or other  
1236 attributes or variables for the tests described. Release criteria may be refined as product development  
1237 progresses toward the marketing authorisation application.

#### 1238 **P.4.2. Analytical procedures**

1239 Where an excipient is not described in a pharmacopoeial monograph listed under P.4.1, the analytical  
1240 methods used and their suitability should be described.

1241 **P.4.3. Validation of the analytical procedures**

1242 Reference is made to S.4.3.

1243 **P.4.4. Justification of specification**

1244 For non-compendial excipients as listed above in P.4.1, the in-house specifications should be justified.

1245 **P.4.5. Excipients of human or animal origin**

1246 For excipients of human or animal origin, information should be provided regarding adventitious agents  
1247 safety evaluation (e.g. sources, specifications, description of the testing performed) and viral safety  
1248 data according to the Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal  
1249 Products (EMA/CHMP/BWP/398498/05) in Appendix A.2. Furthermore, compliance with the TSE  
1250 guideline (EMA/410/01, current version) should be documented in section A.2.

1251 If human albumin or any other plasma derived medicinal product is used as an excipient, information  
1252 regarding adventitious agents safety evaluation should follow the relevant chapters of the *Guideline on*  
1253 *Plasma-Derived Medicinal Products* (EMA/CHMP/BWP/706271/2010). If the plasma derived component  
1254 has already been used in a product with a marketing authorisation then reference to this can be made.

1255 **P.4.6. Novel excipients**

1256 For excipient(s) used for the first time in a medicinal product or by a new route of administration, full  
1257 details of manufacture, characterisation and controls, with cross references to supporting safety data  
1258 (non-clinical and/or clinical), should be provided according to the active substance format (details in  
1259 A.3).

1260 **P.5. Control of the investigational medicinal product**

1261 **P.5.1. Specification**

1262 Quality control tests should be performed at the drug product level, unless appropriate justification can  
1263 be provided based on release testing at the drug substance level. Tests on attributes which are specific  
1264 to the formulated product in its final container and quality attributes which may have been impacted  
1265 by the formulation steps should be included in the release testing.

1266 The same principles as described for setting the active substance specification should be applied for the  
1267 medicinal product. In the specification, the tests used as well as their acceptance criteria should be  
1268 defined for the batch(es) of the product to be used in the clinical trial to enable sufficient control of  
1269 quality of the product.

1270 Tests for contents, identity and purity are mandatory. Tests for sterility and endotoxin are mandatory  
1271 for sterile products. Mycoplasma testing is required for CBIMPs. A potency test should be included  
1272 unless otherwise justified (see S.4.1).

1273 Acceptance criteria for medicinal product quality attributes should take into account safety  
1274 considerations and the stage of development. Since the acceptance criteria are normally based on a  
1275 limited number of development batches and batches used in non-clinical and clinical studies, their  
1276 nature is inherently preliminary. They may need to be reviewed and adjusted during further  
1277 development.

1278 The analytical methods and the limits for content and bioactivity should aim to ensure a correct dosing.

1279 Upper limits, taking safety considerations into account, should be set for the impurities. For the  
1280 impurities not covered by the active substance specification, upper limits should be set, taking safety  
1281 considerations into account.

1282 As knowledge and experience increases the addition or removal of parameters and modification of  
1283 analytical methods may be necessary. Specification and acceptance criteria set for previous trials  
1284 should be reviewed for confirmatory clinical trials and, where appropriate, adjusted to the current  
1285 knowledge and stage of development.

1286 In certain circumstances, namely with autologous cell products, limited amount of final product might  
1287 not allow for extensive release testing. In such circumstances it may be possible to rely on  
1288 intermediate product release criteria, provided these have been shown to be representative of the final  
1289 product based on sufficient process evaluation/ validation data.

1290 In some specific cases (for example due to the short shelf-life), it may be needed to release the drug  
1291 product batch prior to all results of specification testing is available. This approach needs to be justified  
1292 and supported by performed risk analysis. The procedure that is taken when out of specification test  
1293 results are obtained after the release of the product need to be described.

#### 1294 **P.5.2. Analytical procedures**

1295 The analytical methods should be described for all tests included in the specification.  
1296 For some complex or innovative pharmaceutical forms, a higher level of detail may be required.

1297 For further requirements refer to S.4.2.

#### 1298 **P.5.3. Validation of analytical procedures**

1299 For requirements refer to S.4.3.

#### 1300 **P.5.4. Batch analysis**

1301 For requirements refer to S.4.4

#### 1302 **P.5.5. Characterisation of impurities**

1303 Additional impurities and degradation products observed in the ATIMP, such as those resulting from the  
1304 interaction of the cells with the scaffold, but not covered by section S.3.2, should be identified and  
1305 quantified as necessary.

1306 The final product should be tested for residual manufacturing reagents with known or potential  
1307 toxicities and the test procedure described. Limits need to be included in the specifications, unless  
1308 otherwise justified

#### 1309 **P.5.6. Justification of specification**

1310 A justification for the quality attributes included in the product specification should be provided mainly  
1311 based on the active substance specification the composition of the DP and the MoA of the final product.  
1312 Stability indicating quality attributes should be considered. The proposed acceptance criteria should be  
1313 justified.

1314 **P.6. Reference standards or materials**

1315 The parameters for characterisation of the reference standard should be submitted, where applicable.  
1316 Section S.5 - Reference Standards or Materials - may be referred to.

1317 **P.7. Container closure system**

1318 The intended primary packaging to be used for the IMP in the clinical trial should be described and  
1319 compatibility with the product should be justified. Where appropriate, reference should be made to the  
1320 relevant pharmacopoeial monograph. If non-compendial materials are used, description and  
1321 specifications should be provided. For any device used in / as the container closure system, evidence  
1322 of CE mark for the intended use should be provided. If the product is packed in a non-certified  
1323 administration device, a description and specifications should be provided. For parenteral products with  
1324 a potential for interaction between product and container closure system more details regarding  
1325 biocompatibility may be needed. Where applicable, information on the sterilisation procedures of the  
1326 container and the closure should be provided.

1327 **P.8. Stability**

1328 The same requirements as for the active substance are applied to the medicinal product, including the  
1329 stability protocol, stability results, shelf-life determination, including extension of shelf-life beyond the  
1330 period covered by real-time stability data and stability commitment. The storage conditions including  
1331 temperature range should be defined and stability studies should provide sufficient assurance that the  
1332 IMP will be stable during the intended storage period. The stability protocol for the ATIMP should take  
1333 into account the knowledge acquired on the stability profile of the active substance.

1334 Transportation and storage conditions should be supported by experimental data with regard to the  
1335 maintenance of cell integrity and product stability during the defined period of validity. Where  
1336 applicable, product-specific methods for freezing and thawing should be documented and justified.

1337 For preparations intended for use after reconstitution, dilution or mixing, a maximum shelf life needs to  
1338 be defined and supported by in-use stability data.

1339 The stability of the non-cellular components should be assessed in the presence and absence of cellular  
1340 components in order to determine whether the non-cellular component undergoes degradation, or  
1341 physico-chemical alterations (e.g. aggregation, oxidation) that may impact on the quality of the  
1342 product by affecting cellular behaviour and survival. The effect of the cellular component or of the  
1343 surrounding tissues on the degradation (rate and, if appropriate, products) or stability of the structural  
1344 component should be assessed, considering also the effect of the non-cellular components throughout  
1345 the expected lifetime of the product.

1346 Bracketing and matrixing approaches may be acceptable, where justified.

1347 **A.1. Facilities and equipment**

1348 Not applicable.

1349 **A.2. Adventitious agents safety evaluation**

1350 All materials of human or animal origin used in the manufacturing process of both the active substance  
1351 and the medicinal product, or such materials coming into contact with active substance or medicinal  
1352 product during the manufacturing process, should be identified. Information assessing the risk with

1353 respect to potential contamination with adventitious agents of human or animal origin should be  
1354 provided in this section.

1355 The contamination of an ATIMP could originate from the starting or raw materials, or adventitiously  
1356 introduced during the manufacturing process. A thorough testing for the absence of bacteria, fungi and  
1357 mycoplasma shall be performed at the level of finished product. In cases where the short shelf life of  
1358 the CBIMP is prohibitive for the testing of absence of bacteria under the Ph.Eur. requirements in  
1359 chapters 2.6.1, alternative validated testing methods (as in Ph.Eur 2.6.27) are recommended.

1360 In what concerns viral safety, a risk assessment should be performed as indicated in Ph.Eur. 5.1.7 to  
1361 evaluate the possibility of viral contamination or reactivation of cryptic (integrated, quiescent) forms of  
1362 adventitious agents. Appropriate viral testing should be performed with validated methods. When a  
1363 continuous cell line is used in production, testing for presence of adventitious viruses should be  
1364 conducted according to the principles of ICH guideline Q5A and Ph.Eur. 5.2.3 should be followed.

### 1365 ***TSE agents***

1366 Detailed information should be provided on the avoidance and control of transmissible spongiform  
1367 encephalopathy agents. This information can include, for example, certification and control of the  
1368 production process, as appropriate for the material, process and agent.

1369 The *Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy*  
1370 *Agents via Human and Veterinary Medicinal Products* (EMA/410/01) in its current version is to be  
1371 applied.

### 1372 ***Viral safety***

1373 Where applicable, information assessing the risk with respect to potential viral contamination should be  
1374 provided in this section. Risk assessment should be performed according to Ph.Eur 5.1.7. General Text  
1375 on Viral Safety. The documentation should comply with the requirements as outlined in the Guideline  
1376 on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products  
1377 (EMA/CHMP/BWP/398498/05).

1378 Both contaminating extraneous viruses and residues of viruses used during production, such as  
1379 production viruses and helper viruses should be controlled. Bacteriophages are relevant contaminating  
1380 viruses for vectors which are produced on bacterial substrates.

1381 The risk of contamination of the drug substance or drug product by extraneous viruses should be  
1382 minimised by testing seed and cell banks in preparation for early phase trials; intermediates and end  
1383 products testing should also be established over time.

## 1384 **A.3. Excipients**

1385 For novel excipients, information as indicated in section S of the CTD should be provided in line with  
1386 the respective clinical phase.

## 1387 **A.4. Solvents for reconstitution and diluents**

1388 For solvents for reconstitution and diluents, the relevant information as indicated in section P of the  
1389 CTD should be provided as applicable.

1390

## 1391 **5. Non-clinical documentation**

### 1392 **5.1. General aspects**

1393 The non-clinical development pathway for ATMPs may be significantly different from the one for other  
1394 medicinal products including the timing of studies. The sequential non-clinical development in which  
1395 the amount of data required and the duration of dosing increase by the phase of clinical development  
1396 and by the number of patients, is not generally applicable for ATMPs. Instead, in many cases, the  
1397 majority of non-clinical data may need to be available before human exposure.

1398 In general, the non-clinical data supporting the safe use of an ATMP in humans should provide  
1399 information for the estimation of the safe and biologically effective dose(s) to be used in clinical trials,  
1400 support the feasibility of the administration route and the appropriate application procedure, identify  
1401 safety concerns and target organs for potential toxicity, and identify safety parameters to be followed  
1402 in the clinical trials.

1403 This guideline intends to provide recommendations for the non-clinical data requirements before first  
1404 dosing in humans and to give insights into the points where potential flexibility can be applied. The  
1405 extent of the non-clinical data needed to support initiation of clinical development and further clinical  
1406 development are dependent on the perceived risks related to the product itself, previous scientific  
1407 knowledge and clinical experience with similar type of products. It should be determined on a case-by-  
1408 case basis depending on the type of cells, extent of their manipulation, vector type, transgene  
1409 expression, genetic modification, availability of appropriate animal models, and the intended clinical  
1410 use. The extent and duration of exposure significantly affect the anticipated risks related to the clinical  
1411 use of an ATIMP. For example, if the product is intended to be used locally or kept isolated by physical  
1412 or biological means, the need for evaluation of systemic effects is rather low. Similarly, if the product is  
1413 anticipated to persist short-term in the body and is not expected to induce long-lasting effects, the  
1414 duration of non-clinical safety evaluation can be adapted accordingly. The risk-based approach can be  
1415 applied to identify the necessary non-clinical data on a case-by-case basis.

1416 The administration route and the application procedure should as closely as possible mimic those used  
1417 in the clinic. The dose levels tested in the non-clinical studies should provide information on the  
1418 minimal effective and the optimal dose levels to achieve the appropriate therapeutic effect in patients.  
1419 The chosen animal models should allow meaningful and predictive extrapolation from these species to  
1420 humans. Products used in non-clinical studies should be sufficiently characterised to provide  
1421 reassurance that the non-clinical studies have been conducted with material that is representative of  
1422 the product to be administered to humans in clinical studies. Differences between the non-clinical test  
1423 article and the clinical material resulting from product development should be highlighted and its  
1424 potential impact on efficacy and safety of the product should be discussed.

1425 The non-clinical studies can be carried out as stand-alone or as combined studies. Combining relevant  
1426 safety endpoints and biodistribution analysis in a proof of concept study can be done if feasible and  
1427 scientifically justified.

1428 The selection of suitable control groups should be carefully considered.

1429 In the case, where the risks related to the clinical use of the product are well understood and known  
1430 from previous clinical experience with related products, the non-clinical program may be adapted  
1431 accordingly provided that the perceived risks are manageable and adequately mitigated in the clinical  
1432 trial.

## 1433 **5.2. Animal models**

1434 The utility of animal models for non-clinical proof of concept studies and safety testing should be  
1435 carefully considered, and the relevance of selected models justified. The chosen animal model should  
1436 reproduce the disease or condition of the patients as close as possible with ideally similar  
1437 pathophysiology as in patients. Appropriate animal models may include naturally occurring  
1438 spontaneous or experimentally induced disease models, transgenic knock-out or knock-in disease  
1439 models, as well as specifically humanised animal models. Healthy animals are normally used for  
1440 standard toxicity studies. However, for ATIMPs, standard toxicity studies are not always appropriate to  
1441 address safety as a whole in the context of its therapeutic use. Instead, disease models can provide  
1442 clinically meaningful safety data.

1443 Small animal models such as rodents are often useful and widely employed since they are readily  
1444 available and easy to manipulate e.g. for the generation of transgenic models. However, if  
1445 extrapolation from small animal models to human becomes challenging due to e.g. a short or reduced  
1446 lifespan of the animal model or differences in the body size and anatomy that may preclude certain  
1447 administration procedures and devices in small animal models, large animal models may be needed.  
1448 Moreover, the use of homologous animal models is encouraged, wherever such models are expected to  
1449 provide more reliable data than a non-homologous model.

1450 The testing of human cells or a gene therapy vector in animal species may be impeded by immune  
1451 responses against the foreign cells or the viral vector (or its products), or by the lack of necessary  
1452 factors to support survival of human cells in the host, resulting in a premature and rapid elimination of  
1453 the administered product. In such cases, homologous animal models using the respective cells from  
1454 the same animal species and/or an orthologous transgene or a species-specific vector can be used. The  
1455 nature and characteristics of the homologous product as well as the manufacturing should be  
1456 representative of the product to be used in humans. If certain differences in the manufacturing cannot  
1457 be avoided, their potential impact on the predictability of non-clinical data needs to be carefully  
1458 considered.

1459 Generally, non-clinical studies should be done with the most appropriate pharmacologically relevant *in*  
1460 *vitro* and *in vivo* models available. The use of the same animal model in both the toxicology  
1461 investigations and the pharmacokinetic studies may be beneficial, as it allows correlation of the  
1462 biodistribution of the ATIMP with observed toxicity signals. In case a single animal model might not  
1463 suffice to address all relevant aspects, alternative animal models should be employed. For additional  
1464 guidance on the selection of animal species for GTIMPs, see Guideline on quality, non-clinical and  
1465 clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014).

1466 It is acknowledged that appropriate animal models are not always available. For example, in the case  
1467 where functional immune system of the host is needed to achieve the therapeutic effect e.g. correct  
1468 HLA matching or MHC molecule presentation, testing in animal species may not produce meaningful  
1469 information. In such cases, an alternative approach is needed to build up the weight of evidence  
1470 supporting the safe clinical use. Such an approach may include *in vitro* and *ex vivo* cell and tissue-  
1471 based models, *in silico* analyses, literature-based evidence and clinical experience with related  
1472 products.

1473 *In vivo* animal studies should be carefully planned to ensure generation of robust data while  
1474 considering the 3Rs (reduction, replacement, refinement) principles. Any animal testing resulting in  
1475 inconclusive data should be avoided. Where appropriate, animal testing could be replaced by *in vitro* or  
1476 *ex vivo* studies. To this end, the development and use of cell- and tissue-based models including 2D  
1477 and 3D tissue-models, organoids and microfluidics, are encouraged, especially for evaluating the mode  
1478 of action.



1479 **5.3. Pharmacology studies**

1480 • *Proof of concept*

1481 Data to demonstrate proof of concept are normally needed before human exposure in order to provide  
1482 functional evidence of the relevant biological activity to support the therapeutic rationale and clinical  
1483 testing of the product in the treatment of the intended disease or condition.

1484 Generally, animal disease models or experimentally induced models mimicking the condition to be  
1485 treated are considered most relevant for demonstrating the proof of concept. In addition, *in vitro* and  
1486 *ex vivo* cell and tissue-based models can be used to supplement or substitute *in vivo* animal studies to  
1487 demonstrate the proof of concept.

1488 The route and mode of administration should mimic the clinical use as closely as possible. In the  
1489 absence of clinical experience from the administration procedure and application devices, the feasibility  
1490 and safety of the application procedure and application devices should be tested in animal models  
1491 before clinical use.

1492 The dose levels for proof of concept should allow estimation of biologically effective dose and  
1493 meaningful extrapolation to establish the clinical starting dose. It is expected to determine an effective  
1494 dose without toxic effects of the product which exerts the desired pharmacological activity in the most  
1495 suitable animal model.

1496 • Transduction and expression

1497 In the case of GTIMPs, transduction and subsequent expression of transgene product is important for  
1498 interpretation of potential therapeutic effects observed in proof of concept studies. Differences in  
1499 tropism of a gene therapy vector between the animal species and human should be considered when  
1500 extrapolating the results from animals to humans. Therefore, the duration of the transgene expression  
1501 and the therapeutic effect, associated with the nucleic acid sequence, shall be described. The  
1502 relationship with the proposed dosing regimen in the clinical studies should be evaluated.

1503 When designing integrating vectors, applicants should take into account that epigenetics could  
1504 interfere with the efficacy and safety of the final GTMP. Therefore, it is encouraged, where applicable,  
1505 to investigate these issues further by performing *in vitro* analysis of genomic distribution of integrating  
1506 vectors in human cells. This will provide crucial information about 'host-on-vector' influences based on  
1507 the target cell genetic and epigenetic state during early development.

1508 If a replication-competent vector/virus is administered, the detection of viral sequences in non-target  
1509 sites by nucleic acid amplification technology (NAT) techniques should result in quantitative infectivity  
1510 assays in order to evaluate the infectious potential of the detected nucleic acid.

1511 Genome integration studies (*ex vivo* tissue culture or *in vivo* studies) should be performed for GTMPs  
1512 that are intended for integration in the host genome. For more information, see *Guideline on quality,  
1513 non-clinical and clinical aspects of gene therapy medicinal products*.

1514 • Pharmacokinetic studies

1515 Pharmacokinetics for ATIMPs depend on the type of the ATMP and include biodistribution (distribution  
1516 and migration), as well as elimination parameters (persistence and clearance).

1517 For cell-based ATIMPs, including genetically modified cells, distribution, migration and persistence of  
1518 the cells should be understood in order to identify relevant risks related to unwanted biodistribution,  
1519 and to focus the non-clinical safety studies to the aspects that are relevant for the intended clinical

1520 use. These data should also enable adequate design of the safety studies in terms of duration of the  
1521 follow-up and the target organs.

1522 Information on the persistence of cells within the host should guide the selection of relevant safety  
1523 studies as well as the study design and duration of follow-up in order to ensure sufficient monitoring to  
1524 capture both acute and late or delayed effects, and also, to avoid unnecessary testing in the case of  
1525 short-term transient persistence of the administered cells.

1526 The need for biodistribution studies is dependent on the administration route as well as the structural  
1527 or physiological containment of the cells. If cells are administered using an administration route that  
1528 enables distribution of the cells from the site of administration leading to systemic exposure,  
1529 biodistribution data are needed to identify potential target organs. In contrast, the distribution  
1530 potential of the cells is considered limited if the cells are either structurally or physically contained i.e.  
1531 grown onto a matrix or a scaffold, or applied to a confined space closed for example with a membrane  
1532 to prevent distribution of the cells. In such cases, biodistribution data may not be needed. The  
1533 structural integrity of the containment method at the site of administration needs to be demonstrated  
1534 to ensure that there is no unintended leakage of the cells.

1535 For the GTIMPs, the distribution profile of the gene therapy vector is important for an interpretation of  
1536 the therapeutic effects in the proof of concept studies, and it is therefore necessary prior to first  
1537 exposure to humans. A globally harmonised view on expectations for biodistribution analysis of GTIMPs  
1538 and considerations for study design, assay methodology and vector modification has been described in  
1539 the IPRP *Reflection Paper on Expectations for biodistribution (BD) assessments for gene therapy (GT)*  
1540 *products*)<sup>7</sup>.

1541 The dosing used for biodistribution studies should mimic the clinical use with appropriate safety  
1542 margins. The route of administration and the treatment regimen (frequency and duration) should be  
1543 representative for the clinical use with appropriate safety margins. In addition, evaluation of  
1544 biodistribution of the GTIMP after a single administration may add information on the clearance of the  
1545 administered GTIMP. If the administered vector is replication competent, biodistribution studies should  
1546 be designed to cover a second viremia as a result of replication of the vector/virus *in vivo*.

1547 Pharmacokinetic studies should additionally focus on clearance and mobilisation of the GTIMP.

1548 The risk of germline transmission should also be explored before the first use in humans (according to  
1549 the *Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors*).  
1550 The extent of studies will depend on the type of GTIMP, and can be based on a risk-based approach  
1551 (i.e. no risk in gene modified cells with integrating vectors or replication incompetent vector).

1552 • *Shedding*

1553 Shedding data are normally needed for the development of the clinical data and to safeguard the third  
1554 parties that may be exposed to the GTIMP. This information can be based on human data, published  
1555 data and/or a justification. Non-clinical shedding studies are not mandatory for GTIMPs if sufficient  
1556 information on potential sources of unintended exposure is available. For novel types of GTIMPs, non-  
1557 clinical shedding data are needed before clinical trials.

#### 1558 **5.4. Toxicity studies**

1559 Normally, non-clinical general safety or toxicity data are needed to support clinical testing. The need  
1560 for additional toxicity studies e.g. genotoxicity, tumourigenicity, reproductive and developmental

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<sup>7</sup> [http://development.iprp.backend.dev6.penceo.com/sites/default/files/2018-09/IPRP\\_GTGW\\_ReflectionPaper\\_BD\\_Final\\_2018\\_0713.pdf](http://development.iprp.backend.dev6.penceo.com/sites/default/files/2018-09/IPRP_GTGW_ReflectionPaper_BD_Final_2018_0713.pdf)

1561 toxicity, and immunotoxicity studies should be determined on a case by case basis taking into  
1562 consideration the risks related to the nature and characteristics of the particular class of ATMP and the  
1563 intended clinical use.

1564 The safety studies should be designed to generate clinically meaningful and predictive data to support  
1565 safe use of the product in the intended clinical indication. Safety studies in non-relevant species may  
1566 be misleading and are discouraged. For toxicology studies appropriate dose level(s), route and  
1567 methods of administration should be chosen to represent clinical use with appropriate safety margins.  
1568 The mode and schedule of administration shall appropriately reflect the clinical dosing. If the first-in-  
1569 human trial will include repeated dosing, this should be supported by repeat-dose toxicity data unless  
1570 otherwise justified (e.g. advanced cancer indication or immunogenicity restricts repeat-dosing in  
1571 animals).

1572 For ATIMPs intended for single administration, single-dose toxicology studies with an appropriately  
1573 extended post-dose observation period shall be performed to capture relevant safety concerns, e.g.  
1574 ectopic tissue formation or tumour formation. Multiple dose studies are needed only when repeated  
1575 dosing in patients is foreseen. The duration of follow-up should cover the time of persistence of  
1576 administered cells. However, in the case where administered cell-based product is intended to replace  
1577 a tissue and become an integral part of the body, the duration of non-clinical safety evaluation needed  
1578 to support the first human exposure should be determined on a case by case basis.

1579 The safety data can in many cases be collected from disease models to mimic the clinical use and to  
1580 capture safety concerns related to the product and the administration procedure. Separate stand-alone  
1581 safety/toxicity studies may not be needed if adequate safety endpoints are included in proof of concept  
1582 studies. In justified cases *in vitro* and/or *ex vivo* data can be used to replace or supplement *in vivo*  
1583 animal data. The overall safety evaluation should include analysis of cell persistence and biodistribution  
1584 pattern.

1585 One animal species can be considered sufficient if the model is considered predictive. However,  
1586 multiple animal species or strains may be needed to cover all relevant safety aspects on a case by case  
1587 basis.

1588 GLP

1589 It is generally expected that pivotal non-clinical safety studies are carried out in conformity with the  
1590 principles of GLP. However, it is recognised that, due to the specific characteristics of ATMPs, it would  
1591 not always be possible to conduct these studies in full conformity with GLP. The considerations for  
1592 application of GLP for ATMPs are described in the document: [Good laboratory practice \(GLP\) principles  
1593 in relation to ATMPs](#) (EMA, 26 January 2017).

## 1594 **5.5. Minimum non-clinical data requirements before first-in-human studies**

1595 The *Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with  
1596 investigational medicinal products* (EMA/CHMP/SWP/28367/07 Rev. 1) excludes ATMPs. However, the  
1597 principles described in the guideline may be followed where applicable.

1598 Due to specific characteristics of ATMPs, the majority of non-clinical safety data may need to be  
1599 available before first administration to humans. The extent of the non-clinical data package is  
1600 determined on a case-by-case basis taking into consideration the risks, or the lack of risks, associated  
1601 with the product and the intended clinical use, the availability of animal models and publicly available  
1602 information from similar type of products. In exceptional cases, where appropriate *in vitro*, *ex vivo* or  
1603 *in vivo* data with predictive value cannot be generated, a comprehensive risk assessment addressing

1604 risks related to the ATIMP and its clinical use should be provided, and measures to mitigate the risks  
1605 should be described.

1606 At a minimum, the following information should be available before human exposure:

- 1607 – demonstration of proof of concept in a relevant model;
- 1608 – support for the use of administration route, application procedure and application devices;
- 1609 – support of the selection of safe and biologically effective starting dose with adequate safety  
1610 margins for clinical use;
- 1611 – appropriate safety data.

- 1612 • *Proof of concept*

1613 Proof of concept studies can include *in vivo* models mimicking the disease or condition to be treated and  
1614 *in vitro* and *ex vivo* studies to demonstrate mode of action and functionality of the cells and/or the  
1615 expression of the transgene. In cases where the time needed to demonstrate therapeutic effect is very  
1616 long i.e. > 1 year, it is justified to provide an interim analysis of non-clinical proof of concept data of  
1617 shorter duration to support the exploratory clinical study. The duration of such study should be sufficient  
1618 to demonstrate relevant functionality of the product that can be considered predictive of therapeutic  
1619 effect (e.g. formation of a repair tissue for tissue engineered products). The long-term proof of concept  
1620 data can be provided at later stage of development.

- 1621 • *Safety pharmacology*

1622 Safety pharmacology data are not routinely needed for ATIMPs. When potential effects on major vital  
1623 physiological functions i.e. cardiovascular, central nervous system, or respiratory function are  
1624 anticipated, appropriate safety pharmacology data should be available before human exposure. Safety  
1625 pharmacology endpoints can be incorporated in the toxicity studies, if feasible.

- 1626 • *Biodistribution*

1627 These data should be available to provide information on the persistence, duration of effect, and target  
1628 organs in order to support the design and duration of safety study(ies). Extrapolation of information  
1629 which has been obtained from similar type of products using the same route of administration can be  
1630 justified in certain cases e.g. for adenoviral or adeno-associated viral vectors and be used to support  
1631 initiation of clinical development. In contrast, for some products such as replication-competent viral  
1632 vectors, extrapolation from other products may not be appropriate and non-clinical biodistribution  
1633 studies are expected to be conducted to support the first clinical trial.

1634 Information derived either from non-clinical shedding studies or from other sources on potential  
1635 shedding via excreta should be available for investigational gene therapy medicinal products before  
1636 human exposure to safeguard the third parties.

1637 Validation of the bioanalytical methods may not be needed before first clinical study. However,  
1638 sufficient information on the suitability of the used method e.g. specificity and sensitivity (limit of  
1639 detection) should be provided. Further validation can be conducted for biodistribution analyses to  
1640 support later phase clinical development.

- 1641 • *Safety/toxicity*

1642 General safety/toxicity studies should provide information for estimation of safe starting dose, dosing  
1643 regimen and identify relevant safety concerns in the intended clinical use. It may be acceptable to use

1644 safety information collected from a well-designed proof-of-concept study(ies) incorporating adequate  
1645 safety endpoints to support first-in-human studies.

1646 • *Genotoxicity*

1647 For integrating GTMP vectors, insertional mutagenesis shall be evaluated carefully in relevant *in vitro*  
1648 and/or *in vivo* models before exposing humans. The requirement for genotoxicity studies of GTIMPs  
1649 involving host-DNA integration will depend on the way the final product will be delivered (local versus  
1650 systemic), to which tissue/organ the GTIMP will be targeted and the biological status of the cells to be  
1651 targeted. Standard genotoxicity assays are generally not appropriate but may be required to address a  
1652 concern about a specific impurity or a component of the delivery system.

1653 • *Tumourigenicity*

1654 Generally, the risk of tumour formation needs to be addressed before exposing humans. Standard  
1655 lifetime rodent carcinogenicity studies are usually not required. However, depending on the type of  
1656 product, the tumourigenic and oncogenic potential shall be investigated in relevant *in vitro/in vivo*  
1657 models for neoplasm signals, oncogene activation or cell proliferation index. Publically available data  
1658 can be used in support of risk assessment. The extent of non-clinical data is dependent on the  
1659 perceived risk of tumour formation, and should be based primarily on *in vitro* and *ex vivo* analyses  
1660 which in some cases may need to be supplemented with *in vivo* data.

1661 • *Immunogenicity and immunotoxicity*

1662 Delivery of ATIMPs can result in immune responses of the innate and adaptive immune systems. These  
1663 aspects should be considered during the non-clinical development as part of the overall toxicology  
1664 assessment of the product including e.g. histological analysis of immune system activation both locally  
1665 and systemically. The impact of an unwanted immune response on the fate of an administered ATIMP  
1666 needs to be addressed before human exposure.

1667 **5.6. Non-clinical data that can be provided at later stages of development**

1668 For ATIMPs that are expected to persist in the body for extended period of time, interim or short term  
1669 safety data can be used to support first-in-human study. In such cases, long term safety data can be  
1670 provided to support later phase development.

1671 Generally, repeat-dose toxicity data are needed to support multiple administrations in humans.  
1672 However, a clinical study with multiple administrations could be initiated without repeat-dose toxicity  
1673 data provided that such data are available before multiple dosing in humans commences. This approach  
1674 might be justified e.g in the case where dosing interval is very long or when the ATIMP has been  
1675 eliminated from the body before subsequent administrations.

1676 In the case where preliminary biodistribution data have been provided to support the first-in-human  
1677 study or where there are sufficient data from similar type of products, definitive biodistribution data  
1678 including migration or distribution to target and non-target organs, and long-term persistence can be  
1679 provided before exposing larger patient populations.

1680 For tumourigenicity, a comprehensive risk assessment including karyotype, genomic stability and  
1681 possible literature data from similar type of products, should always be available before exposing  
1682 humans. Tumourigenicity data can in some cases e.g. advanced cancer or when administered to a  
1683 location where long-term persistence can easily be monitored, be provided before exposing larger  
1684 patient populations. Although stand alone *in vivo* tumourigenicity studies are not normally necessary,  
1685 relevant information can be gained in adequately designed long-term safety (or proof of concept) study.

1686 The need to address any safety concerns arising from previous clinical study(ies) should be considered  
1687 and addressed before exposing further human subjects.

1688 Where needed, immunogenicity assessment, if not available from the previous proof of concept or safety  
1689 studies, can be provided at later stages.

1690 If effects on reproductive function and/or development are anticipated relevant reproductive and  
1691 developmental toxicity studies should be conducted before exposing larger patient populations.

## 1692 **5.7. Combined ATMPs**

1693 The final combined ATMP needs to be tested in non-clinical experiments.

1694 Non-clinical data needed for the device component alone:

1695 - For medical device components that are CE-marked for the intended use, the non-clinical safety data  
1696 that is evaluated and accepted by a Notified Body should be provided.

1697 - For medical device components that are not CE-marked or that are CE-marked for another use, non-  
1698 clinical safety data in accordance with the Medical device legislation are needed before clinical use.

1699

## 1700 **6. Clinical documentation**

### 1701 **6.1 General aspects**

1702 In general, for ATIMPs the same principles as for other IMPs apply for the clinical development and the  
1703 protocol should be structured according to Annex I of Regulation 536/2014.

1704 However the distinctive characteristics and features of ATMPs are expected to have an impact on the  
1705 trial design, specifically with regards to early phase trials and dose selection, pharmacodynamics,  
1706 pharmacokinetics/biodistribution, while the general principles in late phase trials to demonstrate  
1707 efficacy and safety in the specific therapeutic area are less affected and are essentially the same as for  
1708 other products.

1709 Distinctive features of ATMPs include:

- 1710 • complexity of products, product characteristics and manufacturing considerations, e.g.  
1711 difficulties in the collection and handling of source material, differences between allogeneic vs.  
1712 autologous origin of the cells;
- 1713 • collection procedures, e.g. apheresis, and concomitant medication, e.g. lymphodepleting  
1714 chemotherapy;
- 1715 • limitations to extrapolate from animal data: starting dose, biodistribution, immunogenicity, on-  
1716 and off-target effects and tumourigenicity;
- 1717 • uncertainty about frequency, duration and nature of side effects, persistence in humans and  
1718 immunogenicity;
- 1719 • uncertainty about transformation, genotoxicity, tumourigenicity e.g. in case of integrating  
1720 vector;
- 1721 • risk of shedding and germ line transmission;

- 1722 • the need for long-term efficacy and safety follow-up, based on prolonged biological activity  
1723 and/or persistence of cells;
- 1724 • administration procedures/delivery to target site.

### 1725 **6.1.1 Anticipated benefits and risks for trial subjects**

1726 According to Directive 2001/20/EC and Regulation 536/2014, the known and potential risks and  
1727 benefits for the patient including an evaluation of the anticipated benefit and risk should be included in  
1728 the trial protocol.

1729 Potential benefits and risks include:

- 1730 • the anticipated effect;
- 1731 • the trial population (adult/paediatric);
- 1732 • available treatment options and medical need;
- 1733 • differences of trial-related interventions to normal clinical practice and existing therapies,
- 1734 • additional trial interventions, e.g.
  - 1735 ○ apheresis, conditioning regimen or lymphodepletion,
  - 1736 ○ infusion of DMSO,
  - 1737 ○ surgical or implantation procedures, e.g. in case of tissue engineered products;
- 1738 • potential risks related to the ATIMP itself, e.g.
  - 1739 ○ risks related to quality, manufacturing, supply chain,
  - 1740 ○ risks identified in non-clinical studies, or theoretical risks related to off-target events  
1741 and/or not identified in non-clinical studies (e.g. genome editing ATMPs),
  - 1742 ○ for ATMPs based on viral vectors: the risk of shedding, replication-competence and  
1743 possibility of reactivation of endogenous viruses or complementarity with endogenous  
1744 viruses;
- 1745 • risks of insertional mutagenesis in case of GTMPs;
- 1746 • risks related to immune reactions.

1747 Sponsors should outline in the benefit-risk assessment how expected and potential risks are addressed  
1748 and minimized. Respective risk minimisation measures should be implemented in the trial protocol.

### 1749 **6.1.2. Trial population**

1750 Clinical trials involving ATIMPs are usually conducted in patients and not in healthy volunteers.

1751 The rationale and justification for the choice of the study population should be discussed in the  
1752 protocol.

1753 The population should be selected based on an acceptable balance of risks and anticipated benefits of  
1754 treatment with the ATIMP. Other considerations when choosing a trial population may include pre-  
1755 existing immunity to the product or active substance and the potential that some ATIMPs may have an  
1756 effect on future treatment options (e.g. organ transplants) due to a long lasting effect or



1757 immunogenicity. The stage of disease and the ability of subjects with late stage disease to tolerate the  
1758 treatment may also be considered when choosing a trial population.

1759 For paediatric indications, prior studies in adults should be considered if feasible for the condition i.e.  
1760 unless the disease affects children exclusively or if the phenotypical presentation in adult differs from  
1761 that in children.

### 1762 **6.1.3. Contraceptive measures**

1763 Contraception for clinical trials involving ATIMPs should follow the General principles of the  
1764 *Recommendations related to contraception and pregnancy testing in clinical trials*<sup>8</sup>.

1765 The length of exposure to the ATMP may be lifelong. Some ATIMPs may have the potential to cross the  
1766 placenta. There may be no or limited data available from non-clinical studies about potential  
1767 reproductive toxicity effects. In cases where there is a strong suspicion of human  
1768 teratogenicity/foetotoxicity in early pregnancy based on non-clinical data the inclusion of women of  
1769 childbearing potential requires use of highly effective contraceptive measures. The protocol or  
1770 investigators brochure (IB) should include an evaluation of the period of potential risk and a  
1771 justification for the duration of contraceptive measures. Contraceptive measures should be continued  
1772 during treatment and until the end of the period of potential risk.

1773 In the case of male subjects who are treated with a gene therapy, at least two methods of  
1774 contraception including male barrier protection should be used during the time the virus is shed in the  
1775 semen and for a period of three months after there is no virus shed.

## 1776 **6.2 Exploratory clinical trials**

### 1777 **6.2.1 General considerations**

1778 For exploratory trials, especially for the First-in-human trials, the primary objectives are the safety and  
1779 tolerability.

1780 The design of exploratory trials of ATIMPs often involves consideration of clinical safety issues different  
1781 from other medicinal products (including extended or permanent adverse effects, e.g. long-term or  
1782 delayed safety issues, such as infections, immunogenicity/immunosuppressant, integration into the  
1783 genome of some GTIMPs, ectopic tissue formation and malignant transformation).

1784 Other objectives of exploratory trials are:

- 1785 • pharmacokinetics and biodistribution;
- 1786 • identification and characterisation of the manufacturing and administration issues that can  
1787 influence the product development;
- 1788 • assessment of pharmacodynamics, early measurement of drug activity e.g. gene expression,  
1789 cell engraftment;
- 1790 • assessment of the feasibility of recruitment, treatment approach and the use of the ATMP;
- 1791 • dose selection and determination of recommended dose for confirmatory studies.

1792 First-in-human (FIH) studies are a subset of exploratory studies, when the ATIMP is the first time  
1793 translated from non-clinical studies to humans. The design of FIH clinical trials with ATIMPs deserves

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<sup>8</sup> [http://www.hma.eu/fileadmin/dateien/Human\\_Medicines/01-About\\_HMA/Working\\_Groups/CTFG/2014\\_09\\_HMA\\_CTFG\\_Contraception.pdf](http://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf)

1794 specific considerations. For example, the extrapolation from non-clinical pharmacodynamic,  
1795 pharmacokinetic/biodistribution and toxicity data to the human situation may be limited, depending on  
1796 the relevance of the non-clinical animal model. This may hamper, amongst others, the prediction of a  
1797 safe starting dose for FIH trials and the prediction of target organs of toxicity. Thus, although  
1798 Advanced Therapies are exempt from the scope of the *Guideline on strategies to identify and mitigate*  
1799 *risks for first-in-human and early clinical trials with investigational medicinal products* the outlined  
1800 principles to mitigate risk are applicable.

1801 Exploratory studies with ATIMPs are often designed as phase I/II trials, combining features of phase I  
1802 and phase II design. Examples are trials with GTMPs in patients with monogenetic disease, where dose  
1803 escalation and determination of a recommended dose is followed by an extension phase, to include  
1804 additional patients on the recommended dose level and to further explore the efficacy of the GTMP.  
1805 The trial protocol should define the methodology to move from the dose-escalation phase to the  
1806 extension phase, and how this is captured in a substantial amendment.

1807 In case that major manufacturing process changes are implemented, these should be implemented and  
1808 evaluated clinically before starting confirmatory trials (see also sections S.2.6 and P.2).

### 1809 **6.2.2. Safety and tolerability objectives**

1810 As with other medicinal products, assessment of safety should be the focus of exploratory studies and  
1811 included as a main objective. The ATIMP dose to be administered is either derived from non-clinical  
1812 studies with the product, suggesting safe use in humans, or from data of related products. The use of  
1813 literature data is expected to be more difficult in cases where the product has been extensively  
1814 manipulated, or where a product contains a non-cellular component which may pose additional safety  
1815 concerns. In this case the safety of both components needs to be addressed prior to entering clinical  
1816 development.

1817 Factors to consider in the risks assessment of ATIMPs are related especially to the mode of action, the  
1818 nature of the target, the study population, previous experience in humans with the product or the  
1819 same class of products, if any, and/or the relevance of animal models (see also section 6.1.1).

1820 Increased risk can be expected in ATIMPs with mode of action on multiple systems; in cases, when  
1821 amplification of an effect might not be sufficiently controlled by a physiologic feedback mechanism  
1822 (e.g. immune system; blood coagulation system); when insufficient knowledge on the mode of action  
1823 or on biodistribution is available and in cases with questionable relevance of animal species/models.

1824 The risk of the therapeutic procedure as a whole, e.g. the required surgical procedures to administer  
1825 the ATIMP (e.g. multiple injection, intra cerebral application), the use of general or regional anaesthesia  
1826 or the use of immunosuppressive therapy, shall be evaluated and used to justify the clinical studies  
1827 and the choice of the target patient population. When a surgical procedure is involved, as is the case  
1828 for implantation of chondrocyte-containing products, or intramyocardial injection in the case of cardiac  
1829 indications, potential problems associated with variability of the surgical implantation procedure among  
1830 centres and surgeons should be taken into account. Standardization of the administration procedure  
1831 prior to entering clinical studies is recommended.

1832 All safety issues arising from the non-clinical development should be addressed, especially in the  
1833 absence of an animal model of the treated disease or in the presence of physiologic differences limiting  
1834 the predictive value of homologous animal model.

1835 Particular attention should be paid to those biological processes including immune response, infections,  
1836 ectopic tissue formation, malignant transformation and concomitant treatment during development and  
1837 post-marketing phase of ATIMPs. For trials involving paediatric populations, specific issues such as

1838 requiring preliminary safety data in adults, effects on reproductive health or germline expression may  
1839 arise.

1840 In case of an anticipated risk including events with a late onset (e.g. tumourigenicity), measures to  
1841 detect the signal and to mitigate this risk should be implemented.

1842 Special consideration should be taken in the design of the clinical study and risk evaluation when  
1843 medical devices are used for the delivery or implantation of a ATIMP. Information regarding the safety  
1844 and compatibility of the delivery system should be provided. This information is in general derived  
1845 from quality and non-clinical studies that have been designed to assess performance of the delivery  
1846 system.

### 1847 **6.2.3. Dose finding and dose escalation**

1848 A rationale for the selected starting dose, dose escalation scheme and dosing schedule is required in  
1849 the trial protocol. The predictive value of non-clinical studies for a safe starting dose in humans varies  
1850 considerably, and is influenced by different factors, like ATMP class, type and schedule of  
1851 administration/implantation, type of disease and availability of relevant animal models. In case of  
1852 GTMPs consisting of viral vectors, non-clinical studies in relevant animal models with measurable levels  
1853 of the transgene product (protein or enzyme) may allow more precise prediction of the starting dose,  
1854 compared to cell-based products.

1855 The goal of selecting a starting dose is to identify a dose that is expected to have a pharmacological  
1856 effect and is safe to use. The assessment of a safe and minimal effective dose should be followed by  
1857 further dose exploration. Also, the correlation between exposure and effect should be evaluated with  
1858 the goal to establish an effective dose range and recommended dose. The recommended dose of the  
1859 ATIMP can then be further evaluated, either in expansion cohorts or in separate subsequent clinical  
1860 trials. If appropriate, a maximum tolerated dose should be assessed, for example in oncology and  
1861 haematological indications.

1862 The rationale for dose and schedule is based on the totality of non-clinical data. Differences in  
1863 engraftment, differentiation, persistence and immunogenicity between animals and humans may limit  
1864 the predictive value of non-clinical dose-finding studies, as in the case of e.g. genetically modified  
1865 CD34 positive cells for treatment of severe immune deficiencies. Aspects to take into account for  
1866 selecting dose and schedule are product-specific attributes like cell type and origin (autologous versus  
1867 allogeneic), transduction efficiency, number of transduced cells versus non-transduced cells, mean  
1868 number of vector copies per cell and cell viability, potency and biologic activity, type of co-stimulatory  
1869 molecule, and transgene expression. In case a concomitant preceding conditioning regimen is required,  
1870 the initial dosing can be derived from haematopoietic transplantation, taking into account the necessity  
1871 to apply a minimum dose of CD34 positive cells required to ensure engraftment, and to avoid  
1872 prolonged bone marrow suppression.

1873 A rationale for the schedule of administration, e.g. single or repeated administration should be  
1874 provided, depending on the type of ATIMP, biodistribution, persistence, and ATIMP induced immune  
1875 reaction.

### 1876 **6.2.4. Staggered enrolment**

1877 In FIH studies the treatment of several patients of a dosing cohort or escalating the dose without  
1878 assessing acute and delayed adverse events may put study subjects at risk.

1879 Thus, the first patient in a FIH trial should be intensively monitored for adverse events, taking into  
1880 consideration also delayed adverse events (related to IMP or related to procedure). A waiting period

1881 between treatment of first and subsequent patients in the same dosing cohort should be implemented  
1882 to allow assessment of acute and subacute toxicities, and implementation of stopping rules to halt the  
1883 trial or prevent further patient recruitment.

1884 The choice of the waiting period should take into consideration the time course and nature of acute and  
1885 subacute toxicities in animals and previous experience in humans, if any, with related/similar ATIMPs.  
1886 In addition, study drug administration in the next cohort should not occur before participants in the  
1887 previous cohort have been treated and data/results from those participants have been reviewed in  
1888 accordance with the protocol/drug safety monitoring board (DSMB) charter.

#### 1889 **6.2.5. Pharmacokinetic objectives**

1890 Assessment of pharmacokinetics is another objective of the exploratory clinical trials. Classical  
1891 pharmacokinetic assessment of absorption, distribution, metabolism and excretion (ADME) may not be  
1892 possible or relevant for some types of ATIMPs.

1893 For cell based therapies where conventional ADME assessment cannot be conducted, pharmacokinetic  
1894 assessment should be conducted where feasible to monitor viability, proliferation/differentiation,  
1895 tumourigenicity, immunogenicity, body distribution, ectopic foci, tissue tropism/migration, and  
1896 functionality during the intended viability of the cells/products.

1897 If appropriate, conventional pharmacokinetic assessment, including as a minimum determination of  
1898 (plasma) concentration and half-life, should be performed for the therapeutic transgene product (i.e.  
1899 therapeutic protein) using appropriate and up-to-date bioanalytical assays.

#### 1900 **6.2.6. Pharmacodynamic objectives**

1901 Pharmacodynamic (PD) assessments are intended to substantiate the proof-of-concept. The selected  
1902 PD outcome measures should support the activity of the ATIMP.

1903 In case of GTIMPs, PD assessments are performed to study the expression and function of the gene  
1904 expression product (e.g. as a protein or enzyme, including conversion of prodrugs by therapeutic  
1905 enzymes or induction of immune response) while in other cases the effect of the vector itself is  
1906 addressed (e.g. recombinant oncolytic virus). Appropriate and up-to-date bioanalytical assays should  
1907 be used.

1908 In case of an investigational somatic cell therapy product with immunological function e.g. a cancer  
1909 immunotherapy, PD readouts include cellular and humoral immune response. In case of an  
1910 investigational tissue engineered product where the intended use is to restore/replace cell/tissues, with  
1911 an expected lifelong functionality, structural/histological assays may be potential pharmacodynamic  
1912 markers.

### 1913 ***6.3 Confirmatory phase clinical trials***

#### 1914 **6.3.1 General considerations**

1915 Confirmatory studies should be in accordance to the existing general guidelines for the specific  
1916 therapeutic area.

#### 1917 Clinical trial design

1918 The main points to address in the designs are: choice of target population and of control group,  
1919 blinding, choice of primary and secondary endpoints, study duration, sample size estimation, and  
1920 statistical design.

1921 A description and a justification of the type/design of trial should be provided as well as a schematic  
1922 diagram of trial design and procedures.

1923 Randomized controlled, comparative trials are preferable over single arm trials, or trials with external,  
1924 historical controls, as they eliminate confounding baseline variables, reduce bias and are better  
1925 suitable to obtain an unbiased estimate of the treatment effect. Where reference therapies are not  
1926 available comparison to best supportive care or treatment based on investigator's choice is expected to  
1927 provide evidence of efficacy and is preferred over single arm trials.

1928 For indications in orphan conditions the planning of confirmatory trials should take into account the  
1929 principles outlined in the *Guideline on clinical trials in small populations* (CHMP/EWP/83561/2005).

1930 For some indications a comparator treatment may not be available or it may be unethical to conduct a  
1931 trial using placebo as a comparator. In cases that standard of care, historic/prospective controls or  
1932 data from a disease registry are used, a sound rationale needs to be provided, including a justification  
1933 on the validity of the registry data. Using a sham procedure may also be considered as a comparator,  
1934 dependent upon the additional risks posed to the patient.

1935 For some ATIMPs an intra-subject control with an appropriate run-in phase might be a useful approach.

1936 The trial design should include instructions to ensure blinding of the trial when appropriate and feasible  
1937 e.g. where the person involved at the clinical site in the preparation of the ATIMP cannot be blinded,  
1938 but the health care professional administering the product is blinded. If single or double blinding is not  
1939 possible, this should be appropriately justified, e.g. when surgical procedures are involved. In this case  
1940 the person assessing of the primary efficacy endpoint should be blinded to treatment and act as  
1941 independent reviewer.

### 1942 **6.3.2 Efficacy**

1943 Clinical efficacy endpoints as defined in specific guidance for the studied indication or disease are the  
1944 basis for the clinical evaluation of ATIMPs. The primary objective is to demonstrate, or confirm  
1945 therapeutic benefit. For investigational TEP, additional cell- and tissue-specific endpoints may be  
1946 required such as biochemical, morphological, structural and functional parameters, which are relevant  
1947 for the targeted therapeutic claim. These endpoints can be used as co-primary or secondary variables,  
1948 and are expected to support the clinical primary efficacy variable. In cases where long-term efficacy is  
1949 expected, the endpoints should also focus on the duration of the response. As for any conventional  
1950 medicinal product, any non-validated endpoint or surrogate endpoint, such as novel biomarkers, would  
1951 have to be validated in a prospective study before being used in confirmatory clinical trials.

1952 Sometimes, the desired clinical endpoint, such as prevention of arthrosis, can be observed only after a  
1953 long follow-up. In such cases, additional surrogate endpoints might be included in the trial to support a  
1954 later marketing authorisation. If the efficacy is dependent on the long-term persistence of the product,  
1955 a long-term follow-up plan of the patients should be provided.

### 1956 **6.3.3 Clinical safety**

1957 The detection of the risks should continue during confirmatory phase clinical trials in order to prevent  
1958 and/or minimise the risks. The information regarding the detected (important and potential) risks  
1959 contained in the Development Safety Update Reports could provide the basis for the Risk Management  
1960 Plan (see ICH E2F on development safety update report). Regarding the possible risks in relation to  
1961 ATIMPs, reference is made to the Risk Based Approach methodology as well as the risks listed in  
1962 section 5.1 of the revised *Guideline on safety and efficacy follow-up and risk management of Advanced  
1963 Therapy Medicinal Products* (EMA/149995/2008 rev.1).

1964 In case of an anticipated risk including events with a late onset (e.g. tumourigenicity), measures to  
1965 detect the signal and to mitigate this risk should be implemented.

1966 The safety database should be large enough to predict the safety profile of the ATMP, to implement  
1967 appropriate risk mitigation activities ensuring its safe use post-authorisation.

#### 1968 **6.4 Long term efficacy and safety follow-up**

1969 Long term efficacy and safety follow-up and long term monitoring of patients treated with an ATIMP  
1970 needs to take into account the nature of the ATIMP and its persistence.

1971 The ATMP developers should ensure that patients enrolled in clinical trials (starting with FIH trials) are  
1972 appropriately followed-up in order to generate long-term efficacy and safety data sufficient to support  
1973 the marketing authorisation application. The need for, the duration and the type of follow-up should be  
1974 described in the clinical trial protocol.

1975 The duration of efficacy and safety follow-up should be identified during the exploratory clinical trials,  
1976 also taking into consideration results from non-clinical studies.

1977 The long-term efficacy and safety monitoring should be appropriately designed (e.g. sampling plan,  
1978 sample treatment, analytical methods, endpoints) in order to maximize information output especially  
1979 when invasive methods are used. This is of specific importance when the ATIMP is intended to provide  
1980 life-long persistence of biological activity and treatment effects but also because some ATIMPs have  
1981 high potential for immunogenicity or relatively invasive procedures are needed to administer them.  
1982 Product persistence is assessed by looking for evidence of the presence of cells, vector, or virus in  
1983 biological fluids or tissues. Activity might be assessed by looking for e.g. gene expression or changes in  
1984 biomarkers.

1985 Follow-up of patients should be more intensive in first two years after treatment and for CBIMP and  
1986 GTIMP with increased risk of late onset of adverse reactions (e.g. tumourigenicity) this follow-up period  
1987 should be extended.

## 1988 **Definitions**

## 1989 **References**

1990 Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to  
1991 ATMPs (EMA/CAT/CPWP/686637/2011)

1992 Guideline on strategies to identify and mitigate risks for First-in-Human Clinical Trials with  
1993 Investigational Medicinal Products (Doc. Ref. EMEA/CHMP/SWP/294648/2007)

1994 Reflection paper on classification of advanced therapy medicinal products (EMA/CAT/600280/2010  
1995 rev.1) [https://www.ema.europa.eu/documents/scientific-guideline/reflection-paper-classification-  
1996 advanced-therapy-medicinal-products\\_en-0.pdf](https://www.ema.europa.eu/documents/scientific-guideline/reflection-paper-classification-advanced-therapy-medicinal-products_en-0.pdf)

1997 Guideline on the environmental risk assessment of medicinal products for human use  
1998 (EMA/CHMP/SWP/4447/00 corr 2)

1999 Guideline on environmental risk assessments for medicinal Products consisting of, or containing,  
2000 genetically modified organisms (GMOs) (EMA/CHMP/BWP/473191/2006 - Corr)

2001 Guideline on scientific requirements for the environmental risk assessment of gene therapy medicinal  
2002 products (EMA/CHMP/GTWP/125491/2006)



- 2003 Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal  
2004 Products [https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-](https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf)  
2005 [4/2017\\_11\\_22\\_guidelines\\_gmp\\_for\\_atmps.pdf](https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf)
- 2006 Guideline on the sterilisation of the medicinal product, active substance, excipient and primary  
2007 container (EMA/CHMP/CVMP/QWP/BWP/850374/2015)
- 2008 Guideline on human cell-based medicinal products (EMA/CHMP/410869/2006)
- 2009 Guideline on xenogeneic cell-based medicinal products (EMA/CHMP/CPWP/83508/2009)
- 2010 Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products  
2011 (EMA/CAT/80183/2014)
- 2012 Guideline on process validation for the manufacture of biotechnology-derived active substances and  
2013 data to be provided in the regulatory submissions (EMA/CHMP/BWP/187338/2014)
- 2014 Guideline on Strategies to Identify and Mitigate Risks for First-In-Human Clinical Trials with  
2015 Investigational Medicinal Products (EMA/CHMP/SWP/28367/07)
- 2016 Guideline on Plasma-Derived Medicinal Products (EMA/CHMP/BWP/706271/2010)
- 2017 Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents  
2018 via Human and Veterinary Medicinal Products (EMA/410/01)
- 2019 Good laboratory practice (GLP) principles in relation to  
2020 ATMPs [https://www.ema.europa.eu/documents/other/good-laboratory-practice-glp-principles-relation-](https://www.ema.europa.eu/documents/other/good-laboratory-practice-glp-principles-relation-advanced-therapy-medicinal-products-atmps_en.pdf)  
2021 [advanced-therapy-medicinal-products-atmps\\_en.pdf](https://www.ema.europa.eu/documents/other/good-laboratory-practice-glp-principles-relation-advanced-therapy-medicinal-products-atmps_en.pdf)
- 2022 Guideline on Clinical Trials in small populations (CHMP/EWP/83561/2005)
- 2023 Guideline on safety and efficacy follow-up and risk management of Advanced Therapy Medicinal  
2024 Products (EMA/149995/2008 rev.1)
- 2025 Guideline on follow-up of patients administered with gene therapy medicinal products  
2026 (EMA/CHMP/GTWP/60436/2007)
- 2027 Guideline ICH E2F on development safety update report (EMA/CHMP/ICH/309348/2008)
- 2028 Guideline ICH E6 (R2) for Good Clinical Practice (EMA/CHMP/ICH/135/1995)
- 2029 Guideline ICH E7 on studies in support of special populations: geriatric (CPMP/ICH/379/95)
- 2030 Guideline ICH E8 on general considerations for clinical trials (CPMP/ICH/5746/03)
- 2031 Guideline ICH E9 on statistical principles for clinical trials (CPMP/ICH/363/96)
- 2032 Detailed guidelines on good clinical practice specific to advanced therapy medicinal Products (under  
2033 revision). Eudralex volume 10.  
2034 [https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/2009\\_11\\_03\\_guideline.pdf](https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/2009_11_03_guideline.pdf)