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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

Draft

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30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

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 October 2014 the CHMP and CVMP published a draft 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 draft 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. In so doing, it is hoped that the document may stimulate future submissions for CHMP advice on the regulatory acceptance of new 3Rs approaches.

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.

In addition, the following Working Party also develops guidance that may involve animal testing:

- 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 or argumentation to scientifically demonstrate that the alternative 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 an alternative methodology, the applicant can use the scientific advice procedure to obtain advice on the acceptability of its proposed approach. In addition, where appropriate, published literature can be used as a substitute for new tests.

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 will become out of date.

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)* *test also applicable to biological products	European Pharmacopoeia (Ph.Eur.) 2.6.8	Amikacin-sulfate, Bacitracin zinc, Calcium levulinate dehydrate, Colistimethate sodium, Chloramphenicol, Chloramphenicol sodium succinate, Dicloxacillin sodium, Flucloxacillin sodium, Glucose anhydrous, Glucose monohydrate, Kanamycin acid sulphate, Kanamycin monosulfate, Polymyxin B sulphate, Sodium citrate. Besides the ASs in the table, the test is used in case of derived medicinal products and some older products.	According to specific monographs, this test should be used if the AS is intended for administration by spraying into internal body cavities (Bacitracin zinc) or use in the manufacture of parenteral preparations without further appropriate procedure for the removal of pyrogens. So in practice, the pyrogen test is seldom performed on the active substances.	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).
Bacterial Endotoxins (amoebocyte lysate from	European Pharmacopoeia 2.6.14.	Active substances of endotoxin-free grade and most of medicinal products intended for parenteral	Often used as an alternative to the pyrogen test. The BET is used to detect or quantify endotoxins from Gram-negative bacteria using Limulus	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Limulus polyphemus or *Tachypleus tridentatus)*		administration.	Amoebocyte Lysate obtained from blood cells (amoebocytes) of horseshoe crabs (*Limulus polyphemus*, *Tachypleus tridentatus*). As invertebrates, horse shoe crabs do not fall under the scope of Directive 2010/63/EU.	
Abnormal Toxicity Test (ATT) (Mice)	European Pharmacopoeia 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”.	Communicate that test should not be used routinely since manufacturing methods should be suitably validated to assure compliance "if tested". Ph.Eur. expert groups are proposing the removal of the abnormal toxicity test pending the outcome of the public consultation. Submit relevant data to the Ph. Eur. to support this change.
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	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,	According to the monograph the test should be avoided whenever possible.	To communicate that the test is not really justified and should be deleted from the specification.

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	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 indication of the suitability for the intended purpose").	Technetium (99m TC) macrosalb injection, Technetium (99m TC) medronate injection, Technetium (99m TC) microspheres injection, Technetium (99m TC) succimer injection.		

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74 ## *2.2. CHMP Safety Working Party*

75 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)) Guideline on repeated dose toxicity (CPMP/SWP/1042/99 Rev 1 Corr)	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.	One species could be acceptable on a case by case approach, and if clearly justified.	Inclusion e.g. of safety pharmacology or genotoxicity endpoints: need for retrospective data analysis to expand concept beyond ICH S9 Exposure-based setting of the maximum tolerated dose (MTD): is a 25-fold exposure sufficient?
Repeated dose toxicity: reversibility	Q&A to the 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))	ICH M3(R2) states the following in Section 1.4, General Principles: "The goals of the non-clinical safety evaluation generally include a characterisation of toxic effects with respect to target organs, dose dependence, relationship to exposure, and, when appropriate, potential reversibility."	Recommendations to avoid unnecessary studies: A toxicity study that includes a terminal non-dosing period is generally not warranted when the toxicity: - can be readily monitored in humans at an early stage before the toxicity becomes severe; or - is known to be irrelevant to humans (e.g., rodent Harderian gland toxicity); or - is only observed at high exposures not considered clinically relevant; or	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
			- is similar to that induced by related agents, and the toxicity based on prior clinical experience with these related agents is considered a manageable risk. A reversibility study is generally not warranted to support clinical trials of a duration equivalent to that at which the adverse effect was not observed non-clinically. If a particular lesion is demonstrated to be reversible in a short duration (e.g., 2 weeks or 1 month) study, and does not progress in severity in longer term studies, repeating the reversibility assessment in longer term toxicity studies is generally not warranted.	
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) as part of repeated dose toxicity study; no stand-alone in vivo genotoxicity	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
		study requested. Follow up of in vitro positives: a single combined in vivo genotoxicity study (e.g. MN blood & comet liver).		
Carcinogenicity	Note for Guidance on Carcinogenicity: Testing for Carcinogenicity of Pharmaceuticals (CPMP/ICH/299/95; ICH S1B)	rat 2 year carcinogenicity testing and ; 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) Study for effects on embryo-foetal development: rat and rabbit. Study for effects on pre- and postnatal development,		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.

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		including maternal function: rat (or mouse).		
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 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.		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 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)) Note for Guidance on Pharmacokinetics: Repeated Dose Tissue Distribution Studies	Information on pharmacokinetics (PK) (e.g., absorption, distribution, metabolism and excretion), in test species and in vitro biochemical information relevant to potential drug interactions.	Standard in vitro models for comparison of in vitro metabolism across species, effect on enzyme P450 activity, protein binding, absorption using Caco-2 cells Standard in vivo models for single dose pharmacokinetic studies in rodent and non-rodent.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	(CPMP/ICH/385/95; ICHS3B)	Repeated dose tissue distribution studies in rodent or non-rodents (case-by-case).	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 Non-rodents: up to 9 months (see ICH M3 for 'when to use')		
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). No need for fertility studies (effect on reproductive organs from repeat dose toxicity studies). No need for pre- and post-natal development studies if embryo-foetal development study is positive, no confirmatory study in 2nd species is needed.	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
			Inclusion of safety pharmacology endpoints in repeat dose toxicity studies (ECG in non-rodents). No need for non-rodent studies for initiation of clinical trials with cytotoxic pharmaceuticals. Need for recovery in general 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). Reduction of animal numbers with one treated group and a control group can be accepted based on scientific justification. No need for stand-alone fertility studies in non-human primates if additional relevant endpoints are included in repeat dose toxicity	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
			studies. Use of only one relevant species for chronic toxicity studies (similar toxicity findings from biologicals in the same class and findings understood from mode of action). Recovery group sufficient at one (justified) dose level. No need for two-year carcinogenicity studies. Use of a surrogate in order to avoid use of non-human primates e.g. for reproductive toxicity testing, if justified.	
Safety pharmacology	Note for Guidance on the Non-clinical Evaluation of the Potential for Delayed Ventricular Repolarisation (QT IOnterval 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;	"Core battery tests" of CNS and cardiovascular/respiratory	Integration of safety pharmacology parameters in repeated dose toxicity studies	Inclusion of safety pharmacology endpoints: need for retrospective data analysis to expand concept beyond ICH

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	ICHS7A)	function (mainly non-rodent).	(see ICH S9).	S9.
Immunotoxicity	Note for Guidance on Immunotoxicity Studies for Human Pharmaceuticals (CHMP/167235/2004; ICH S8)	Non-clinical assessment of unintended immune supression or enhancement.	Specific studies only when standard toxicity studies indicate a cause for concern (weight of evidence approach).	
Phototoxicity	Note for Guidance on Photosafety testing (CPMP/SWP/398/01 - 2002) Q&A on the NfG on photosafety testing (EMA/CHMP/SWP/336670/2010) ICH guideline S10: Guidance on photosafety evaluation of pharmaceuticals (EMA/CHMP/ICH/752211/2012)	Integrated process that can involve an evaluation of photochemical characteristics, data from non-clinical studies and human safety information.	Use of photo-chemical evaluation and in vitro tests in combination with in vivo non-clinical or clinical data if deemed necessary based on a weight of evidence approach. 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. In vitro local tolerance testing and /or integration of appropriate endpoints into general toxicity studies highly recommended.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
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 properties and potential to cause withdrawal phenomena is necessary.	Specific studies only when standard non-clinical studies indicates a cause for concern (weight of evidence approach).	
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) Q&A on the guideline on the	Basic framework for environmental risk assessment of human pharmaceuticals (Phase II,	Revision of the ERA guideline ongoing at the level of EMA SWP. 3Rs principles optimisation regarding the testing strategy	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	environmental risk assessment of medicinal products for human use (EMA/CHMP/SWP/44609/2010)	Tier A): Fish toxicity (Fish Early Life Stage Toxicity test OECD 210) Phase II, Tier B Fish bioaccumulation (OECD 305)	and methodology will be considered.	
Qualification of impurities	ICH Guideline Q3A(R2): Note for guidance on impurities testing: impurities in new drug substances (CPMP/ICH/2737/99) ICH Guideline Q3B(R2): Note for guidance on impurities in new drug products (CPMP/ICH/2738/99)	A general toxicity study (one species, usually 14 to 90 days), if data are unavailable for qualification.		Discussion on use of animal-free alternatives (e.g., read-cross approaches).

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77 ## *2.3. CHMP Biosimilar Medicinal Products Working Party*

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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. 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. 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.	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
		It is included in the current guideline that the number of 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	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	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		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.	information obtained. The principles of the 3Rs (replacement, refinement, reduction) according to Article 4 of Directive 2010/63/EU should be 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. Data from at least one 4 week repeat dose toxicity study (including local	Information on the erythrogenic activity may be obtained from the described repeat dose toxicity study or from a specifically designed assay (e.g. the European Pharmacopoeia normocythaemic mouse assay; data may be already available from quality-related bioassays).	Guideline will be revised (in 2017) to include a 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
		tolerance data) should be provided.		
Biosimilar LMWH	Non-clinical and clinical development of similar biological medicinal products containing low-molecular-weight heparins (EMEA/CHMP/BMWP/118264/2007)	The pharmacodynamic activity of the similar and the reference LMWH should be quantitatively compared in an appropriate in vivo model. Data from at least one 4 week repeated dose toxicity study (including local tolerance data) should be provided.	Guideline under revision to include a stepwise approach (see EMEA/CHMP/BMWP/42832/2005-Rev.1) - current proposal for the revision: If physicochemical and biological characterisation of the biosimilar and the reference LMWH performed using sensitive state-of-the-art methods convincingly demonstrates close similarity, *in vivo* studies are not required as part of the comparability exercise. Generally, separate repeated dose toxicity studies are not required.	Guideline expected to be finalised in 2017.
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	Guideline under revision to include a stepwise approach (see EMEA/CHMP/BMWP/42832/2005-Rev.1)	Guideline expected to be finalised in 2017.

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		dose toxicity study (including local tolerance data) should be provided.		
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 data) should be provided.	Guideline under revision to include a stepwise approach (see EMEA/CHMP/BMWP/42832/2005-Rev.1)	Guideline expected to be finalised in 2017.
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.		Guideline will be revised (in 2017) to include a 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
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. Generally, separate repeated dose toxicity studies are not required.	Guideline recently revised including the stepwise approach (see EMEA/CHMP/BMWP/42832/2005 -Rev.1).	

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80 *2.4. CHMP Biologics Working Party*

81 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 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. Guideline updated to remove reference to the production of monoclonal antibodies from ascites fluid. 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 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. 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 CHMP guideline on the quality of biological active substances produced by stable transgene expression in	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	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	higher plants (CPMP/BWP/48316/06)		that may also be used.	
Manufacture, characterisation and control of the drug substance	Annex I of Directive 2001/83/EC 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 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. 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 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 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	The “where appropriate” allows justification of alternative approaches.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		transplanted human tissues or systems may be suitable for this purpose.		
Manufacture, characterisation and control of the drug substance	Directive 2001/83/EC 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 ICH Topic Q6B: 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 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.	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Plasma derived medicinal products	Directive 2001/83/EC 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 use of its natural animal host (duck or primary duck cells) for titration."	Primary duck cells may be used rather than live animals.	
Plasma derived medicinal products	Directive 2001/83/EC 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 an alternative to rabbit pyrogen testing.	The monocyte activation test (MAT; 2.6.30, Ph.Eur.) provides an alternative to the rabbit pyrogen test.
Plasma derived medicinal products	Directive 2001/83/EC 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.		
Vaccines	Directive 2001/83/EC CPMP Note for guidance on the development of vaccinia virus based	Possible animal use includes for preparation of vaccine seed lots, characterisation of seed lot material, infectivity titre (in vivo growth, animal		

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	vaccines against smallpox (CPMP/1100/02)	model), testing for adventitious agents, testing final bulk, testing for, pharmacodynamics characterisation, virulence testing, neurovirulence testing, reproductive function testing.		
Stability	Directive 2001/83/EC 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 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 ICH Topic Q5A (R1): Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or	Animal testing is needed for detection of some viruses.		

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	animal origin (CPMP/ICH/295/95)			
Adventitious agents, safety evaluation, viral safety	Directive 2001/83/EC 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 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 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. 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. 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. 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. 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) Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009) Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009) 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).	

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
Secondary pharmacodynamics	Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006) Guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009)	Potential undesirable physiological effects of 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) Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009) Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009) 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	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
		duration of monitoring should be appropriate to detect possible adverse 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) 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) Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009) Reflection paper on in-vitro cultured chondrocyte containing products for	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)		

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	cartilage repair of the knee (EMA/CAT/CPWP/568181/2009) Guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009)	recommended. For stem cells, studies on biodistribution, differentiation and possible ectopic tissue 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) Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009)	Risk of tumourigenicity arising from the cell product or due to neoplastic transformation of host cells should be considered on a case-by-	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

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
	Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009) Guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009)	case basis. For stem cells, evaluation of tumour formation including in vitro and/or in vivo studies is essential.		vitro studies are normally sufficient, in vivo studies only if in vitro assays indicate an increased risk for tumour formation. Discussion on the need for animal studies to address the risk of tumour formation of all cell-based products initiated at EMA (SWP and CAT).
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) Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009) 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.		

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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) Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006) Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (EMA/CAT/GTWP/671639/2008) 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) 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) Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006) 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. 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).

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
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.	
Biodistribution - *kinetics, persistence, migration*	Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99) Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006) 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.	Conflicting requirements regarding biodistribution studies in different guidelines; harmonisation might be needed.

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) 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) 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) 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.	

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) Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006) Reflection paper on management of clinical risks deriving from insertional mutagenesis (EMA/CAT/190186/2012) 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		

Topic	Regulatory provision	Animal testing requirements	Implemented 3Rs opportunities	Newly identified opportunities for 3Rs implementation
		perinatal toxicity studies 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) Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006) 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) Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006) Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors (EMEA/273974/2005) 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) 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.		

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