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# Reflection paper providing an overview of the current regulatory testing requirements for medicinal products for human use and opportunities for implementation of the 3Rs

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|---|---|
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# Reflection paper on providing an overview of the current regulatory testing requirements for medicinal products for human use and opportunities for implementation of the 3Rs

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# 1. Introduction

In December 2016 the CHMP and CVMP published a guideline on regulatory acceptance of 3R (replacement, reduction, refinement) testing approaches (EMA/CHMP/CVMP/JEG-3Rs/450091/2012). The current reflection paper has been developed as a follow up to that guideline and provides an overview of the main animal tests required for the regulatory testing of medicinal products for human use (a parallel document has been developed in relation to veterinary medicinal products – EMA/CHMP/CVMP/JEG-3Rs/740772/2015). It includes information on opportunities for limiting animal testing that can already be implemented, where appropriate, as well as information on opportunities that may become available in the future. The latter comprises areas, which are currently under investigation and will necessitate data review and further discussion before a definite impact on 3Rs can be appraised. This document should encourage sponsors to develop new 3Rs methodologies and submit them for regulatory review and acceptance.

The information is presented in tabular format and divided into sections based on the main working party responsible for development of relevant guidance. Separate tables are provided for guidance developed by:

- the joint CHMP/CVMP Quality Working Party (QWP), which develops guidance on quality testing for medicinal products for human and veterinary use;
- the CHMP Safety Working Party (SWP-H), which develops guidance on non-clinical testing;
- the CHMP Biologics Working Party (BWP), which develops guidance on quality and safety testing for biological and biotechnological medicinal products;
- the CHMP Vaccines Working Party (VWP), which develops guidance relating to the development of vaccines, including guidance on non-clinical requirements for vaccines;
- the Committee for Advanced Therapies (CAT) responsible for assessing the quality, safety and efficacy of advanced-therapy medicinal products (ATMPs) and following scientific developments in the field;
- the CHMP Biosimilar Medicinal Products Working Party (BMWP), which develops guidance on non-clinical and clinical matters relating to biosimilar medicinal products.

It is important to note that for the tests enumerated in the tables below applicants may deviate from guidelines as long as they are able to provide data (new data or published literature) or argumentation to scientifically demonstrate that the 3R approach provides an equivalent level of quality, safety or efficacy. If an applicant considers that a particular test is not necessary or would like to use a 3R methodology, the applicant can use the scientific advice procedure to obtain advice on the acceptability of its proposed approach.

The current reflection paper provides a snapshot of the testing requirements at the time of publication. It is to be expected that, over time, new testing approaches will become accepted and the tables should be considered accordingly.

In reviewing these tables the reader should remember that the fundamental responsibility of the CHMP is to ensure the quality, safety and efficacy of medicinal products and so to safeguard patient health. While the CHMP is committed to encouraging use of 3Rs approaches wherever possible, these cannot be accepted at the expense of safety and efficacy for patients.

# 2. Overview of testing requirements

## *2.1. CHMP/CVMP Quality Working Party*

Overview of animal testing requirements for active substances (ASs) of synthetic, semi-synthetic, fermentation origin as well as medicinal products and radiopharmaceutical preparations (Quality Working Party - CHMP/CVMP)

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Pyrogens (Rabbits)*<br><br>*test also applicable to biological products | European Pharmacopoeia (Ph.Eur.) 2.6.8 | Amikacin-sulfate, Calcium levulinate dihydrate, Colistimethate sodium, Chloramphenicol sodium succinate, Dicloxacillin sodium, Flucloxacillin sodium, Glucose, Glucose monohydrate, Kanamycin acid sulphate, Kanamycin monosulfate, Polymyxin B sulphate, Sodium citrate. Besides the active substances in the table, the test is used in case of derived medicinal products and some older products. | According to specific Ph. Eur. monographs, this test should be used if the active substance is intended for use in the manufacture of parenteral preparations without further appropriate procedure for the removal of pyrogens.<br>Therefore, in practice, the pyrogen test is seldom performed on the active substances.<br><br>In addition, the latest version of Chapter 2.6.8 (published in edition 9) includes the following text:<br><br>'In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, tests must be carried out in such a way as to use the minimum number of animals and to cause the | To communicate that the test shall be used only in the justified and authorised cases when neither the Monocyte-activation test (MAT, 2.6.30, Ph.Eur.) nor the Bacterial Endotoxins test (BET, 2.6.14, Ph.Eur.) can be performed (see Ph.Eur. general monograph Substances for pharmaceutical use and Chapter 2.6.30).<br><br>For new applications for marketing authorisation of medicinal products, the MAT and BET should be considered as the first choice for validation and submission. In the case of older products the pyrogen test should be replaced after demonstration of suitability of MAT or BET for the product via variation procedures. |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | | | least pain, suffering, distress or lasting harm. Wherever possible and after product specific validation, the pyrogen test is replaced by the monocyte-activation test (2.6.30).' | |
| Bacterial Endotoxins (amoebocyte lysate from *Limulus polyphemus* or *Tachypleus tridentatus)** <br><br> *test also applicable to biological products | Ph. Eur. Chapter 2.6.14. | Active substances of endotoxin-free grade and most of medicinal products intended for parenteral administration. | Often used as an alternative to the pyrogen test. The BET is used to detect or quantify endotoxins from Gram-negative bacteria using Limulus Amoebocyte Lysate obtained from blood cells (amoebocytes) of horseshoe crabs (*Limulus polyphemus, Tachypleus tridentatus*). | BET assays based on recombinant Factor C, a non-animal derived reagent, are available. Their use is referred to in Ph.Eur. chapter 5.1.10, "Guidelines for Using the Test for Bacterial Endotoxins", Section 12.2 states: *The use of alternative reagents such as recombinant factor C as a replacement to the amoebocyte lysate eliminates the use of a reagent extracted from live animals. Replacement of a rabbit pyrogen test or a bacterial endotoxin test prescribed in a monograph by a test using recombinant factor C reagent or any other reagent as a replacement of the amoebocyte lysate is to be regarded as the use of an alternative method in the replacement of a pharmacopoeial test, as described in the General Notices.* |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Abnormal toxicity test (ATT) (Mice)*<br><br>*test also applicable to biological products | Ph. Eur. Chapter 2.6.9. | Dihydrostreptomycin Sulphate, Streptomycin sulphate, Griseofulvin, Kanamycin acids sulphate, Kanamycin monosulfate, Nystatin, Rifamycin sodium. | This test is included in the Production section of the Ph.Eur. monographs of some active substances, therefore its performance is not required as a routine, since the Ph.Eur. establishes that "the method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test". | At its session in November 2017 the Ph.Eur. Commission adopted the deletion of the Abnormal Toxicity test completely from all Ph.Eur. monographs and the European Pharmacopoeia 2.6.9. general chapter on ATT was withdrawn. Revised texts are published in the Ph. Eur. 9.6 (July 2018) and will come into force on 1 January 2019. |
| Physiological distribution (Usually rats or mice) | European Pharmacopoeia General Monograph on Radiopharmaceutical Preparations (human) (01/2014:0125: this test may be required "if the test for identity and for radiochemical purity are not adequate to completely define and control the radiochemical species in a radiopharmaceutical preparation". "The distribution pattern of radioactivity observed in specified organs, tissues or other body compartments of an appropriate animal species can be a reliable | Medicinal Products: Technetium (99m TC) colloidal rhenium sulphide injection, Technetium (99m TC) colloidal sulphur injection, Technetium (99m TC) colloidal tin injection, Technetium (99m TC) etifenin injection, Technetium (99m TC) gluconate injection, Technetium (99m TC) humani albumin injection, Technetium (99m TC) macrosalb injection, Technetium (99m TC) medronate injection, Technetium (99m TC) microspheres injection, Technetium (99m TC) succimer injection. | The test should be avoided whenever possible.<br><br>According to the scientific development and improved methods of synthesis in the past years these animal studies are dispensable for new radiopharmaceuticals prepared according to the European Pharmacopoeia General Monograph on Radiopharmaceutical Preparations (human). | To communicate that the test is not justified anymore and should be deleted from the specification. |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | indication of the suitability for the intended purpose"). | | | |

## *2.2. CHMP Safety Working Party*

Overview of animal testing requirements for non-clinical studies for human pharmaceuticals (SWP Working Party - CHMP)

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Repeated dose toxicity | Note for Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals (CPMP/ICH/286/95; ICH M3(R2))<br><br>Guideline on repeated dose toxicity (CPMP/SWP/1042/99 Rev 1 Corr)<br><br>EMA/CHMP/ICH/731268/1998: Guideline on preclinical safety evaluation of biotechnology–derived pharmaceuticals ICH S6(R1)<br><br>EMA/CHMP/ICH/507008/2011: ICH M3(R2))<br><br>CHMP/ICH/646107/2008: ICH S9<br><br>EMA/CHMP/ICH/126642/2008: ICH S2 | The recommended duration of repeated-dose toxicity studies to support clinical trials and/or marketing depends on the duration of the indicated treatment and ranges from 2 weeks up to 9 months (see ICH M3(R3)). | One species could be acceptable on a case-by-case approach, and if clearly justified.<br><br>Inclusion of additional *in vivo* endpoints in repeat dose toxicity studies in order to reduce animal use is accepted if scientifically justified (e.g. by integration of safety pharmacology or genotoxicity endpoints). | Expansion of the concept of integration of additional endpoints in repeat dose toxicity studies if equivalent safety information is supported by retrospective data analysis and/or when sufficient experience has been acquired.<br><br>Regarding the exposure-based setting of the maximum tolerated dose (MTD) further discussion on the scientific rationale for the required exposure margin is needed. |
| Repeated dose toxicity: reversibility | Guideline on repeated dose toxicity (CPMP/SWP/1042/99 Rev 1 Corr)<br><br>ICH M3(R2))<br><br>ICH guideline M3 (R2) – questions | ICH M3(R2) states the following in Section 1.4, General Principles:<br>“The goals of the non-clinical safety evaluation generally | Guidelines, in particular ICH M3 and ICH S6 give recommendations on the purpose of reversibility and interpretation of toxicity findings after a | Broader application of the concepts regarding reversibility as described in ICH guideline M3 (R2) – questions and answers document should be considered. |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| | and answers (EMA/CHMP/ICH/507008/2011;<br>EMA/CHMP/ICH/731268/1998: Guideline on preclinical safety evaluation of biotechnology–derived pharmaceuticals ICH S6(R1) | include a characterisation of toxic effects with respect to target organs, dose dependence, relationship to exposure, and, when appropriate, potential reversibility." | recovery period. The ICH guideline M3 (R2) - questions and answers document explains in details reasons where reversibility is not warranted. This Q&A states also that "If a reversibility study is warranted, it is efficient to conduct it as part of a chronic study so that all toxicities of concern can be assessed in a single study provided that it is not critical to conduct it earlier to support a specific clinical trial." | |
| Genotoxicity | ICH Guideline S2(R1) on genotoxicity testing and data interpretation for pharmaceuticals intended for human use (EMA/CHMP/ICH/126642/2008) | Standard test battery: *In vivo* genotoxicity measurement (e.g. MN) can be integrated into repeated dose toxicity study, when feasible; otherwise a stand-alone *in vivo* genotoxicity study is requested.<br>Follow up of *in vitro* positives: A single combined *in vivo* genotoxicity study (e.g. MN blood & comet liver) is acceptable, if possible. | Standard battery without extra animal study is recommended (*in vitro* tests plus genotoxicity integrated in repeated dose toxicity study). | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Carcinogenicity | Note for Guidance on Carcinogenicity: Testing for Carcinogenicity of Pharmaceuticals (CPMP/ICH/299/95; ICH S1B) | rat 2 year carcinogenicity testing and ;<br>mouse 1.5 year carcinogenicity testing or mouse 26 weeks TG bioassay (p53+/-, Tg ras H2, Tg.AC). | | ICH Guideline S1 - Regulatory notice on changes to core guideline on rodent carcinogenicity testing of pharmaceuticals (EMA/CHMP/51230/2013): new testing paradigm under evaluation based on a more comprehensive and integrated weight-of-evidence approach to address the risk of human carcinogenicity of small molecule pharmaceuticals, and to define conditions under which 2-year rat carcinogenicity studies could be omitted. |
| Reproductive toxicity | Note for Guidance on the Detection of Toxicity to reproduction for Medicinal products & Toxicity to Male Fertility (CPMP/ICH/386/95; ICH S5(R2)) | Study of fertility and early embryonic development to implantation: rat (or mouse)<br>Study for effects on embryo-foetal development: rat and rabbit.<br>Study for effects on pre- and postnatal development, including maternal function: rat (or mouse). | | ICH S5(R2) is currently under revision. Aspects under consideration include evaluation of novel *in vitro* methodologies for embryo-foetal development testing within an integrated testing strategy and potential to replace one *in vivo* species. |
| Toxicokinetics | Note for Guidance on Toxicokinetics: a Guidance for Assessing Systemic Exposure in Toxicology Studies (CPMP/ICH/384/95; ICHS3A) | Toxicity studies which may be usefully supported by toxicokinetic information include single and repeated-dose toxicity studies, reproductive, genotoxicity | | Draft ICHS3A Q&A currently in public consultation: this Q&A document focuses on points to consider before incorporating the microsampling method in TK studies acknowledges its benefits (and some limitations) for assessment of TKs in main |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | | and carcinogenicity studies. Normally, samples for the generation of toxicokinetic data may be collected from main study animals, where large animals are involved, but satellite groups may be required for the smaller (rodent) species. | | study animals and its overall important contribution to the 3Rs benefits by reducing or eliminating the need for TK satellite animals. |
| Pharmacokinetics | Note for Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals (CPMP/ICH/286/95; ICH M3(R2))<br><br>Note for Guidance on Pharmacokinetics: Repeated Dose Tissue Distribution Studies (CPMP/ICH/385/95; ICHS3B) | Information on pharmacokinetics (PK) (e.g., absorption, distribution, metabolism and excretion), in test species and *in vitro* biochemical information relevant to potential drug interactions.<br><br>Repeated dose tissue distribution studies in rodent or non-rodents (case-by-case). | Standard *in vitro* models for comparison of *in vitro* metabolism across species, effect on enzyme P450 activity, protein binding, absorption using Caco-2 cells<br><br>Standard *in vivo* models for single dose pharmacokinetic studies in rodent and non-rodent. Guideline on the investigation of drug interaction (CPMP/EWP/560/95-Rev.1 Corr.): *in vitro* approaches are preferred. | |
| Duration of chronic toxicity studies | Duration of Chronic Toxicity Testing in Animals (Rodent and Non-Rodent) Toxicity Testing (CPMP/ICH/300/95; ICHS4) | Rodents: 6 months<br><br>Non-rodents: up to 9 months (see ICH M3 (R2): In the EU, studies of 6 months duration | | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | | in non-rodents are generally acceptable) | | |
| Non-Clinical Evaluation of Anticancer Pharmaceuticals | Note for Guidance on Non-clinical Evaluation for anticancer Pharmaceuticals (EMEA/CHMP/ICH/646107/2008; ICHS9) | Basic framework for non-clinical evaluation of anticancer pharmaceuticals. | 3-month data sufficient for marketing authorisation application (previously 6-month chronic toxicity study needed).<br><br>No need for fertility studies (effect on reproductive organs from repeat dose toxicity studies).<br><br>No need for pre- and post-natal development studies if embryo-foetal development study is positive, no confirmatory study in 2nd species is needed.<br><br>Inclusion of safety pharmacology endpoints in repeat dose toxicity studies (ECG in non-rodents).<br><br>No need for non-rodent studies for initiation of clinical trials with cytotoxic pharmaceuticals.<br><br>Need for recovery in general | Q&A related to ICH S9 guideline currently in preparation. Aspects under consideration include clarification of the scope which may result in further decrease of the conduct of toxicology animal studies in the development of this product class. |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | | | toxicity studies based on scientific rationale. | |
| Safety testing of biologicals | ICH guideline S6 (R1) – preclinical safety evaluation of biotechnology-derived pharmaceuticals (EMA/CHMP/ICH/731268/1998) | Basic framework for non-clinical safety evaluation of biologicals. | Enhanced Pre- & Post-Natal Development study design: Reduction of the need for 2 separate studies (embryo-foetal development and peri-postnatal development studies).<br><br>Reduction of animal numbers with one treated group and a control group can be accepted based on scientific justification.<br><br>No need for stand-alone fertility studies in non-human primates if additional relevant endpoints are included in repeat dose toxicity studies.<br><br>Use of only one relevant species for chronic toxicity studies (by default the lowest phylogenetic species) generally acceptable (e.g. similar toxicity findings from biologicals in the same class and findings understood from mode of action).<br><br>Recovery group sufficient at one | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | | | (justified) dose level.<br><br>No need for two-year carcinogenicity studies unless concern.<br><br>Use of a surrogate product in order to avoid use of non-human primates e.g. for reproductive toxicity testing, only if necessary and scientifically justified. | |
| Safety pharmacology | Note for Guidance on the Non-clinical Evaluation of the Potential for Delayed Ventricular Repolarisation (QT Interval Prolongation) by Human Pharmaceuticals (CPMP/ICH/423/02; ICH S7B) | *In vivo* and *in vitro* tests as complementary approaches to assess the potential for QT interval prolongation. | Integrated test strategy including *in vitro* tests (e.g. hERG assay) for assessment of QT-prolongation (ICH S7B). | ICH S7B guideline is currently scheduled for revision. Aspects under consideration will be advances in the science and methods as currently discussed in the Comprehensive *In vitro* Pro-arrhythmia Assessment (CIPA) initiative. |
| | Note for Guidance on Safety Pharmacology Studies for Human Pharmaceuticals (CPMP/ICH/539/00; ICHS7A) | "Core battery tests" of CNS and cardiovascular/respiratory function . | Integration of safety pharmacology parameters in repeated dose toxicity studies (see ICH S9). | Inclusion of safety pharmacology endpoints: need for retrospective data analysis to expand concept beyond ICH S9. |
| Immunotoxicity | Note for Guidance on Immunotoxicity Studies for Human Pharmaceuticals (CHMP/167235/2004; ICH S8) | Non-clinical assessment of unintended immune suppression or enhancement. | Specific studies only when standard toxicity studies indicate a cause for concern (weight of evidence approach). | |
| Phototoxicity | ICH guideline S10: Guidance on photosafety evaluation | Integrated process that can involve an evaluation of | Use of photo-chemical evaluation and *in vitro* tests in combination | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | of pharmaceuticals (EMA/CHMP/ICH/752211/2012) | photochemical characteristics, data from non-clinical studies and human safety information. | with *in vivo* non-clinical or clinical data if deemed necessary based on a weight of evidence approach.<br><br>No photocarcinogenicity test (see ICHM3(R2)) and no *in vivo* photo-allergy test. | |
| Local Tolerance | Guideline on non-clinical local tolerance testing (EMA/CHMP/SWP/2145/2000-Rev.1); Updated in 2016 (effective 01/05/2016). | Local tolerance testing should be included as part of the general toxicity studies; "stand-alone" studies on local tolerance are generally not required. | Extra animal studies are generally not required.<br><br>*In vitro* local tolerance testing and /or integration of appropriate endpoints into general toxicity studies highly recommended. | |
| Dependence Potential | Guideline on the non-clinical investigation of the dependence potential of medicinal products (EMEA/CHMP/SWP/94227/2004) | Two-tiered approach to investigate the dependence potential of new CNS active substances. In the first tier, studies reveal the pharmacological profile of the active substance. Based on data from the first tier and other early indicators it should be decided whether subsequent *in vivo* behavioural studies investigating the reinforcing | Specific studies only when standard non-clinical studies indicate a cause for concern (weight of evidence approach). | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | | properties and potential to cause withdrawal phenomena is necessary. | | |
| Testing in Juvenile Animals | Guideline on the Need for Non-Clinical Testing in Juvenile Animals of Pharmaceuticals for Paediatric Indications (EMEA/CHMP/SWP/169215/2005) | Juvenile animal studies are needed only if safety concerns cannot be adequately assessed in the adult population or in standard non-clinical studies. | Specific studies only when standard non-clinical studies and clinical safety information from adult population indicates a cause for concern (weight of evidence approach). | ICH S11 in preparation for better guidance to avoid unnecessary animal studies. |
| Environmental studies | Environmental risk assessment of medicinal products for human use (CPMP/SWP/4447/00)<br><br>Q&A on the guideline on the environmental risk assessment of medicinal products for human use (EMA/CHMP/SWP/44609/2010) | Basic framework for environmental risk assessment of human pharmaceuticals (Phase II, Tier A):<br><br>Fish toxicity (Fish Early Life Stage Toxicity test OECD 210)<br><br>Phase II, Tier B Fish bioaccumulation (OECD 305) | Revision of the ERA guideline ongoing at the level of EMA SWP.<br><br>3Rs principles optimisation regarding the testing strategy and methodology will be considered. | |
| Qualification of impurities | ICH Guideline Q3A(R2): Note for guidance on impurities testing: impurities in new drug | A general toxicity study (one species, usually 14 to 90 days), if data are unavailable for qualification (new | | Discussion on use of animal-free alternatives (e.g., read-cross approaches, SARs, literature based assessments). |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | substances (CPMP/ICH/2737/99)<br><br>ICH Guideline Q3B(R2): Note for guidance on impurities in new drug products (CPMP/ICH/2738/99)<br><br>ICH guideline Q3C (R6) on impurities: guideline for residual solvents (EMA/CHMP/ICH/82260/2006 | impurity) and guideline criteria are met. | | |

## *2.3. CHMP Biosimilar Medicinal Products Working Party*

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Similar biological medicinal products | Similar biological medicinal products (CHMP/437/04-Rev.1) and similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005-Rev.1) | A stepwise approach is recommended for evaluation of the similarity of the biosimilar and the reference product. Analytical studies and *in vitro* pharmaco-toxicological studies should be conducted first and a decision then made as to the extent of what, if any, *in vivo* work in animal studies will be required. | If an *in vivo* evaluation is deemed necessary, the focus of the study/studies (PK and/or PD and/or safety) depends on the need for additional information.<br><br>Animal studies should be designed to maximise the information obtained. Depending on the endpoints used, it may not be necessary to sacrifice the animals at the end of the study.<br><br>The duration of the study (including observation period) should be justified, taking into consideration the PK behaviour of the reference medicinal product and its clinical use. | |
| Biosimilar FSH | Similar biological medicinal products containing recombinant follicle-stimulating hormone (CHMP/BMWP/671292/2010) | The Steelman-Pohley assay needs to be performed to establish the *in vivo* potency of both the biosimilar and the reference product.<br><br>It is included in the current guideline that the number of | Guideline recently revised including the stepwise approach (see EMEA/CHMP/BMWP/42832/2005 Rev.1) | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | | different assays performed may be reduced by a study design in which the biosimilar and the reference medicinal product are compared and simultaneously calibrated against the reference standard. This reduces inter-assay variation and is more economical with regard to reagents and animals used. | | |
| Biosimilar IFN-beta | Similar biological medicinal products containing interferon beta (CHMP/BMWP/652000/2010) | Generally, *in vivo* studies in animals are not recommended. | Guideline recently revised including the stepwise approach (see EMEA/CHMP/BMWP/42832/2005-Rev.1) | |
| Biosimilar mAbs | Similar biological medicinal products containing monoclonal antibodies: non-clinical and clinical issues (EMA/CHMP/BMWP/403543/2010) | A stepwise approach is recommended for evaluation of the similarity of the biosimilar and the reference product. Analytical studies and *in vitro* pharmaco-toxicological studies should be conducted first and a decision then made as to the extent of what, if any, *in vivo* work in animal studies | If an *in vivo* evaluation is deemed necessary, the focus of the study/studies (PK and/or PD and/or safety) depends on the need for additional information. Animal studies should be designed to maximise the information obtained. The principles of the 3Rs (replacement, refinement, reduction) according to Article 4 of Directive 2010/63/EU should be | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | | will be required. | considered when designing any *in vivo* study. Depending on the endpoints used, it may not be necessary to sacrifice the animals at the end of the study. The duration of the study (including observation period) should be justified, taking into consideration the PK behaviour of the reference medicinal product and its clinical use. | |
| Biosimilar EPO | Similar biological medicinal products containing recombinant erythropoietins (EMEA/CHMP/BMWP/301636/08) | The erythrogenic effects of the similar biological medicinal product and the reference medicinal product should be quantitatively compared in an appropriate animal assay.<br><br>Data from at least one 4 week repeat dose toxicity study (including local tolerance data) should be provided. | Technical update to reflect current best practise with regard to implementation of 3Rs approaches including the stepwise approach (see EMEA/CHMP/BMWP/301636/08) | |
| Biosimilar LMWH | Non-clinical and clinical development of similar biological medicinal products containing low-molecular-weight heparins | The pharmacodynamic activity of the similar and the reference LMWH should be quantitatively compared in | Guideline recently revised including the stepwise approach (see EMEA/CHMP/BMWP/118264/2007-Rev.1) | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | (EMEA/CHMP/BMWP/118264/2007) | an appropriate *in vivo* model.<br><br>Data from at least one 4 week repeated dose toxicity study (including local tolerance data) should be provided. | | |
| Biosimilar INF-alpha | Non-clinical and clinical development of similar medicinal products containing recombinant interferon alpha (EMEA/CHMP/BMWP/102046/2006) | The pharmacodynamic activity of the similar and the reference medicinal product could be quantitatively compared in an appropriate animal model. Data from at least one 4 week repeated dose toxicity study (including local tolerance data) should be provided. | Reflection paper under revision to include a stepwise approach (see EMEA/CHMP/BMWP/102046/2006) | Reflection paper expected to be finalised in-2019 |
| Biosimilar GCSF | Non-clinical and clinical issues - Guidance on biosimilar medicinal products containing recombinant granulocyte-colony stimulating factor (EMEA/CHMP/BMWP/31329/2005) | *In vivo* rodent models, neutropenic and non-neutropenic, should be used to compare the pharmacodynamic effects of the test and the reference medicinal product. Data from at least one 4 week repeated dose toxicity study (including local tolerance | Guideline under revision to include a stepwise approach (see EMEA/CHMP/BMWP/42832/2005-Rev.1) | Guideline expected to be finalised in 2019 |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
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| | | data) should be provided. | | |
| Biosimilar somatropin | Non-clinical and clinical issues - Guidance on similar medicinal products containing somatropin (EMEA/CHMP/BMWP/94528/2005) | An appropriate *in vivo* rodent model should be used to quantitatively compare the pharmacodynamic activity of the similar biological medicinal and the reference medicinal product. Data from at least one 4 week repeated dose toxicity study (including local tolerance data) should be provided. | Technical update to reflect current best practise with regard to implementation of 3Rs approaches including the stepwise approach (see EMEA/CHMP/BMWP/94528/2005). | |
| Biosimilar recombinant human insulin and insulin analogues | Non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues (EMEA/CHMP/BMWP/32775/2005 Rev.2) | Comparative *in vivo* studies of pharmacodynamic effects would not be anticipated to be sensitive enough to detect differences not identified by *in vitro* assays, and are not required as part of the comparability exercise.<br><br>Generally, separate repeated dose toxicity studies are not required. | Guideline recently revised including the stepwise approach (see EMEA/CHMP/BMWP/42832/2005 Rev.1). | |

## *2.4. CHMP Biologics Working Party*

Overview of animal testing requirements for biological medicinal products (Biologics Working Party (BWP) - CHMP)

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Manufacture, characterisation and control of the drug substance | Annex I of Directive 2001/83/EC<br><br>CHMP guideline on development, production, characterisation and specifications for monoclonal antibodies and related substances (CHMP/BWP/157653/07) | The biological activity should be assessed by *in vitro* and/or *in vivo* assays as appropriate. | The option of using *in vitro* assays already exists.<br><br>Guideline updated to remove reference to the production of monoclonal antibodies from ascites fluid.<br><br>An *in vitro* assay should be used to monitor the biological activity of the monoclonal antibody unless thoroughly justified. | |
| Manufacture, characterisation and control of the drug substance | Annex I of Directive 2001/83/EC<br><br>CHMP guideline on potency testing of cell based immunotherapy medicinal products for the treatment of cancer (EMEA/CHMP/BWP/271475/2006) | Potency testing may be performed by means *in vivo* or *in vitro* tests. | The option of using *in vitro* assays already exists.<br><br>Guideline has been updated to stress that for routine testing an adequate *in vitro* assay is the preferred option. | |
| Manufacture, characterisation and control of the drug substance | Annex I of Directive 2001/83/EC<br><br>CHMP guideline on the quality of biological active substances produced by stable transgene expression in higher plants (CPMP/BWP/48316/06) | Strategies for control of virus and viroid adventitious agents may include *in vitro* and *in vivo* tests for the absence of such material. | The option of using *in vitro* assays already exists. In addition, the guideline identifies a number of other approaches that may also be used. | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Manufacture, characterisation and control of the drug substance | Annex I of Directive 2001/83/EC<br><br>CHMP guideline on development and manufacture of lentiviral vectors (CPMP/BWP/2458/03) | In relation to delivery of lentiviral vectors, *in vitro* and/or *in vivo* experiments are needed to assess construct of characteristics including risk of replication competent lentivirus generation. | The option of using *in vitro* approaches already exists. | |
| Manufacture, characterisation and control of the drug substance | Annex I of Directive 2001/83/EC<br><br>CHMP Note for guidance on production and quality control of animal immunoglobulins and immunosera for human use (CPMP/BWP/3354/99) | Potency testing may be performed in animals. | The existing text encourages the use of *in vitro* methods.<br><br>A cell based *in vitro* potency assay has been included in the guideline as an example of an *in vitro* assay. | |
| Manufacture, characterisation and control of the drug substance | Annex I of Directive 2001/83/EC<br><br>CHMP guideline on allergen products: production and quality issues (CHMP/BWP/304831/07) | In relation to stability testing, if it is not possible to perform potency tests, *in vivo* immunogenicity tests or validated alternative *in vitro* tests should be performed in the at the beginning and end of the proposed shelf-life period. | The option of using *in vitro* assays already exists. | |
| Manufacture, characterisation and control of the drug substance | Directive 2001/83/EC<br><br>Guideline on gene therapy product quality aspects in the production of vectors and genetically modified somatic cells (3AB6A) | Where appropriate and for vectors intended for direct *in vivo* application, biological potency tests in animal tissues maintained ex vivo or in whole animals should be carried out. Transgenic animals or animals with transplanted human tissues or systems may be suitable for this purpose. | The “where appropriate” allows justification of alternative approaches. | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Manufacture, characterisation and control of the drug substance | Directive 2001/83/EC<br><br>Guideline on use of transgenic animals in the manufacture of biological medicinal products for human use (3AB7A) | Provides guidance on the use of transgenic animals. | Alternative approaches can equally be used - there is no requirement to use transgenic animals in the manufacture of biological medicinal products. | |
| Specifications | Directive 2001/83/EC<br><br>ICH Topic Q6B:<br>Note for guidance on specifications - test procedures and acceptance criteria for biotechnological/ biological products (CPMP/ICH/365/96) | Biological activity should be assayed, either by animal-based assays, cell culture-based assays, biochemical assays or other procedures. | The use of non-animal approaches is referred to in the guideline. | |
| Specifications | Directive 2001/83/EC<br><br>Guideline on test samples of biological origin (3AB11a) | In relation to the criteria for validation of test procedures, the guideline indicates that “Each test procedure should be validated for each type of biological sample and each species (animal, human). If the same test procedure has been used during the development of the medicinal product (*in vitro*) and during routine tests (*in vivo*), a revalidation is necessary. | There is not a requirement for an *in vivo* test. | |
| Plasma derived medicinal products | Directive 2001/83/EC<br><br>CHMP Guideline on plasma-derived medicinal products (EMA/CHMP/BWP/706271/2010) | In relation to hepatitis B virus validation the guideline indicates that “An animal virus model, the duck hepatitis B virus (DHBV), may be used as a model of human HBV. However, it requires the | Primary duck cells may be used rather than live animals. | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| | | use of its natural animal host (duck or primary duck cells) for titration. In consequence, there is no general requirement to include DHBV in the virus panel. However, in some specific situations where the efficacy of new inactivation procedures are highly virus dependent among enveloped viruses and for which inactivation/removal efficacy cannot be extrapolated from limited number of model viruses, the use of DHBV could be requested." | | |
| Plasma derived medicinal products | Directive 2001/83/EC<br><br>CHMP Guideline on the replacement of rabbit pyrogen testing by an alternative test for plasma derived medicinal products (CHMP/BWP/452081/07) | The guideline specifically relates to the implementation of an alternative to rabbit pyrogen testing. | The guideline specifically relates to the implementation of the bacterial endotoxin test as an alternative to rabbit pyrogen testing. | The monocyte activation test (MAT; 2.6.30, Ph.Eur.) also provides an alternative to the rabbit pyrogen test. |
| Plasma derived medicinal products | Directive 2001/83/EC<br><br>CPMP Guideline on the investigation of manufacturing processes for plasma-derived medicinal products with regard to VCJD risk (CPMP/BWP/5136/03) | Infectivity assays in animals are accepted as the gold standard for the detection of TSE agents as there are no generally applicable *in vitro* tests available to identify presence of infectivity and to quantify the infectivity level. | The use of a biochemical assay for detection of $PrP^{sc}$ could be acceptable subject to demonstration of correlation between infectivity in a bioassay and the detection of $PRP^{SC}$ in the biochemical assay. | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Vaccines | Directive 2001/83/EC<br><br>CPMP Note for guidance on the development of vaccinia virus based vaccines against smallpox (CPMP/1100/02) | Possible animal use includes for preparation of vaccine seed lots, characterisation of seed lot material, infectivity titre (*in vivo* growth, animal model), testing for adventitious agents, testing final bulk, testing for, pharmacodynamics characterisation, virulence testing, neurovirulence testing, reproductive function testing. | | |
| Stability | Directive 2001/83/EC<br><br>Guideline on quality of biotechnological products: stability testing of biotechnological/biological products (3AB5A, CPMP/ICH/138/95, ICH Topic 5QC) | Potency testing may be performed in animals. | There is not a requirement for potency testing to take place in animals – other approaches can also be accepted. | |
| Drug product | Directive 2001/83/EC<br><br>CPMP annex to Note for Guidance on Development Pharmaceutics (CPMP/QWP/155/96) - Development Pharmaceutics for Biotechnological and Biological Products - Annex to Note for Guidance on Development Pharmaceutics (CPMP/BWP/328/99) | Potency (biological activity) may be tested in animals. | There is not a requirement for potency testing to take place in animals – other approaches can also be accepted. | |
| Adventitious agents, safety evaluation, viral safety | Directive 2001/83/EC<br><br>ICH Topic Q5A (R1): Quality of biotechnological products: viral safety | Animal testing is needed for detection of some viruses. | | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| | evaluation of biotechnology products derived from cell lines of human or animal origin (CPMP/ICH/295/95) | | | |
| Adventitious agents, safety evaluation, viral safety | Directive 2001/83/EC<br>CPMP Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses (CPMP/BWP/268/95) | Animal testing is needed for the detection of some viruses. | | |
| Investigational Medicinal Products | Directive 2001/83/EC<br>CHMP Guideline on requirements for quality documentation concerning biological investigational medicinal products in clinical trials (EMA/CHMP/BWP/534898/08) | Potency testing may take place in animals. | There is not a requirement for potency testing to take place in animals – other approaches can also be accepted. | |
| Investigational Medicinal Products | Directive 2001/20/EC<br>CHMP Guideline on virus safety evaluation of biotechnological investigational medicinal products (EMEA/CHMP/BWP/398498/05) | Tests for infectious retroviruses and *in vivo* tests may be needed depending on the cell type used in manufacture.<br>Testing for viruses may use animals. | Alternatives to the use of animals may be available. | |

## *2.5. CHMP Vaccines Working Party*

Overview of animal testing requirements for vaccines (Vaccines Working Party (VWP) - CHMP)

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Non-clinical testing of adjuvants | Guideline on adjuvants in vaccines for human use (EMEA/CHMP/VEG/134716/2004) | The increased immunological response to the adjuvant/antigen combination should be shown in a relevant animal model.<br><br>Toxicity program in general similar to toxicity program for a vaccine, with the combination of adjuvant and antigen. In addition, studies on adjuvant should be performed.<br><br>Toxicity studies with adjuvant alone should be performed in two species unless otherwise justified. | | One species sufficient for toxicity testing. Toxicity studies with the adjuvant alone may not be needed. |
| Non-clinical testing of influenza vaccines | Guideline on influenza vaccines. Non-clinical and clinical module. (Draft CHMP guideline) | In addition to safety testing, in accordance with the guideline on non-clinical testing of vaccines, animal studies on protection are required for some vaccines. The most appropriate animal model for these studies is the ferret. | None. | None. |

## *2.6. Committee for Advanced Therapies (CAT)*

Overview of animal testing requirements for non-clinical studies for cell-based and gene therapy medicinal products

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Risk-based approach as defined in the Annex I, Part IV of Directive 2001/83/EC can be applied for the non-clinical regulatory testing for the authorisation of Advanced Therapy Medicinal Products (ATMPs). ATMPs include both cell-based medicinal products and gene therapy medicinal products.<br><br>The following guideline should be consulted: Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to Advanced Therapy Medicinal Products (EMA/CAT/CPWP/686637/2011). | | | | |
| **Cell-based medicinal products** | | | | |
| Pharmacodynamics - Proof of concept | Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006)<br><br>Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009)<br><br>Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009)<br><br>Guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009) | Suitable *in vitro* and/or *in vivo* models should be used. Homologous models or immunocompromised models can be used. Small animal models usually not sufficient for proof of concept for *in vitro* cultured chondrocyte products. An orthotopic large animal model should be used. | If relevant animal models cannot be developed, *in vitro* studies may replace animal studies. Use of 3D cell culture models can be used. Clinical experience might substitute for some parts of the non-clinical development on a case-by-case basis (EMA/CAT/CPWP/568181/2009). For stem cells, *in vitro* models may provide additional and/or alternative ways to address some specific aspects (EMA/CAT/571134/2009). | |
| Secondary pharmacodynamics | Guideline on human cell-based medicinal products | Potential undesirable physiological effects of | | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| | (EMEA/CHMP/410869/2006)<br><br>Guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009) | cells and their bioactive products should be evaluated in an appropriate animal model on a case-by-case basis. | | |
| General safety - Toxicity *Single dose toxicity Repeated dose toxicity* | Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006)<br><br>Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009)<br><br>Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009)<br><br>Guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009) | Framework for testing requirements for cell-based medicinal products acknowledging that conventional pharmacology and toxicology studies may not be appropriate. Single and/or repeated toxicity studies depending on the intended clinical use (single administration or multiple administrations). Relevant animal models should be used. The number of animals, gender, frequency and duration of monitoring should be appropriate to detect possible adverse | Risk-based approach as defined in the Annex I, Part IV of Directive 2001/83/EC can be applied. Non-clinical testing should be proportional to the risk expected to be associated with clinical use. If relevant animal models cannot be developed, *in vitro* studies may replace animal studies. Can be combined with proof of concept or efficacy studies, and with safety pharmacology endpoints and local tolerance. | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| | | effects. Due to species-specificity more than one animal species or strains may be needed to address all safety aspects related to stem cells. | | |
| Safety pharmacology | Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006)<br><br>Guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009) | Should be considered on a case-by-case basis. | Can be combined with safety or proof of concept studies. | |
| Biodistribution - *kinetics, persistence, migration* | Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006)<br><br>Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009)<br><br>Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009)<br><br>Guideline on xenogeneic cell-based medicinal products | Tissue distribution, viability, trafficking, growth, phenotype or any alteration of phenotype due to factors in the new environment should be evaluated. Biodistribution studies in small animals (rodents) recommended. For stem cells, studies on biodistribution, differentiation and possible ectopic tissue | | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| | (EMEA/CHMP/CPWP/83508/2009) | formation are required. Biodistribution studies might not be necessary when cells are physically retained. | | |
| Genotoxicity | Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006) | Not required unless the nature of any expressed product indicates an interaction directly with DNA or other chromosomal material. | | |
| Carcinogenicity | Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006) | Conventional carcinogenesis studies not feasible. | | |
| Tumourigenicity | Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006)<br><br>Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009)<br><br>Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009)<br><br>Guideline on xenogeneic cell-based medicinal products | Risk of tumourigenicity arising from the cell product or due to neoplastic transformation of host cells should be considered on a case-by-case basis. For stem cells, evaluation of tumour formation including *in vitro* and/or *in vivo* studies is essential. | Tumourigenicity assessment can be integrated in chronic disease or toxicity models. | A step-wise testing strategy for MSCs is proposed in a publication of common effort of scientists in the field and the regulators (Barkholt et al, 2013: Cytotherapy. Risk of tumorigenicity in mesenchymal stromal cell-based therapies - bridging scientific observations and regulatory viewpoints). *In vitro* studies are normally sufficient, *in vivo* studies only if *in vitro* assays indicate an increased risk for tumour formation. |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| | (EMEA/CHMP/CPWP/83508/2009) | | | |
| Reproductive and developmental toxicity | Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006) | Generally not needed, should be considered on a case-by-case basis. | | |
| Local tolerance | Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006) | May be required. | Tissue compatibility and tolerance to excreted substances can be evaluated in single or repeated dose toxicity (safety) studies. | |
| Immunogenicity, immune response | Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006)<br>Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009)<br>Guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009) | Possible immunogenicity should be considered. For xenogeneic products, studies addressing the immunologic response of the host with or without suppression to the xenogeneic cells, including their bioactive products, are needed. | | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| **Gene therapy medicinal products** | | | | |
| Pharmacodynamics - Proof of concept | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)<br><br>Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006)<br><br>Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (EMA/CAT/GTWP/671639/2008)<br>Oncolytic viruses (EMEA/CHMPICH/607698/2008) | The nature and extent of pharmacological and toxicological evaluation considered on a case-by-case basis. Relevant animal models should be used; i.e. should be permissive for the viral vector and/or mimic the disease or condition to be treated. For genetically modified cells, *in vitro* models can be used when appropriate animal models are not available. | | |
| Secondary pharmacology | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)<br><br>Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006) | Should be considered on a case-by-case basis. | Endpoints can be included in other pharmacological and/or safety studies. | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| General safety – *Toxicity, Single dose toxicity, +Repeated dose toxicity* | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)<br><br>Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006)<br><br>Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (EMA/CAT/GTWP/671639/2008) | The nature and extent of pharmacological and toxicological evaluation considered on a case-by-case basis. Relevant animal models should be used; i.e. should be permissive for the viral vector and/or mimic the disease or condition to be treated. One relevant species usually sufficient. The duration and gender of animals in line with the ICH M3. Single or repeated dosing mimicking the clinical dosing.<br><br>For genetically modified cells, *in vitro* models can be used when appropriate animal models are not available. | Risk-based approach as defined in the Annex I, Part IV of Directive 2001/83/EC can be applied. Non-clinical testing should be proportional to the risk expected to be associated with clinical use. In cases where there is extensive experience (preclinical and/or clinical) with the particular vector by a particular route of administration, information from the literature could be used to replace some studies. Can be combined with proof of concept or efficacy studies, and with safety pharmacology or other endpoints. | Concept paper on the revision of the Note for guidance on the quality, pre-clinical and clinical aspects of gene transfer medicinal products (EMA/CHMP/GTWP/BWP/234523/2009). |
| Safety pharmacology | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99) | Should be considered on a case-by-case basis. | Can be included in toxicity/safety studies. | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Biodistribution - *kinetics, persistence, migration* | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)<br><br>Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006)<br><br>Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors (EMEA/273974/2005) | Biodistribution of gene therapy vector to all organs listed in the Annex to the Guideline on repeated dose toxicity (CPMP/SWP/1042/99-Rev & Corr*) should be evaluated including persistence, mobilisation and shedding. Distribution, exposure to, clearance and transcription of the nucleic acid should be investigated. Biodistribution studies in at least two species, one of which should be a non-rodent species, with two dose levels at minimum, should be conducted (EMEA/273974/2005). | Can be included in toxicity/safety studies. | Ongoing revision of the "Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)" will allow harmonisation of requirements between different guidelines. |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Genotoxicity | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)<br><br>Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal product (EMEA/GTWP/125459/2006) | Conventional genotoxicity studies generally not needed. | | |
| Carcinogenicity | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)<br><br>Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal product (EMEA/GTWP/125459/2006) | Conventional carcinogenicity studies generally not needed. | | |
| Tumourigenicity/ oncogenicity | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)<br><br>Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal product (EMEA/CHMP/GTWP/125459/2006) | Tumourigenic potential of expressed transgene product may need to be evaluated. Oncogenic potential to be addressed in silico, if potential identified it should be evaluated in *in vivo*/*in vitro* models. | Use of alternative non-animal methods is recommended. | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Insertional mutagenesis | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)<br><br>Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006)<br><br>Reflection paper on management of clinical risks deriving from insertional mutagenesis (EMA/CAT/190186/2012)<br><br>Reflection paper on quality, non-clinical and clinical issues related to the development of recombinant adeno-associated viral vectors (EMEA/CHMP/GTWP/587488/2007-Rev.1) | Required for integrative gene therapy vectors. *In vitro* and/or *in vivo* evaluations needed. For rAAV vectors, *in vitro* studies to address vector integration are preferable. | | |
| Reproductive and developmental toxicity | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99) | The need to be decided based on the possible distribution of gene therapy product to the gonads. Effects on fertility and general reproductive function may be needed. Embryo-foetal and perinatal toxicity studies | | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| | | may be required if WOCBP are to be exposed. | | |
| Local tolerance | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99) | May be required, in one species. | | |
| Immunogenicity, immune response | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)<br><br>Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006)<br><br>Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (EMA/CAT/GTWP/671639/2008) | Should be considered on a case-by-case basis. | Immunotoxicity endpoints can be integrated in the toxicity studies. | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Germ line transmission | Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)<br><br>Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006)<br><br>Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors (EMEA/273974/2005)<br><br>Reflection paper on quality, non-clinical and clinical issues related to the development of recombinant adeno-associated viral vectors (EMEA/CHMP/GTWP/587488/2007-Rev.1) | Non-clinical germline transmission studies are mandatory unless otherwise justified prior to first administration to humans. One animal species may be sufficient. | | |
| Shedding | General principles to address virus and vector shedding (EMEA/CHMP/ICH/449035/2009)<br>Oncolytic viruses (EMEA/CHMP/ICH/607698/2008) | Assessment of virus/vector shedding to tissues and excreta should be conducted in animals to guide the clinical shedding monitoring plan. | Non-clinical evaluation of shedding can be integrated into other animal studies. | |

| Topic | Regulatory provision | Animal testing requirements | Implemented 3Rs opportunities | Newly identified opportunities for 3Rs implementation |
|---|---|---|---|---|
| Reactivation and latency of virus | Reflection paper on quality, non-clinical and clinical issues related to the development of recombinant adeno-associated viral vectors (EMEA/CHMP/GTWP/587488/2007-Rev.1) | Maintenance and potential for reactivation or induction of latency should be evaluated in non-clinical studies. | | |