Regulatory requirements and actual data outcomes from EPARs on the

Non-clinical Studies of ATMPs (Covering Gene Therapy, Somatic Cell Therapy), and Recombinant Proteins other than Monoclonal Antibodies

based on Review of EPARs (12/2009-12/2019)

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I List of Abbreviations

3R	Reduce, Refine, Replace
AAV	Adeno-associated virus
ADME	Absorption, Distribution, Metabolism and Excretion
ATMPs	Advanced Therapy Medicinal Products
AUC/AUEC	Area under the plasma (effective) concentration-time curve
C1NH	C1 inhibitor
CAR T	Chimeric antigen receptor T
CAT	Committee for Advanced therapies
CBMP	Cell-based medicinal products
CHMP	Committee for Medicinal Products for Human Use
Cmax	Maximum plasma concentration
CMV	Cytomegalovirus
CPMP	Committee for Propriety Medicinal products
CTD eCTD	Common Technical Document/ Electronic Common Technical Document
DART	Developmental and Reproductive Toxicity
EEA	European Economic Area
EFD	Embryonic and Foetal Development
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	European Medicines Agency
EPAR	European Public Assessment Report
FEED	Fertility and Early embryonic development
FIH	First in Human
GLP	Good Laboratory Practices
GMO	Genetically Modified organisms
GTMP	Gene therapy medicinal products
IA	intraarterial
IP	intraperitoneally
IV	Intravenously

ICH	International Conference of Harmonisation
IGF	Insulin-like growth factor
IVT	intravitreal
JAS	Juvenile Animal Studies
M	Multidisciplinary Guidelines
MA	Marketing authorisation
MoA	Mode of Action
MPs	Medicinal products
NOAEL	No observed adverse effect level
PAD	Pharmacologically Active Dose
PASS	Post-authorisation safety studies
PD	Pharmacodynamics
PDDI	Pharmacodynamic drug-drug interactions
PEG	Poly ethylene glycol
PK	Pharmacokinetics
PKDI	Pharmacokinetic drug-drug interactions
RDTS	Repeat dose toxicity studies
RMP	Risk management plan
RoA	Route of administration
SC	Subcutaneous(ly)
SDTS	Single dose toxicity studies
SmPC	Summary of Product Characteristics
TCR	Tissue Cross Reactivity Study
Tmax	Time to maximum plasma concentration

1. Objective

In the present thesis, the focus of literature-based research is to analyse the non-clinical studies of biopharmaceuticals which have been authorised in the EU in the past 10 years. The scope of the thesis covers ATMPs (gene and somatic cell therapy products) and recombinant proteins excluding monoclonal antibodies. The non-clinical studies submitted for the purpose of MA by the MAA can be evaluated from the European Public Assessment Reports (EPARs), which are accessible online on the EMA website. As the key guideline for conducting preclinical safety evaluation of biotech-based pharmaceuticals, the ICH S6(R1) guideline does not recommend a uniform standard for the conduct of non-clinical studies, but allows a case-by case, science based approach to addressing the non-clinical issues, the information in EPARs is expected to be specific to the product.

With the present literature-based research, it is expected to

- Compare and contrast the requirements and the actual data submitted versus the requirements for non-clinical studies;
- be used as a reference for devising an appropriate regulatory strategy on non-clinical program for ATMPs and recombinant proteins.

The structure and presentation of the results section, as far as possible, will be representative of the common technical document (CTD), as is also the case for EPARs.

2. Introduction

2.1. European Public Assessment Reports (EPAR)

Decisions on the evaluation process of the marketing authorisation applications of medicinal products (MPs) are published by the EMA on its webpage (www.ema.europa.eu). The scientific rationale for additional requirements, if any; inclusion of information in the risk management plan (RMP) or the Summary of Product Characteristics (SmPc) or the decision to refuse applications for marketing authorisation (MA) as referred in Article 13 (3) of Regulation 726/2004 [1] are also made public in the EPARs. Furthermore, EPARs are continuously updated to reflect the latest scientific knowhow on the MP throughout its lifecycle and summarize the current state of the art on the safety and efficacy of the MP. Structure and content of EPARs follow the internationally agreed format for the submission of dossier content during the MAA [2]. EPARs summarize the complete evaluation process of the MP from the point of view of the assessors. The content is discussed with respect to the compliance of the submitted data and results to the regulatory guidelines applicable to the MP. EPARs are essentially the CHMP assessment report (without annexes and commercially confidential information) and follow a format, where the subsections are in line with the CTD subsections and the non-clinical aspects are covered in the section 2.3 of the EPARs.

2.2. Regulatory framework

As EMA is a member of the International Conference on Harmonisation (ICH), both ICH and EMA scientific guidelines are applicable for the MA of new drug products in the EU centralised authorisation procedure. The ICH guidelines are classified into four groups:

- Quality Guidelines (Q) for harmonised standards in quality and manufacturing processes;
- Efficacy Guidelines (E) on design, conduct, safety and reporting of clinical trials;
- Safety Guidelines (S) to detect potential risks like carcinogenicity, genotoxicity and reproduction toxicity during the non-clinical testing phase and
- Multidisciplinary Guidelines (M) which are applicable to all aspects of the drug development procedure (https://www.ich.org/page/ich-guidelines)

The EMA scientific guidelines shed light on the interpretation of ICH guidelines and the practical aspects to generate evidence of quality, safety and efficacy of the drug product. While the guidelines are not legally binding, any deviations have to be appropriately justified.

2.3. Non- clinical studies for Marketing Authorization Applications (MAA)

The marketing authorization (MA) of biotechnology-derived pharmaceuticals requires a comprehensive pre-clinical data set to be presented in Module 4 of the CTD [2]. Non-clinical data provides the primary understanding of the product's mode of action (MoA), allows a prediction of the safety profile of the product, and can be used as tools while standardizing a manufacturing process or in case of a significant change in the manufacturing or the route of administration (RoA).The scope and objectives of the non-clinical studies are predetermined and referred in ICH-M3 (R2) *Guideline on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals [3]*. Detailed requirements for the conduct of non-clinical studies for biopharmaceuticals are discussed in the following section.

2.3.1. Non-clinical requirements for Advanced Therapy Medicinal Products (ATMPs)

The legal definitions of ATMPs and the terms ‘gene therapy medicinal product’ and ‘somatic cell therapy product’ are laid down by Directive 2001/83/EC, as amended [4] and Regulation(EC) No 1394/2007 [5]. Although ATMPs are biotechnology derived pharmaceuticals, the scope of the guideline ICH S6(R1) [6] for preclinical evaluation of biotech derived pharmaceuticals specifically excludes cell and gene therapies. Therefore, the non-clinical requirements for ATMPs are laid down by EMA guidelines. These include the overarching guideline for human GTMPs “*Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products”* [7] and “The *Guideline on human cell-based medicinal products* “[8]. If the cells are genetically modified, the *Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells* [9]

has to be considered for the non-clinical program. However, all ATMP specific EMA guidelines recommend reference to the ICH guidelines. Therefore, both ICH guidelines and EMA specific guidelines will apply to ATMPs.

The EMA website defines Advanced therapy medicinal products (ATMPs) as ''medicinal products that are based on genes, tissues or cells''. ATMPs can be further classified as:

1. Gene therapy medicinal products (GTMPs), where the intended therapeutic, prophylactic or diagnostic effect of the medicinal product (MP) is derived from genes. Gene therapy works by insertion of a 'recombinant' gene into the body, to treat genetic disorders, cancer or long-term diseases;
2. MPs based on somatic-cell therapy (CBMPs), where cells or tissues are manipulated to alter their biological characteristics or cells or tissues not intended to be used for the same essential functions in the body. CBMPs can be used to cure, diagnose or prevent diseases;
3. MPs based on tissue-engineered products contain modified cells or tissues for use in repair, regeneration or replacement of human tissue.

Other than the above, there are combined ATMPs, which may contain one or more medical devices as an integral part of the MP.

2.3.2. Non-clinical requirements for Gene Therapy Medicinal Products (GTMPs)

The overarching guideline for human GTMPs is the *Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products* [7]. The parameters to be considered while designing the non-clinical program for GTMPS are discussed in the following sections

General principles

The aim of non-clinical studies in the development of GTMPs is to provide enough evidence to enable benefit-risk assessment for the use in humans. The nature and extent of non-clinical program depends on the nature of the GTMP, availability of relevant models, clinical use, intended population and the route of administration A risk-based approach is allowed in the non-clinical design.

Characterisation of the GTMP

Sufficient characterization of the products used in the non-clinical studies is required to assure that the studies have been conducted with a representative material that would be administered in humans in clinical studies. The extrapolation of the impact of any changes to the manufacturing process and the test article during the development to humans should be considered

Methods of analysis

Methods used in the non-clinical program should be technically validated with the test article. justification for the selection of assays and their specificity and sensitivity Should be provided.

Species/model selection

Non-clinical studies should be conducted the best available pharmacologically relevant in vitro and in vivo models with the rationale for the choice of the models. If no appropriate animal models are available, animal models should be developed or in vitro systems reflecting the disease state should be used. In case a single animal model is not sufficient to address all aspects, more than one animal model should be considered.

1 Pharmacodynamics (PD) for GTMPs

Proof of concept studies: In vitro and in vivo studies should detail the mechanism of action (MoA) in relation to the therapeutic use (pharmacodynamic "proof of concept" studies) in relevant animal species and models suitable to demonstrate that the nucleic acid sequence reaches its intended target (organ or cells) and is functional (with respect to the expression level and functional activity). PD studies with gene therapy products/ GM cells should demonstrate evidence supporting the potential clinical effect or information on the related biological effect/molecular MoA. The expected effects of genetic modification, such as cell differentiation and/or proliferation induced by the gene product or recovery. When the GTMP is intended to have a selective or target-restricted function, studies to confirm the specificity of this function in target cells and tissues should be performed.

Safety Pharmacology (SP) studies may be required to investigate the potential undesirable PD effects of the GTMP on vital physiological functions (CNS, CV system, respiratory system), and other organ systems depending on the product, as recommended in the *ICH S7A [10].* Appropriate SP studies should be conducted or its absence justified and agreed by the competent authorities. Decision to conduct SP studies is on a on a case-by-case basis, depending on the intended route of Administration (RoA) in patients, state of knowledge of the vector class and distribution, and the MoA of the transgene product. SP endpoints should be incorporated in toxicity and biodistribution studies, wherever possible.

2 Pharmacokinetic studies

Pharmacokinetic studies (PK) studies for GTMPs should address the in vivo fate of GM cells with respect to the biodistribution, persistence, clearance and mobilization of the GTMP, and the associated risk of germline transmission. PK studies should be combined with non-clinical safety studies where possible [7, 11]. As PK studies rely on the detection of nucleic acid sequence administered (vector and/or transgene), the studies should include all relevant organs and tissues, targeted and non-targeted. Studies on the shedding of virus should be performed in accordance with the *ICH considerations: General principles to address virus and vector shedding.* [12]. For PK studies, only validated methods should be used to investigate tissue distribution and persistence of the GTMP. Justification for the selection of assays and their specificity and sensitivity should be provided.

Biodistribution, persistence and clearance: The dosing used for biodistribution studies should mimic the clinical use with appropriate safety margins for the doses selected for human use. The RoA and the frequency of use should mimic the use in clinical setting. Biodistribution of the GTMP after a single administration may additionally provide information on the clearance of the administered GTMP.

Intended genomic integration: If the whole vector (e.g. retro/lentiviruses) or part (chimeric vectors with retroviral/lentiviral portions) is intended for integration in the host genome, it should be evaluated by integration studies (ex vivo tissue culture or in vivo). Integration studies should focus on target tissues/organs for vector integration, copy number and localisation of the integrated vector copies in the host genome, structural integrity of the vector/genomic stability of the vector and on/off target integration events.

Risk of Germline Transmission

The possibility of vertical germline transmission of vector DNA should be investigated unless otherwise justified based on the clinical indication and / or patient population. The decision to study potential germline transmission in the context of vector DNA biodistribution should follow the risk assessment of the GTMP with respect to vector type, dose, RoA and clinical purpose on a case-by-case basis, as recommended by the *Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors*.[13] The above aspects should be considered in conjunction with the *Guideline on human cell-based medicinal products* [8], *Note for Guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products* [14], and the *Guideline on non-clinical studies required before first clinical use of gene therapy medicinal products*[15].

Vector shedding: Shedding is defined as the dissemination of vector through secretions and/or excreta and should be addressed in animal model. of the shedding pattern is known, there is no need for additional non-clinical evaluation.

3 Other Pharmacokinetic (PK) studies

The PK behaviour of devices or structural components of a GTMP should be investigated where applicable and the impact of the components on vector distribution should be analysed.

4 Toxicology of GTMPs

Toxicity should be assessed for the whole GTMP and for the transgene product such that the unwanted consequences of the distribution and persistence of the vector, its infection / transduction /transfection, the expression and biological activity of the therapeutic gene(s) and vector genes are evaluated. Unwanted pharmacological effects and immunogenicity should be assessed. The extent of non-clinical safety assessment and the study design should be determined on a case by case basis, depending on the type of the product and tissue tropism/biodistribution and persistence of the GTMP.

Study design for toxicology studies

Single and repeat dose toxicity studies: for GTMPs intended for single administration, single dose toxicology studies (SDTS) with extended observation period should be performed.

Repeated-dose toxicity studies (RDTS) when multiple dosing in humans is intended, RDTS should be conducted using the route and schedule of administration that reflects the clinical dosing. The duration of the SDTS and RDTS may be longer than standard toxicity studies for other bio-pharmaceuticals, depending on the persistence of the GTMP, level and site of expression and the anticipated risks. The duration of the studies should be justified and the recovery period should rely on the persistence of the vector and the transgene expression. The use of one relevant species for SDTS and RDTS may be sufficient unless specific safety concerns require the use of a second animal species.

5 Genotoxicity studies for GTMPs

The need for genotoxicity studies depends on the kind of product and the objectives should cover the following:

1) Investigation of gene modification and any changes in cell behaviour
2) Evaluation of toxicity issues related to insertional mutagenesis and the mechanism of action
3) Evaluation of off target toxicity, when the product is meant for an on-target approach.
4) Identification of genomic integration sites (IS) and evaluate possible cross-talk between the transgenic and neighbouring sequences

Insertional mutagenesis studies can be performed in vitro and/or in vivo. Established cell lines, primary cells, or animal models should be considered to investigate the safety profile of any GTMP

The risk of insertional mutagenesis should be analysed based on the knowledge on the insertional profile of the vector and the enhancer and promoter sequences used for transactivation to enable the expression of the transgene; the proliferative potential of the target cells, and the knowledge on the resistance of the target cells towards transformation. The possibility of the release of the transfer vector by GM cells i*n vivo* should be investigated, including the potential for interactions with other infectious agents or disease-related drugs, when applicable. The extent of these studies depends on the transfer vector used, its replication capacity and its integration status in the cells.

Vector specific considerations: The potential for integration of the transgene expression cassette into the host genome should be investigated and discussed, for cases where it is intended (retroviral/lentiviral vectors are used) and in cases where integration is not intended (adenoviral, adeno-associated viral or plasmid vectors are used). Requirement for genotoxicity studies of GTMPs with vectors that have a potential for integration to the host genome depends on the manner that the final product is delivered (local versus systemic) and the target tissue/organ system and the biological of the cells to be targeted.

Cells transduced with integrating vectors such as γ-retroviral or lentiviral vectors should be examined for the number of integration sites and characterised to identify adjacent genes and their relevance should be discussed in relation to clinical application. Activation of oncogenes and/or inactivation of tumour suppressor genes and the risk from insertional mutagenesis should be adequately addressed.

6 Tumorigenicity

Standard rodent carcinogenicity studies are usually not required in the non-clinical development. The tumorigenic/oncogenic potential should be investigated in relevant in vivo/in vitro models for neoplasm signals, oncogene activation or cell proliferation index. The decision if the tumorigenic or oncogenic potential of a GTMP needs to be investigated should be guided by the Weight of Evidence approach as per the ICH S6(R1) [6].

7 Other toxicity studies

Immunogenicity and immunotoxicity: Delivery of GTMPs can result in immune responses of the innate (systemic cytokine elevations, multi-organ inflammation) and adaptive immune system. these aspects should be considered in the development of the non-clinical program. The potential for complement activation and the resulting consequences should be considered. Repeat-dose administration can lead to complement activation. Therefore, markers of complement activation should be investigated in animal and human sera.

8 Reproductive and developmental toxicity studies

The potential for reproductive/developmental toxicity has to be determined on a case by case basis, based on the product type, mechanism of action, distribution and shedding profile, and patient population. General principles related to reproductive toxicity are provided in ICH guideline S5 (R2) [16]. If the risk for germline transmission cannot be determined as per the guideline[13], breeding studies should be conducted to address the transmission of the nucleic acid to the offspring. Embryo-foetal and perinatal toxicity studies and germline transmission studies should be provided, unless otherwise justified on the basis of the type of product concerned. If women of childbearing potential are to be exposed to the GTMP, embryo-foetal and perinatal toxicity studies may be required, depending on the clinical use.

9 Local tolerance

Local tolerance studies may be relevant for some GTMPs, depending on their type, route and protocol of administration (e.g. intra-ocular, intramuscular, intravenous, intratumoural). Local tolerance studies are not required if the proposed clinical formulation and route of administration have been examined in other animal studies.

10 Drug interactions

Effects of co-medication should be investigated on a case by case basis if an impact on the vector transduction, tropism and efficacy, expression of the therapeutic gene or biological activity of the expressed proteins and tissue distribution of the vector is expected. Especially, the clearance of the

viral vector and the effects of GTMP may be altered when immunosuppressive treatments are concomitantly.

11 Environmental Risk Assessment for GMOs

Directive 2001/83/EC, as amended [4], and Regulation 726/2004 [1] mandate that the potential risk of the GMO containing MP to the environment must be conducted according to the principles of Annex II of Directive 2001/18/EC [17]. The guidance for MPs containing GMOs is outlined in '*Environmental Risk Assessment for Medicinal Products Consisting of, or containing, Genetically Modified Organisms (GMOs)'* [18] and the *Guideline on scientific requirements for the environmental risk assessment of gene therapy medicinal products.*[19]. As there is no threshold limit for GTMPs to define the risk to the environment, ERA has to be based on the probability of GTMP transmission from the patient to other persons, animals, plants or the environment at large.

2.3.3. Non-clinical requirements for human cell-based medicinal products (CBMPs)

As per the *Guideline on human cell-based medicinal products* [8], the variability of CBMPs should be reflected in the non-clinical studies and the conventional requirements detailed in CTD Module 4 may not be appropriate. If the cells used in CBMPs have been genetically modified, the non-clinical program should comply with the GTMPs [14].

In vitro models can be used if in vivo models cannot be developed. The rationale for the selection of animal model must be justified. Expression level of biologically active molecules, the RoA and the dosages tested should reflect the intended clinical use in humans as per the *ICH S6(R1)* [6].

1 **Pharmacology**: adequate proof-of principle should be provided in suitable in vitro and /or in vivo models. The markers used for testing the biological activity should be reasonably justified and enable identification of pharmacodynamic action of the CBMP in the host. Functional tests to demonstrate the restoration of function should be conducted in case a CBMP is intended to restore the function of deficient cells/tissue (tissue regeneration). If the intended use is an immunological function, data on immunological action of the CBMP should be included with the data on the biological effect. Animal models may include immunocompromised, knockout or transgenic animals. Homologous models might be advantageous, as application of cells or tissues in heterologous models might be altered due to mismatch. Homologous models should also be considered to study stem cell differentiation. In vitro studies should address cell and tissue morphology, proliferation, phenotype, heterogeneity and the level of differentiation as a part of the primary PD analyses [8].

Secondary PD studies should include potential undesirable physiological effects of human CBMP in an appropriate animal model. Migration of cells from their intended location and homing to other organs should be investigated.

Safety pharmacology (SP) should be considered on a case-by-case basis depending on the CBMP characteristics, if the CBMP is expected to secrete pharmacologically active substances affecting the CNS, CV, respiratory, renal or GI functions. ICH S7A *[10]* should also be considered for SP aspects.

2 **Pharmacokinetic evaluations**

Kinetics, migration and persistence should be the focus of PK investigations in CBMPs**.** Tissue distribution, viability, trafficking, cell growth and phenotypic changes due to factors in the new environment should be conducted. For human CBMPs intended for secretion of systemically active biomolecules, the distribution, duration and amount of expression of these molecules should be investigated along with the survival and the functional stability of the cells at target sites.

Interactions of the applied cells or surrounding tissue with the non-cellular structural components, other bioactive molecules and the integration of the CBMP with the surrounding tissue should be monitored.

3 Toxicology: toxicology studies for CBMPs are product specific and conventional toxicology studies may be inappropriate. Toxicity may evolve in case of CBMPs, as an effect of a manufacturing process change, causing an altered excretion patterns and in vivo behaviour. The need for drug interaction studies depends on the intended use and the type of the CBMP and should be discussed. Potential immunogenicity of a CBMP should be considered, especially for excreted substances as per *ICH S6(R1)* and auto-immunity should be considered where cells are used for immunotherapy (e.g. cancer immunotherapy). **Single and repeated dose toxicity studies (RDTS)** should be performed in relevant animal models. If the human cells are not immediately rejected, the studies may be combined with SP, local tolerance, or proof of concept and efficacy studies. Sufficiently characterized analogous animal-derived cells may be used for some allogeneic CBMP, when not immediately rejected. The duration of observations in such studies might be much longer than in standard single dose studies, as cells function for longer time period. The RoA and dosing regimen should reflect the intended clinical use. RDTS are only relevant if the clinical use includes multiple dosing.

4 Local tolerance studies: may be required in an appropriate species. For excreted substances, local tolerance and tissue compatibility can be evaluated in SDTS or RDTS. Risk of tumorigenicity should be addressed on a case-by-case basis.

5 Carcinogenicity and Genotoxicity studies

Carcinogenicity studies may not be feasible and tumorigenesis studies are preferable with cells at the limit, or beyond the of routine cell culturing limit. Genotoxicity studies are not considered necessary for human CBMPs, unless there is a risk of direct interaction of an expressed product with DNA or chromosomal material.

6 The need for reproductive studies for CBMP and should be considered on a case-by-case basis and the recommendations in the *Guideline on human cell-based medicinal products* [8] and *Risk based approach to non-substantially manipulated cell-based ATMPs* [20] should be considered. Deviations from the above principles should be justified and the need for toxicology studies should follow a risk based approach, where safety endpoints may be incorporated into proof of concept studies in justified cases [21].

2.3.4. Non-clinical studies for biotechnology -derived medicinal products-overview

Biopharmaceuticals comprise of a heterogeneous group with respect to their structural properties and biological functions. Conventional approaches used for small molecule drugs are inappropriate for the characterisation of biologics. The ICH regions have therefore agreed on a "flexible, case-by-case, science-based approach" for non-clinical testing for biologics, as stated in the ICH-S6 guideline on preclinical safety evaluation of biotechnology-derived pharmaceuticals and the addendum to this guideline, referred to as ICH S6 (R1) [6]. This guideline along with other relevant guidelines [2, 3] lay down the approach for the evaluation of biologics.

Another aspect in which biologics differ from small molecule non-clinical studies is the requirement for Good Laboratory Practices (GLP), which is mandatory for small molecule drugs but allows for flexibility in case of biologics and the submitted studies may be used wherever feasible *[3]*.

1 Test Material Specifications

Biotechnology derived pharmaceuticals are biologically active molecules and are prone to contamination from the host cells (yeast, bacteria, mammalian cells) in which they are produced. These contaminants can potentially trigger immunogenic reactions, cause viral contamination or carry the risk of integration of foreign DNA into the recipient genome. These risks have to be appropriately mitigated by setting specific criteria that can be evaluated on the basis of biochemical and biological characterisation [6]. The test materials used during the drug development stages have to be comparable in predefined specification parameters and additional characterisation may be required where manufacturing process is changed and, or optimized to ensure consistent quality. The *Guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process: non-clinical and clinical issues* [22] outlines the requirements in such cases. Comparability of all critical non-clinical endpoints should be demonstrated, for example for PD parameters with relevance to clinical application, the PK parameters, and consistencies in the immune response and the observed toxicological profiles [23].

2 Defining the relevant species

Relevant species must be defined before first in human (FIH trials) and their relevance is established on the basis of the functional/pharmacological activity of the candidate drug in species-specific cell

systems in vitro or in vivo. Species relevance can be established if there is a modulation of a known biological response or modulation of a pharmacodynamic marker indicative of functional activity. Following criteria can be used to justify pharmacological relevance of the species used in non- clinical studies:

- In-vitro binding affinity, receptor occupancy, In-vitro bioactivity
- Sequence homology to humans,
- Expression pattern of the target
- In-vivo pharmacological activity.

In the absence of a relevant species, transgenic animals expressing the human receptor or the use of homologous proteins should be considered [6].

3 Primary and Secondary Pharmacodynamics, and Safety Pharmacology (SP)

The aims of pharmacodynamics (PD) is to demonstrate the efficacy of the drug and its modes of action. Pharmacology studies are further classified as:

- Primary PD effects: Investigation of the MoA and effects with respect to the desired therapeutic target,
- Secondary PD effects: mechanism of action (MoA) and effects not related to the desired therapeutic target
- Safety Pharmacology (SP) studies: include investigations on the potential undesirable PD effects of the candidate drug on physiological functions (when exposed in and above the therapeutic range as per ICH S7A *[10]*.

The effects on the cell phenotype/ metabolic status, can be determined *in vitro* using cell lines expressing receptors relevant to humans. Properties like phosphorylation status, receptor binding and activation can be used to establish clinically relevant pathways and signalling effects. As per the ICH-S6 (R1) [6], clinical relevance of biotech-derived active substances depends on their functionality, as defined by the binding to a specific receptor/interaction with other proteins. To evaluate on/off - target effects, in vitro and ex-vivo tissue cross reactivity studies (TCR) should be used [6], which must be performed prior to toxicity studies [24]. Additionally, immunological properties of biotech derived medicines such as “antigenic specificity, complement binding, and any unintentional reactivity and/or cytotoxicity towards human tissues, which is distinct from the intended target, have to be defined.

Safety Pharmacology (SP): SP studies enable detection and investigations on any potential undesirable PD effects of the test substance and help in identification of endpoints to be monitored in the context of toxicity and clinical studies. In vivo SP studies reveal any functional effects on the vital organ systems including the cardiovascular (CV), respiratory, renal and central nervous systems

(CNS), and are defined as the "core battery tests". For biotechnology-derived pharmaceuticals, the endpoints for SP studies can be incorporated in the design of other PD/toxicology studies. In contrast to the products that are not highly specific with respect to the binding to targets, like for small molecule drugs, an extensive SP investigation should be performed for biotechnology-derived pharmaceuticals, as outlined in the ICH-S7A *[10]*.

4 Pharmacokinetics (PK) and Toxicokinetics (TK)

It is important to characterise the PK properties of the biotech-derived products with respect to drug availability. As per the ICH-S6(R1), following studies have to be conducted for biotechnology-based pharmaceuticals:

- PK- single and multiple dose pharmacokinetics
- Toxicokinetics
- Tissue distribution studies in relevant species

Due to diverse nature of biopharmaceuticals the non-clinical testing may require a product-specific study design. TK evaluations extend the data derived from PK studies while implementing higher dosages of the product. For recombinant proteins, the pattern of distribution (D), metabolism (M) and elimination(E) is often predictable, as they are expected to be degraded like endogenous proteins. Therefore, specific interaction studies with tissue components and the possible influence of binding proteins may be essential to understand the pharmacological behaviour of the active substance. Non-specific or target mediated proteolysis is a common elimination pathway for protein-based products and oxidative hepatic metabolism is generally not expected. Of importance is the difference in PK among the test species used as well as the immune mediated clearance mechanisms, which may impact the kinetic profiles of the drug candidate, affecting the predictive value of drug use in humans. All potential factors affecting the kinetics should be taken into account[6].

Route of Administration (RoA): The RoA and frequency of administration used in non-clinical animal studies should mimic the intended clinical use and any RoA used for the non-clinical investigations other than the clinically intended should be justified [6].

Requirements for FIH Dose Selection: Dosages tested in relevant species provide information on the dose-response relationship. The addendum to the ICH-S6 gives the advice to determine "*a dose which provides the maximum intended pharmacological effect in the non-clinical species*" and "*dose which provides an approximately 10-fold exposure multiple over the maximum exposure to be achieved in the clinic*" [23]. The higher of these two doses should be chosen for the high dose group in non-clinical toxicity studies. This approach allows for the identification of a toxic dose and a no observed adverse effect level (NOAEL), which is a requirement of the ICH-S6. NOAEL is a generally accepted as a reference for the determination of FIH safe starting dose. This is used to determine a

maximum exposure that is not to be exceeded in the study subjects [25]. In case a hypothetical risk factor is identified, the use of minimal anticipated biological effect level (MABEL), estimated pharmacologically active dose (PAD) and anticipated therapeutic dose range (ATD) approaches are recommended. The calculation of MABEL should be performed using all in vitro and in vivo information available from PK/PD studies. Using a MABEL approach is suited for biopharmaceuticals acting on the immune system, as stated by the ICH-S9 guideline [26].

5 Toxicology- Single and Repeated Dose Toxicity Studies

Single dose/acute toxicity studies establish the relationship between the dosage (low-high) to systemic and/or local toxicity in representative animal species. The treatment regimen in repeat-dose toxicity studies (RDTS) should be specified on the basis of toxicokinetic (TK) findings, single dose toxicity studies and the PD data [6]. The duration of RDTS, the RoA and the frequency of administration should cover the intended duration of clinical exposure and should be scientifically justified. As per the ICH-S6 (R1) [6], for biologics intended for single or short-term use (under 7 days), up to 2-week RDTS are considered sufficient to support MA. Pharmaceutical products intended for long-term use (i.e. for chronic indications) generally have to submit RDTS of 6 months duration, or shorter, if appropriately justified. In this case, ICH-S6 (R1) refers to experience rather than instructions and generally, studies between 1-3 months have been accepted [6]. **Recovery monitoring period**, is used to evaluate the reversibility or worsening of occurred events along with the identification of delayed toxic effects, and should generally be included in study designs. For MPs with prolonged pharmacological/toxicological effects, recovery group animals should be observed until reversibility is demonstrated [6]. **Selection of Relevant Species for toxicology studies:** Species specificity is an important challenge for biotechnology-derived products to allow for a relevant extrapolation of toxicity findings for human use. The use of two species (rodent and non-rodent) for toxicological assessment in short-term toxicology studies is mandated by regulatory guidance [6, 27] for both small and large molecules. Long-term general toxicity studies (chronic toxicity studies) in one species are sufficient. Since the use of non-relevant species may lead to false interpretations regarding drug safety, it must be avoided. Furthermore, the principles of 3Rs should be followed to achieve a reduction of the number of animals used as per the *Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches.*[28].

6 Genotoxicity and Carcinogenicity Studies

Genotoxicity is generally not expected from biotechnology-derived recombinant proteins as they do not interact directly with DNA. In case of conjugated MPs with an organic linker molecule, genotoxic evaluation may be required. In general, according to the ICH-S1A, carcinogenicity studies are required for pharmaceutical expected to be in clinical use for more than 6 months [29].For biotech-derived pharmaceuticals, standard carcinogenicity studies are inappropriate and the decision to

conduct carcinogenicity studies should be product specific [6] as per the ICH S1A [29]. If an assessment of carcinogenicity is warranted, weight of evidence approach should be used based on literature evidence on animal disease models, class effects, detailed information on target biology and mechanism of action, in vitro data, data from chronic toxicity studies and clinical data. The product-specific assessment of carcinogenic potential is used to communicate risk and provide input to the risk management plan along with labelling proposal. Carcinogenicity studies are not recommended for therapeutics intended for treatment of advanced cancers and biosimilar products as per the guideline on biosimilar biotech-derived products [30].

7 Reproductive and Developmental Toxicity (DART) and JAS Studies

DART of human pharmaceuticals is regulated by the ICH-S5(R2) guideline [16]. Reproduction toxicity studies aim to identify the effects of drug use on mammalian reproductive system and its function. As per the guideline, all treatment associated and abnormalities should be adequately evaluated, to allow a reliable assessment of the risks to human fertility and all developmental stages. Any treatment or observational gaps should be avoided. The development stages are defined as the following:

A Premating to conception; **B** Conception to implantation; **C** Implantation to closure of the hard palate; **D** Closure of the hard palate to the end of pregnancy; **E** Birth to weaning; **F** Weaning to sexual maturity.

DART studies are of significance only when conducted in a pharmacologically relevant species. For products directed against exogenous agents (example vaccines) (bacteria, virus, other drugs), no reproductive toxicity studies are required [29], unless novel excipients or adjuvant systems are used. Furthermore, as per ICH S6 (R1), the need for DART studies is dependent upon the product, clinical indication and intended patient population [6].

Fertility and Early Embryonic Development (FEED) studies should be designed to adequately address the detection of the impact of the candidate drug on stages A-B (in point 7 above). Extrapolation of the impact of the drug reproductive hormone levels, oestrous cycle, sperm count, morphology and motility, mating behaviour, conceptions and implantations issues (i.e. early embryonic development) should be possible [16]. These evaluations may be done as a part of RDTS, in which case the RDTS should be of at least 3 months duration and include sexually mature animals.

Embryo-Foetal Development (EFD) Studies: EFD studies should address the detection of the impact of the drug on pregnant females and on the development of the embryo and foetus in stage C (in point.7). If the embryo-foetal exposure is expected to be low because of the placental barrier, as is the case for large molecules, the EFD study-reports can be submitted with the MAA [6]. Fusion proteins with properties similar to monoclonal antibodies (those containing Fc elements) have their own active membrane-anchored transport mechanism via FcRn-mediated uptake of Fc fragment of

IgG. This transport does not reach the critical extent during organogenesis [31]. Studies aiming to detect any possible secondary effects on the maternal or placental toxicity should be performed [6] While GLP studies are recommended, highest scientific conditions are to be used in case GLP studies are not possible [6]

Studies for Effects on Prenatal and Postnatal Development, Including Maternal Function (PPND)

PPND studies evaluate the effect of drug exposure in females from implantation through weaning and its effect on pregnant/lactating female and the offspring. Observations should cover the period including sexual maturity of the offspring (stages C-F listed in point 7 above) to enable the detection of delayed effects.

Juvenile Animal Studies (JAS): Data on juvenile animals is not mandatory to include paediatric population in clinical program. If deemed necessary, JAS should be available before the initiation of paediatric clinical studies, as per the *Guideline on the need for non-clinical testing in juvenile animals of pharmaceuticals for paediatric indications [32].* The other guidelines referring to the requirement for JAS include ICH-M3(R2) [3] and ICH-S5 (R2) [16].

8 Immunotoxicity and Immunogenicity/Antigenicity in Test Animals

Non-clinical immunotoxicity requirements are discussed in the *Guideline on Immunogenicity Assessment of Biotechnology-derived Therapeutic Proteins*[33]. 28-day immunotoxicity tests with consecutive daily dosing in rodents or other standard test batteries are not recommended for biotechnology-derived pharmaceuticals. Measurement of antibodies in non-clinical studies are requested as part of repeated dose toxicity studies (RDTS), so as to aid their interpretation, as stated in the ICH S6-R1[6]. When anti-drug antibody (ADA) measurements are not included in the protocol, blood samples should be stored for future evaluations when warranted. Comparison of the antibody response to the reference product in an animal model may form a part of the comparability exercise for similar biological MPs as per *Guideline on similar biological medicinal products* [30] and for changes in manufacturing processes as per the *Guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process -Non-clinical and clinical issues* [22]. Toxic or autoimmune relevant endpoints can be incorporated in RDTS or DART studies. Anti-drug antibodies (ADA) also have the potential to decrease/ neutralise the levels of circulating drug during multiple dose applications, which are required for chronic, development or reproductive toxicity studies and therefore, careful evaluation of immunogenicity data must be conducted to exclude any misinterpretation of safety-relevant data. Measurement of ADA in animal studies can be included in RDTS and supports their interpretation. For biosimilars, in vivo comparability for immunogenicity testing is generally not recommended [34].

Immunotoxicity and Immunogenicity in Humans: Humanized or fully human proteins (i.e. intrinsic proteins) can induce ADAs in clinical use, which can result from a number of factors like impurities,

sequence variation from the endogenous protein, post-translational modifications, or protein aggregation, which are either product or process dependent. Product dependent immunogenic factors cannot be predicted easily and severe adverse reactions such as anaphylactic shock, and neutralization of an endogenous proteins have been reported for recombinant proteins. In most cases, ADAs result in the impairment of treatment efficacy. While not predictive for immunogenicity in humans, the assessment of immunotoxic potential of biopharmaceuticals in in-vivo animal studies is a valuable supportive tool, especially for immunomodulatory biopharmaceuticals [2].

9 Local Tolerance studies

The *Guideline on non-clinical local tolerance testing of medicinal products* [11] lays down the recommendations for local tolerance testing and is relevant for both, small molecule drugs and biopharmaceuticals. It however also refers to ICH S6-R1[6] for biotechnology-derived products, which does not specify the need for stand-alone studies for local tolerance and recommends incorporating local tolerance endpoints in other toxicity studies [6]. The use of a pharmacologically active species for local tolerance studies is also not mentioned in cases where standalone local tolerance studies are conducted and evaluation in one species and in a single sex is sufficient.

10 Other Toxicity Studies

According the ICH-M4S guideline [2], if the studies on antigenicity; Immunotoxicity; mechanistic studies (if not reported elsewhere); studies on dependence, metabolites and impurities or 'other studies' have been performed, they should be summarised with an appropriate rationale for the conduct of studies in the CTD under section 2.6.6.8 "Other Toxicity Studies". As per the *ICH M4S* [2], the studies on impurities and metabolites in the drug substance do not apply to biotechnology-derived products. However, as per ICH S6 (R1) "*It is preferable to rely on purification processes to remove impurities and contaminants rather than to establish a preclinical testing program for their qualification*" [6].

11 Environmental Risk Assessment (ERA)

The *Guideline on the environmental risk assessment of medicinal products for human use* [35], lays down the recommendations for ERA studies. MPs containing natural peptides or proteins are readily degradable and therefore do not have a requirement to submit ERA. If, however, a MP includes a synthetic protein/peptide, which has been modified to affect the stability or other characteristics, it is recommended to perform an additional screening step to demonstrate that they are readily degradable in the environment. ERA is not required when the non-natural peptide/protein is demonstrated to be excreted in amounts < 10% of the dose [35].

2.3.5. Non-clinical requirements for similar biological medicinal products

As per the *Guideline on similar biological medicinal* products [36] and the *Guideline on similar biological medicinal products containing-biotechnology derived-proteins* [30] a biosimilar is "*a biological medicinal product that contains a version of the active substance of an already authorised original biological medicinal product (reference medicinal product) in the EEA*". The non-clinical program for biosimilars should be guided by the following approach:

Step 1: ***in vitro* pharmaco-toxicological studies** to enable decision on the extent of *in vivo* animal studies required. Non clinical studies should be designed after a clear understanding of the characteristics of the reference product. Results from the physico-chemical and biological characterisation studies (i.e. comparability of the biosimilar to the reference product) should be reviewed from aspect of the potential impact on efficacy and safety.

Binding to target(s): Receptors, antigens, and enzymes known to impart pharmaco-toxicological effects and/or pharmacokinetics of the reference product should be investigated in the in vitro studies. Signal transduction and functional activity/viability of cells of relevance for the pharmaco-toxicological effects of the reference product should be studied in a comparative manner. If *in vitro* studies are considered satisfactory and there are no issues identified that would block direct FIH use, i*n vivo* animal study is usually not considered necessary.

Step 2 Determination of the need for In vivo studies: Presence of relevant quality attributes not detected in the reference product (e.g. new post-translational glycosylations) and when relevant quantifiable differences in quality attributes between the biosimilar and the reference product are present (i.e. differences in formulation, excipient effects), that cannot be sufficiently characterised in vitro, in vivo studies may be necessary. Careful consideration should be made necessary information can be derived from clinical testing in healthy volunteers. In case additional in vivo studies are needed, availability of a relevant animal species or other models (e.g. transgenic animals, transplant models) should be considered. If relevant in vivo animal model is not available, proceeding to human studies is possible, after adopting risk mitigation measures.

Step 3: if an in vivo evaluation is the focus of the study/studies (PK and/or PD and/or safety), depending on the need for additional information, animal studies should be designed to maximise the information obtained using the principles of the 3Rs (replacement, refinement, reduction) as per the *Article 4 of Directive 2010/63/EU [37].*

Studies not required for biosimilars: Safety pharmacology, reproductive and developmental toxicity, carcinogenicity and local tolerance are not required for non-clinical testing of biosimilars.

2.3.6. Requirements for novel and biosimilar insulin analogues

Carcinogenicity of novel insulin analogues: The nonclinical evaluation of carcinogenicity for novel insulin analogues is addressed in the '*Points to consider document on the non-clinical assessment of the carcinogenic potential of insulin analogues'* [38], stating that insulin Asp B10 insulin (also called X10) should be considered as a positive control in the studies along with normal insulin. AspB10 insulin has the B10 histidine residue replaced with an aspartic acid residue, and has a mitogenic activity exceeding that normal human insulin [39]. Native human insulin is weakly mitogenic and it is important to address the mitogenic effect of modified insulin analogues for assessment of safety aspects. Structural modifications of the normal human insulin molecule result in increased mitogenic potency, which can stimulate the growth of pre-existing neoplasms. As insulin analogues are meant for long term use, insulins with an increased mitogenic effect require a thorough assessment of carcinogenic potential.

Biosimilar insulin analogues have special requirements and the *Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues* [40] addresses non-clinical issues specific to biosimilar insulins and insulin analogues for the biosimilarity exercise for insulin analogues:

Pharmacodynamic (PD) studies: In vitro studies are required to address the differences in properties of the biosimilar and the reference MP in a head to head comparison. These include comparative in vitro bioassays for receptor binding and tests for biological activity. In part, this data may be available from bioassays measuring potency in the evaluation of physico-chemical characteristics. Comparative receptor binding on both human insulin receptors (IR-A and IR-B), including on-off kinetics, should be shown. Cells artificially expressing IR-A and IR-B, can be used. If cell lines with endogenous expression of IR-A or IR-B are employed, it has to be demonstrated that only one receptor subtype is present. If other state-of-the-art methods are used to determining binding, the choice of the method should be justified [40].

Biological activity should be compared at two levels:

1) Receptor autophosphorylation and
2) Metabolic activity through assays for glycogen formation, lipogenesis, inhibition of stimulated lipolysis as well as glucose transport. At least 3 different assays of metabolic activity should be performed for confirmation on the agonist properties of the insulin receptors in the biosimilar and the reference product.

Studies not required for biosimilar insulin analogs: Comparative in vivo PD studies are not required for the comparability exercise. Separate repeat dose toxicity studies (RDTS) are generally not required. In cases where novel excipients are introduced, a risk-based approach should be followed

as per the *Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues rev. 1*[30]. Safety pharmacology, developmental and reproductive toxicity studies, carcinogenicity and studies on local tolerance are not required unless new excipients are introduced (not well characterised for the intended RoA).

2.3.7. Requirements for similar medicinal products (MPs) containing recombinant granulocyte-colony stimulating factor (G-CSF).

As per the "*Annex to the guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues: Guidance on similar medicinal products containing recombinant granulocyte-colony stimulating factor "*[41], comparability of the test and the reference should be demonstrated at the receptor level in an appropriate *in vitro* cell-based bioassay or receptor-binding assay. Both, non-neutropenic and neutropenic rodent models should be used to compare the PD effects of the test and reference as per the guideline [41] and the concept paper published on 27/07/2015 [42]. **Toxicology studies**: Data from at least one repeat dose toxicity study should be provided with a minimum study duration of 28 days in a relevant animal species in accordance with '*Note for Guidance on Repeated Dose Toxicity*'[27] and in accordance with the "*Note for Guidance on Toxicokinetics: A Guidance for assessing systemic exposure in toxicological studies* [43]. Importance should be given to immune responses to the MPs. **Local tolerance** in at least one species should be conducted as per guideline [11]. If feasible, local tolerance testing can be a part of RDTS.

Studies not required: safety pharmacology, reproduction and developmental toxicity, genotoxicity and carcinogenic potential are not required for the development of biosimilar G-CSF products.

2.3.8. Requirements for biosimilar Fusion proteins containing Fc element.

For biosimilar fusion proteins containing Fc Elements, in addition to the ICH S6(R1), the *Guideline on similar biological medicinal products containing monoclonal antibodies – non-clinical and clinical issues.*[44] has to be considered, as the Fc Element imparts the properties of monoclonal antibodies. For biosimilars fusion proteins based on IgG, comparison for affinity to the intended target and binding affinity of the Fc to relevant receptors (e.g. FcγR, C1q, FcRn) [45] should be conducted, unless otherwise justified. As the comparability testing is based on the in vitro binding properties to the target molecule, unintended binding to the three isoforms of the relevant Fc-γ receptors, Fab- and Fc-associated cytotoxic or other safety related effects should be conducted [44]. In vitro assays are more specific and sensitive than in vivo studies for the non-clinical comparability exercise. Only if the need for in vivo studies are well justified, some in vivo toxicity studies may be useful. The incorporation of endpoints in one in vivo study should be a preferred for biosimilars and RDTS in non-human primates and the use of non-relevant species in RDTS is not recommended.

2.3.9. Requirements for biosimilar MPs with recombinant human follicle stimulating hormone (r-hFSH)

For biosimilar MPs with r-hFSH, the *Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human follicle stimulating hormone (r-hFSH)* [46] has been issued for this product class and recommends comparative in vivo pharmacodynamic studies for test and reference products. **Studies not required**: Safety pharmacology, reproductive and developmental toxicity, carcinogenicity and local tolerance.

3. Methods

For the selection of medicinal products (MPs) relevant to the scope of the thesis, human medicines data on MPs authorised by EU centralised procedure was downloaded from the following link: (https://www.ema.europa.eu/en/medicines/download-medicine-data). The details on authorised medicines for 1427 human medicines and 251 veterinary medicines (including withdrawn applications) was downloaded. The list of 1427 human medicines cannot be sorted for new recombinant proteins based on the "active substance". Data on biosimilars can directly be sorted and data on 20 biosimilar recombinant proteins were extracted. The list was then filtered using the terms "*Human"* and limiting the marketing authorisation date (December 2009-December 2019) to arrive at 735 human medicines. These were then individually reviewed to extract relevant ATMPs and recombinant proteins. Naturally occurring proteins (for example extracted proteins from blood plasma), synthetic peptides and proteins, monoclonal antibodies and recombinant vaccines are excluded.

In the ATMP category, only products with a classification 'gene therapy' and 'somatic cell therapy' are included. Tissue engineered products and authorised methods for stem cell applications in clinic are excluded. A list of EPARs for 80 MPs was identified for analysis. In cases where sufficient information is available in the EPARs, the data were included in the analysis even if the application is currently withdrawn as the assessment process was completed. For data extraction, the EPARs were reviewed at least twice and the extracted data was compared. The final data extracted from EPARs was used for analysis purposes. For the purpose of the thesis only a 'quantitative evaluation' is performed on the comparison of the regulatory requirements for non-clinical studies and studies actually performed for marketing authorisation. No interpretations are applied on the data that is missing or not discussed in the EPARs.

4. Results and Discussion

4.1. Classification of 80 medicinal products (MPs) into subgroups

In the period from 1st December 2009-31th December 2019, 80 products are identified. 10 products are ATMPs and 70 products are recombinant proteins which are analysed in the present thesis (*Annex II)*.

The group of 70 MPs in the recombinant proteins is further divided into subgroups containing MPs of the same pharma-therapeutic group and /or active substance for the ease of comparison. The identified groups include the following:

1. Insulin Analogs: 9 MPs including combination products
2. Glucagon-like Peptide receptor agonists (GLP) agonists: 5 MPs include GLP-1 and GLP-2 agonists
3. Filgrastim Analogs: 12 MPs with active substance granulocyte-colony stimulating factor (G-CSF)
4. Blood Factors: 17 MPs
5. Parathyroid hormone/analogs: 3 MPs with active substance as human parathyroid hormone.
6. Fusion proteins/chimeric proteins: 6 MPs are chimeric proteins with Fc element.
7. Interferons: 2 MPs with active substance ropeginterferon alfa-2b, peginterferon beta-1a
8. Follicular stimulating hormone (FSH) analogs: 3 MPs with active substance follicle stimulating hormone
9. Unclassified recombinant proteins: remaining 13 MPs with diverse active substances

1 product Lifmior is withdrawn, with no details on the non-clinical studies in the EPAR. Therefore, it is not included in the analysis. In the 'unclassified group', 14 products were initially identified in this group from which 1 product (Invitrolife IVF media) is intended for use in medical devices and excluded from analysis. Both, the EMA classification based on the pharma therapeutic group, and self-annotated classification are indicated in Annex I. The details on all 80 MPs analysed in the present thesis are enlisted in *Annex III*. All results discussion will be described on the basis of self-annotated classification.

4.2. ATMPs

10 MPs are classified as ATMPs of which 2 are cell therapy products (CBMPs: Alofisel, and Provenge). Alofisel is an allogenic somatic -cell therapy product while Provenge comprises of autologous peripheral-blood mononuclear cells with autologous CD54+ cells activated with prostatic acid phosphatase granulocyte-macrophage colony-stimulating factor (G-SCF).

8 ATMPs are classified as gene therapy medicinal products (GTMPs) of which 4 GTMPs (Kymriah, Strimvelis, Yescarta and Zynteglo) are genetically modified autologous cells, 3 GTMPs (Glybera, Imlygic and Luxturna) have gene transfer vectors and 1 GTMP (Zalmoxis) has allogeneic T cells

genetically modified with a retroviral vector encoding truncated nerve growth factor (Annex II). 3 of the 10 ATMPs analysed here have a withdrawn MA (Provenge, Glybera and Zalmoxis).

4.2.1. Pharmacodynamics (PD) studies for ATMPs

Primary PD data is available for 10/10 ATMPs and extensive studies were performed on the in vitro and in vivo characterisation of the model systems used. All 10 ATMPs conducted primary PD studies in in vivo animal models showing the 'proof of concept' (details on species used in *Annex IV*). In case of the allogeneic ATMP Alofisel, the lack of relevant in vivo model was discussed in scientific advice and the use of colitic mice model was agreed. In case of Provenge, surrogate rodent models with species specific variation have been used. Regarding GTMP Kymriah, in vitro and in vivo non-clinical studies were performed using tumour cells from patients with acute lymphoblastic leukemia (ALL), and not from patients with diffuse large B-cell lymphoma (DLBCL) (relevant for the indication). This was considered acceptable to CHMP based on the clinical experience with the indication. For Zalmoxis, humanised mouse model is used to study cell engraftment and efficacy of suicide system (thymidine kinase) in a xenograft model of graft versus host disease.

Secondary PD studies are available for 2 ATMPs Provenge and Zynteglo. For Zynteglo, as the secondary effects were expected from the normalisation of the expression of beta globulin gene to physiological levels, the effects were analysed in primary PD studies. There is no information on the secondary PD studies for 1 ATMP (Luxturna) and the remaining 7 GTMPs did not conduct secondary pharmacology studies. The common reason cited for the omission of secondary PD studies is the nature of the ATMPs, which probably implies that it is difficult to investigate secondary effects in animal models, and has been accepted by the regulatory authorities.

Safety pharmacology (SP) studies are conducted for 2 CBMPs (Alofisel and Provenge). For Alofisel, male and female athymic nude rats are used covering effects on CNS; and SP endpoints are integrated in pharmacodynamics studies for Provenge. 8 GTMPs (Glybera, Imlygic, Luxturna, Kymriah, Strimvelis, Yescarta, Zalmoxis and Zynteglo) did not conduct SP studies. Lack of SP studies is generally acceptable if no adverse effects are observed in the in vivo studies, as the SP parameters are expected to be integrated in the in vivo studies. For Zynteglo, SP studies are not deemed necessary while for Luxturna, as it is intended for ocular use, SP studies are not conducted. Strimvelis cites the lack of model as it is a patient derived product. Details in Annex IV

Specific parameters for CAR-T containing GTMPs (Kymriah and Yescarta)

Studies on on-target/off-tumour effect should be investigated when CAR T cells are used in ATMPs[7]. These are conducted only for Yescarta in the syngeneic mouse model.

Immunophenotyping for the surface marker subtypes has been conducted for Yescarta (CD3, CD4, CD8 and CD45RA). Although immunophenotyping of surface markers has not been conducted for

Kymriah, it is considered acceptable on the basis of the clinical experience with CAR+ T cell properties (Kymriah EPAR).

4.2.2. Pharmacokinetics (PK) studies for ATMPs

No ATMP has submitted data on absorption. 9/10 MPs (except Provenge) have conducted distribution studies (biodistribution and persistence studies) and no ATMP conducted studies for metabolism and elimination (Details on animal models in *Annex V*). Conventional ADME studies applicable for small molecule drugs do not apply to ATMPs because of the nature of the products (cells). Important is the attribute of biodistribution which is discussed in the next sections.

Cell based medicinal products (CBMPs)

Biodistribution has been studied for 1 CBMP (Alofisel). In case of Provenge, lack of biodistribution studies is accepted as clinical experience is available with the product and additionally non-clinical studies would not add to the existing knowledge. Studies on homing/migration of the cells are conducted only for 1 CBMP Alofisel and not conducted for 1CBMP Provenge. Persistence of applied cells has been studies only for 1 CBMP (Alofisel) and no studies are conducted for Provenge. Studies on kinetics of CBMP are not conducted for any of the 2 CBMPs and not discussed in the EPARs (Annex V).

GTMPs

PK parameters relevant for GTMPs include Biodistribution, Persistence, Clearance, Germline transmission and vector shedding

Biodistribution studies are conducted for 8/8 GTMPs. Biodistribution studies are not conducted in detail for Zalmoxis as all organs were not considered for the distribution profile of the administered cells. Cells were tracked only with a focus on lympho-haematopoietic and non-lympho-haematopoietic organs known to be targeted by the graft vs host disease. Organs not generally targeted were excluded from analysis. This is not in line with the annex A in the Note for guidance on repeat dose toxicity [27]. However, the data indicated that the cells could be traced to any tissue, which is expected following IV administration.

The issue of safety margins has not been addressed in any of the EPARs. This was raised as a concern by the CHMP for Strimvelis that no safety margins calculation was made and the applicant was asked to explain, as the administered dose provide only safety margin of approximately half the highest dose administered in humans. The applicant maintained that was not possible to establish a safe viral count numbers and they are kept at the lowest possible number that results in sufficient transduction. Accounting for the proposed indication, i.e. treatment of patients with severe combined immunodeficiency due to adenosine deaminase deficiency, CAT/CHMP considered this

issue resolved. The issue of safety margins with respect to the extrapolation of dosage to humans is extremely important but not discussed for the remaining 6 GTMPs.

Vector persistence: 7/8 GTMPs (except Strimvelis) have conducted studies on persistence of the vector in in vivo studies. For Zynteglo, the persistence studies are only reflective of peripheral blood and bone marrow. For Yescarta, persistence studies in syngeneic mouse lymphoma model have shown viral persistence up to 209th day. Persistence studies establish how long the vector can remain in the transduced cells/organs after single/multiple dosing. While the discussion section acknowledged that persistence was not evaluated, no additional studies were required to be submitted for Strimvelis. Details on persistence studies are indicated in Annex VI.

Vector clearance: Data on vector clearance is available for 3/8 GTMPs (Glybera, Imlygic and Luxturna). For Kymriah, vector clearance studies are not considered feasible and vector clearance is not investigated for Yescarta. 2 GTMPs (Strimvelis and Zynteglo) have no details in the EPAR on clearance studies. In case of Zalmoxis, vector clearance studies have not been conducted as the finished product with integrated retrovirus cannot undergo any elimination/inactivation during the manufacture process.

Risk of germline transmission is investigated for 1 GTMP Glybera as the vector DNA was detected in the gonads. Germline transmission studies are not conducted for 6 GTMPs (Kymriah Luxturna, Strimvelis, Yescarta, Zalmoxis and Zynteglo) and no details on germline transmission studies are available in the EPAR from Imlygic. In cases where cells transduced with a viral vector are used (Kymriah, Yescarta and Zalmoxis), the *Guideline… (EMEA/273974/2005)* [13] is cited stating that '*the risk of germline transmission associated with the administration of GM human cells is considered to be low and, as animal testing of human cells may be difficult or not meaningful, non-clinical germline transmission studies of human GM cells are not recommended*.

Extensive literature data is submitted for Zalmoxis to support the lack of studies on germline transmission. In case of Imlygic, germline transmission is not discussed in EPAR. While no vector DNA was detected in gonads and therefore germline transmission studies would not be necessary for Imlygic, this subject is not discussed either in the main text or in the non-clinical discussion[13] (details in Annex VI). In case of Strimvelis, although vector DNA was detected in testes and ovaries, no germline transmission studies were conducted with a justification that patients would receive a busulfan pre-conditioning which has a known gonadotoxic effects in humans and animals. Furthermore, the risk of germline transfer associated with the administration of genetically modified human cells is considered to be low, as per guideline [13]. Although it was also remarked in EPAR that most ADA-SCID patients are treated within the first few years of life before puberty begins, no further germline transmission studies were conducted, emphasising that a risk-based approach is adopted in case of complex MPs such as GTMPs.

Vector shedding studies are conducted for 1/8 GTMPs (Imlygic). vector shedding studies are not conducted for 4 GTMPs (Kymriah, Luxturna Yescarta and Zalmoxis). For Glybera, and Luxturna, the vector shedding data is available from clinical studies. For Strimvelis extremely low viral count after Strimvelis administration is (below the Lower limit of Quantitation) is cited as a justification for omitting the studies. No information is available in the EPAR from Zynteglo although this parameter is important even from the aspect of environmental risk assessment. (details in Annex VI).

4.2.3. Toxicology -ATMPs

Single dose toxicity studies (SDTS) are conducted for 5/10 ATMPs (1 CBMP Alofisel and 4 GTMPs Glybera, Luxturna, Zalmoxis and Zynteglo). SDTS are not conducted for 5 ATMPs (CBMP Provenge and 4 GTMPs (Kymriah, Imlygic, Strimvelis and Yescarta). Justifications for omitting SDTS include *'patient-derived cells expressing a housekeeping gene at or below physiological levels '*, for Strimvelis. For Kymriah, as it is a patient specific product, it is inappropriate to administer to immune competent animals (details in Annex VII).

Repeat dose toxicity studies (RDTS) are conducted for 3/10 ATMPs (Alofisel, Imlygic and Luxturna). 3 ATMPs (Glybera, Strimvelis and Zynteglo) did not submit RDTS as they are intended for a single use injection. This is in agreement with the overarching guideline [7], based on the duration of use and indication of the MP (Annex VII*)*. The duration of toxicity studies depends on the indication and ranges from 14 days for Alofisel to 7 months for Luxturna.

Of special reference to the case of Provenge, in accordance with Annex I, part IV of Directive 2001/83/EC, applicable to ATMPs, a discussion on the risk-based approach was submitted to justify the lack of several pivotal studies expected in a non-clinical program, especially the lack of PK and toxicity studies. A profiling of the risks associated with unwanted immunogenicity, treatment failure, disease transmission, and toxicity was discussed based on risk-risk factor relationships. Although there were several data gaps identified with the study endpoints and limited data on pharmacology and toxicology aspects, it was decided that the risks were appropriately addressed in the Risk management plan and no additional studies were required. (details in Annex VII).

4.2.4. Genotoxicity and carcinogenicity studies for ATMPs

For autologous CBMPs, as per the Guideline on human cell-based medicinal products (CBMPs) [8], genotoxicity and carcinogenicity studies carry little relevance, but, the risk of tumorigenicity should be addressed. (discussed in section 4.2.6). 1/10 ATMPs (Zynteglo) conducted genotoxicity studies (details in *Annex XLIII).* No ATMP has submitted conventional carcinogenicity studies. For 2 ATMPs (Strimvelis, Yescarta,), exemption of genotoxicity and carcinogenicity studies was agreed upon during scientific advice. All ATMPs have provided justifications for not conducting conventional genotoxicity and 2-year rodent carcinogenicity. In cases where it is known that the administration of the ATMP is

associated with a risk of carcinogenicity, weight of evidence approach has to be used as per ICH S6(R1). This is based on published data from transgenic, knock-out or animal disease models, human genetic diseases and information on class effects, or target biology and mechanism of action [6]. This is observed especially in the cases where integrating viruses are used. (Kymriah, Yescarta and Zalmoxis, detailed in Annex XLIII). For Imlygic, as it is an anti-cancer product, ICH S9 is cited for omitting carcinogenicity studies.

4.2.4.1. Vector specific considerations (for gene therapy medicinal products (GTMPS)

In vivo/in vitro integration site analysis of the vector integration in the genome after transduction has been performed for 5/8 (63%) GTMPs (Glybera, Kymriah, Strimvelis, Zalmoxis and Zynteglo). The decision to conduct integration site analysis is based on the knowledge regarding the vector and transcriptional elements used to drive the expression of the transgene. A case by case approach has to be followed depending on the kind of vector and the transgene cassette used. For Luxturna, the occurrence of integration events is recognised by the CHMP and the risk is considered to be low. The adenovirus used in Luxturna is classified as non-integrating virus as per the guideline on inadvertent germline transmission[13]. However, some integration events were associated with the virus at random sites and it is stated that the risk of integration is greatest at the site of application (retinal pigment epithelial cells), which are considered to be post-mitotic in patients that would be administered Luxturna. As AAV integration requires the cells to be dividing and as a consequence, the risk of insertional mutagenesis seems limited. For Imlygic, previous experience on lack of integration of wild type HSV-1 vector has been cited (literature evidence), and guideline [13] for Kymriah, and the lack of suitable design for Glybera. Although Yescarta contains a γ-retroviral vector, only literature evidence was cited on the resistance of mature mouse T cells to transformation induced by genomic integration and this was accepted during the scientific advice. Yescarta cited the "*lack of availability of any suitable in vitro, ex vivo or in vivo models to appropriately address the toxicological issues*". In case of Zalmoxis, in vitro integration site analysis is conducted along with supportive literature evidence which was considered acceptable (details in Annex IX).

4.2.5. Tumorigenicity (CBMPs) and risk of insertional mutagenesis (GTMPS)/oncogenic potential

The risk of tumorigenic potential (relevant to CBMPs) is evaluated for both CBMPs (Alofisel, and Provenge) (details in Annex VIII). For Alofisel, the risk of ectopic tissue formation and potential for tumorigenicity is recognised and has to followed up in a post authorisation safety study (PASS) and included in the risk management plan. The risk of insertional mutagenesis (relevant for GTMPs) is conducted for 2 GTMPs (Zalmoxis and Zynteglo). For the remaining 6 GTMPs, justifications for not conducting studies on insertional mutagenesis have been provided. In case of Strimvelis, the applicant was recommended to conduct studies on insertional mutagenesis in scientific advice but

the failure of cell engraftment over a long period to study oncogenic potential resulted in a lack of model to study insertional mutagenesis and the EPAR cites that "*from a non-clinical perspective, the carcinogenic potential due to insertional mutagenesis and the potential for subsequent clonal expansion cannot be determined at the time of assessment. Accordingly, the SmPC and RMP have been updated and the applicant has agreed to a long-term follow up of patients in clinical practise (15 years) and to monitoring of potential mutagenicity*". In case of Zynteglo, as the risk was present, the issue of the integration and the potential of oncogenesis upon treatment of patients with Zynteglo-transduced CD34+ cells will be addressed in a post authorisation follow up in the long-term registry. For Kymriah, risk of insertional mutagenesis is included in SmPC section 5.3, as a potential risk. In case of Zalmoxis, in vitro assessment was conducted on the risk of oncogenic potential and it was considered to be low. No in vivo studies were conducted to investigate in vivo risks associated with insertional mutagenesis. Detailed justifications are enlisted in Annex VIII.

4.2.6. Other toxicity studies

Immunogenicity/immunotoxicity studies are conducted for 4/10 ATMPs (2 CBMPs Alofisel and Provenge and 2 GTMPs Luxturna and Zalmoxis) Immune response studies are not conducted for 3 GTMPs (Imlygic, Strimvelis, Yescarta). No information is available in the EPARS from 3 GTMPs on immunogenicity and immunotoxicity studies (Glybera, Kymriah and Zynteglo). Immune response for ATMPs where autologous cells are used are generally not expected. In case of Alofisel, as allogeneic cells are used, it is important to address immunogenic effects of the CBMP. Zynteglo cites that the patients will be immunosuppressed during treatment while for Strimvelis, it contains "*autologous-derived cells with intracellular ADA, in which an immunogenic response of any sort would be unlikely*" (*Annex X*). In case of Provenge, immune response to recombinant rat PAP protein and recombinant human PAP protein was determined in the context of development of anti-drug antibodies. High degree of anti-PAP antibodies was attributed to the homology between rat and human PAP protein.
Other toxicity studies: 5 of the 10 ATMPs (1 CBMP Alofisel and 3 GTMPs Glybera, Kymriah and Imlygic) have conducted product relevant toxicity studies. As Alofisel is an allogeneic cell product, immunogenic potential of expanded cells has been investigated along with the cross talk with natural killer cells and recognition by T cells. Additionally, studies to support the use of colitic mouse model have been submitted (as it is not a representative model for the disease condition). For Glybera, the risk of toxicity due to the presence of baculoviral vector DNA has been addressed. In case of Imlygic, as HSV virus is used in the ATMP, the effect of common antiviral drug acyclovir on the replication of the oncolytic virus has been addressed using plaque assay. Furthermore, studies on tolerability and anti-tumour effects on human colorectal carcinoma (HT-29) tumours in nude mice have been conducted. For Kymriah, as magnetic beads are used for the separation of the virus, solvent and

excipient mediated toxicity has been addressed supported by in vitro toxicity studies. As these studies have to be decided on a case by case basis, the availability or lack thereof is decided on the basis of the product type, manufacturing process and several other considerations that are generally product specific (Annex VII).

4.2.7. Reproductive toxicity studies for ATMPs

No CBMPs (Alofisel and Provenge) have conducted reproductive toxicity studies and they are acceptable based on the guideline on human cell based medicinal products [8]. 2/8GTMPs (Imlygic and Glybera) have conducted FEED studies. 1 GTMP Imlygic conducted EFD studies. No ATMP submitted PPND and JAS. Glybera and Luxturna cited absence of any toxicity to the reproductive system in toxicity studies. For cells modified with viral vectors, the risk of germline transmission is considered to be low and therefore, the studies are omitted. Justifications for not conducting reproductive toxicity studies are indicated in Annex XI.

The decision to conduct or omit reproductive toxicity studies is on a case by case basis depending on the product type, mechanism of action, biodistribution profile of the vector and intended patient population. These issues however have not been adequately discussed and the lack of FEED and EFD studies is not justified in all cases where vector DNA is present in the gonads. As a risk-based approach is taken for the decision to conduct reproductive toxicity studies, any identified risks are communicated through product labeling and adopting risk-mitigation measures. This is observed for all GTMPs.

4.2.8. Local tolerance studies

Local tolerance studies are conducted for 4/10 MPs in ATMP category (CBMP Alofisel and 3 GTMPs, Glybera, Imlygic, and Zynteglo). The reason for the lack of local tolerance studies for Strimvelis included ‘well established therapy’, and autologous cell transplantation does not alter the physicochemical nature of the cells’. Local tolerance studies are decided on a case by case basis for ATMPs (details in Annex XII).

4.2.9. Drug interaction studies

1 GTMP (Glybera) conducted Pharmacodynamic drug-drug interaction (PDDI) studies to support the use of immunosuppressants during treatment. 2 CBMPs (Alofisel and Provenge) and 6 GTMPs (Imlygic, Kymriah, Strimvelis, Yescarta Zalmoxis and Zynteglo) did not conduct PDDI studies. There are no details on drug interaction studies for Luxturna. Justifications included the specific mode of action for Imlygic and lack of suitable model to investigate PDDI for Yescarta. 5 ATMPs did not conduct Pharmacokinetic drug-drug interaction (PKDI) studies (1 CBMP Provenge and 4 GTMPs Imlygic, Kymriah, Yescarta and Zalmoxis). Mode of action is cited as a reason for omitting PKDI

studies for Imlygic. No information is available in the EPAR from 5 ATMPs on PKDI studies (CBMP Alofisel and 4 GTMPs Glybera, Luxturna, Strimvelis and Zynteglo). The need to conduct drug interaction studies is decided on a case by case basis for ATMPs and the guideline recommends discussion on this aspect. As the EPARs do not carry information on PDDI studies and limited information on PKDI studies, any interpretation on the justification for omission is not possible.

4.2.10. Environmental risk assessment (ERA)

8/10 ATMPs have conducted ERA. All 8 GTMPs have conducted ERA and no CBMPs have conducted ERA, where it is justified on the basis that they are not genetically modified cells (details in Annex XII). All GTMPs have conducted ERA following the precautionary principle using the methodology set down in Commission Decisions 2002/812/EC [47] and 2002/623/EC [37] and EMA guideline [35]. Potential hazards have been identified and the likelihood of transmission to the thirds has been discussed in all EPARS.

4.3. Recombinant proteins

4.3.1. Pharmacodynamics, Pharmacokinetics and drug interaction studies

4.3.1.1. Insulin analogues

There are 9 MPs classified as insulin analogues (4 biosimilars: Abasaglar, Insulin lispro, Semglee and Lusduna, and 5 new entities: Fiasp, Ryzodeg, Suliqua, Tresiba, and Xultophy)(Annex XIII).Fiasp is a new formulation of an already approved MP (NovoRapid, approved on 07/09/1999 in the EU) and 1 MP (Lusduna) has a withdrawn MA. However, the data from Lusduna EPAR was used for analysis.

Pharmacodynamics (PD) studies for insulin analogs

PD studies are available for 9/9 MPs (100%). In vitro and in vivo primary PD is conducted for 6/9 MPs (4 new entities Ryzodeg, Tresiba, Suliqua and Xultophy and 2 biosimilars Lusduna and Semglee). Annex XIII). 1 MP (Fiasp) referred to the MA of older formulation. 2 biosimilars (Abasaglar and Insulin lispro Sanofi) conducted primary PD studies in vitro as a part of comparability exercise. All 4 biosimilars (Semglee, Insulin lispro Sanofi, Abasaglar and Lusduna) have conducted primary PD data in comparison to the reference product as a part of the comparability exercise. 2 biosimilars (Lusduna and Semglee) have also conducted in vivo primary PD studies which are not required for biosimilars. as per the guideline on biosimilar medicinal products. This has been specifically mentioned in the EPARs. (Annex XV).

Secondary PD studies are conducted for 6/9 MPs (4 biosimilars Abasaglar, Lusduna, Semglee and Insulin lispro Sanofi; and 2 new entities Ryzodeg and Tresiba). No secondary PD studies are conducted for 3 MPs (3 new entities (Fiasp, Xultophy and Suliqua), (Annex XIII). As Fiasp is a new formulation, it refers to the market authorisation of NovoRapid. Suliqua and Xultophy are combination products and refer to the authorisations of the component active substances.

Secondary PD effects of insulin are measured as the mitogenic activity (undesirable effects), which are sometimes not separately mentioned in the secondary pharmacology effects. This trend was noted in several EPARs where the section 'secondary pharmacology' is completely missing and secondary PD effects are listed together with the primary PD effects (Lusduna EPAR). Safety Pharmacology (SP) studies are conducted for 3/9 MPs (3 new entities Ryzodeg, Suliqua and Tresiba). 4 MPs did not conduct SP studies (2 biosimilars Abasaglar, Insulin Lispro and 2 new entities Fiasp and Xultophy). No information is available from 2 MPs (biosimilars Lusduna and Semglee) (Annex XIII). 2 biosimilars have not conducted SP studies in line with the *guidance on biosimilar medicinal products.* In case of Fiasp and Xultophy, they refer to the MA application of older formulation and components respectively and the lack of studies is acceptable. For 2 biosimilars (Lusduna and Semglee) with no information in the EPARs, assuming no studies were conducted would still be in accordance with the guideline on biosimilar medicinal products as SP studies are not required for biosimilar insulin analogs. (Annex XIV).

Species used: 5/9 MPs used 2 and more species, mimicking type 1 diabetes mellitus in humans, for primary PD data (2 biosimilars Lusduna, Semglee and 3 new entities Suliqua, Ryzodeg, Tresiba). Rats/mice have been used as rodent species and pigs/rabbits/dogs have been used as non-rodent species. All are pharmacologically active species for insulin analogs based on the sequence homology and extensive characterisation in vitro with binding and affinity assays (Annex XIII).

Specific PD requirements for insulin analogues: Biosimilar Insulin analogues have specific requirements laid down by *Guideline* [40] regarding parameters listed in *Table 1*. All 4 biosimilars have submitted data on the receptor binding with IR-A and IR-B receptors (*Table 1)*. Metabolic activity and phosphorylation status are also submitted for 4 biosimilars. However, all the data submitted by Semglee were non-comparative *vs*. reference Lantus, which required submission of raw data for assessment. To evaluate the mitogenic potential of novel insulin analogues, the CHMP ''*Points to consider"* [38] document recommends the use of insulin X10 as a positive control in comparative studies with novel insulin analogs.

Table 1: Data on specific assays for insulin analogues (only for studies conducted).

Active substance insulin analogs		Insulin analog specific requirements					
		Relevant for biosimilars				Relevant for novel insulin analogs	
		Receptor binding with IR A and IR B		Metabolic activity and phosphorylation		Insulin X10 as positive control	
	N	N	%	N	%	n	%
All products	9	8	89	8	89	0	0
New entities	5	4	80	4	80	0	0
Biosimilars	4	4	100	4	100	0	0

In the insulin analogs analysed, Insulin X10 is not used by any new entity as a control and this is raised as an issue by CHMP for Ryzodeg and Tresiba. The justification was provided on the basis of

data on the incidence of spontaneous tumours in Sprague Dawley rats, and its known responsiveness to insulin X10. It was also mentioned that insulin X10 is a rapid-acting insulin analogue with the tolerability and PK profile which is very different from insulin degludec (Tresiba), and therefore, it is not seen as an appropriate positive control. This justification is endorsed by the CHMP. For other MPs however, no concerns were raised and the comparative analysis of mitogenic activity is conducted with normal human insulin for Xultophy and insulin glargine for Suliqua, as a control (data not shown). Fiasp and Suliqua (new entities) referred to individual components of combination in their MAA while no data is available in the EPAR from Xultophy as it refers to the MA of individual components Tresiba and Victoza (*Table 1)*.

Pharmacokinetics (PK) studies for insulin analogs

PK studies are conducted for 9/9 MPs. 9/9 MPs submitted data on absorption (A). 2/9 MPs submitted data on distribution (D) (2 new entity Tresiba and 1 biosimilar Lusduna). 2/9 MPs submitted data on metabolism (M) (1 new entity Tresiba and 1 biosimilar Lusduna). 1/9 MPs submitted data on elimination (E): new entity Tresiba (*Annex XIV*). In case of biosimilars, additional studies are not required if the biocomparability exercise is demonstrated in vitro. Therefore, the lack of in vivo PK studies is acceptable.

Species used for PK studies: Rats, rabbits, Beagle dogs and pigs are the species used for PK studies all species used are pharmacologically active species based on sequence homology. (details on species used for each product in *Annex XIV*).

Drug-drug interaction studies-insulin analogs

Pharmacodynamic drug-drug interaction (PDDI) studies are conducted for 1/9 MPs (new entity Fiasp with reference to Novo rapid) PDDI studies are not conducted for 6/9 MPs (3 new entities: Ryzodeg, Suliqua and Tresiba and 3 biosimilars: Abasaglar, Insulin lispro Semglee) and no information on PDDI studies for 2 MPs (Lusduna and Xultophy). A common justification for the lack of PDDI studies for Ryzodeg and Tresiba is that “Pharmacodynamic interactions are generally not observed for insulin products”. For combination product Suliqua, PDDI studies were not conducted for the combination and it was considered acceptable.

Pharmacokinetic drug-drug interaction (PKDI) studies are available for 2/9MPs (new entities Ryzodeg and Tresiba) and not conducted for 2/9 MPs (biosimilars Insulin lispro and Semglee). No information is available on PKDI studies for 5/9 MPs (3 new entities: Fiasp, Suliqua, Xultophy and 2 biosimilars: Abasaglar and Lusduna) (*details in Annex XIV*).

4.3.1.2. Recombinant proteins-GLP agonists

GLP agonist group has 5 MPs (Eperzan, Ozempic, Revestive, Saxenda and Trulicity) and all are new entities. 1 product (Eperzan) was approved but the MA is currently withdrawn but the data from EPAR has been included in the analysis.

Pharmacodynamics (PD) studies for GLP agonists

In vitro and in vivo primary PD studies are conducted for all 5 MPs (Eperzan, Ozempic, Revestive, Saxenda and Trulicity, Annex XVI. Secondary PD studies are conducted in vitro for 4/5 MPs (Eperzan, Saxenda, Revestive and Ozempic) using a panel of receptors, ion channels and transporters and not conducted for 1 MP (Trulicity), where the justification for omitting of studies is that it is acceptable based on the 'kind of product'. This is however questionable as there are several identified adverse effects of GLP receptor agonists [48], and an analysis of cross-reactivity with other closely related receptors is important, which has also been conducted by other MPs in the group. In vivo safety pharmacology (SP) studies are conducted for all 5 products for the analysis of effects on the vital organ systems. In case of Trulicity, due to the observed cardiovascular effects, additional studies were requested but the applicant cited clinical experience with the product. As the active sequence of the GLP1 receptor is 100% conserved for all of the residues involved in direct ligand interaction between human and cynomolgus monkey receptors and between human and rat receptors, they are all considered as pharmacologically relevant. **Species used**: Mice, rats, rabbits, Guinea pigs and cynomolgus monkeys are pharmacologically active species for GLP agonists. PD studies included normal animals and animal models of disease mimicking the clinical condition (i.e. diet induced obesity (DIO) and knock out mouse models. 2/5 MPs (Saxenda and Trulicity) conducted primary PD in 1 specie (rats and mice respectively). 2/5 MPs (Revestive and Ozempic) conducted primary PD evaluation in multiple species (detailed in Annex XVI). The EPAR for Eperzan mentions that in vivo primary and secondary pharmacology studies were conducted, however, there is no data available on the species used.

Pharmacokinetics (PK) studies for GLP agonists.

All 5 products in the GLP agonist category (Eperzan, Ozempic, Revestive, Saxenda and Trulicity) have detailed information on absorption data (A). Data on distribution is available for 3/5 MPs (Saxenda, Revestive and Ozempic)*, Annex XVIII*. Metabolism studies (M) are conducted for 2 MPs (Saxenda and Ozempic) while data on elimination (E) is available for 3/5 MPs (Saxenda, Revestive and Ozempic). **Species used**: Non-human primates (NHP) are used as at least one specie in PK studies for Eperzan, Ozempic, Saxenda and Trulicity while PK data for Revestive is available in Wistar rats. In all cases, pharmacologically active species are used in the PK studies. Details on the species used for PK studies included in (*Annex XVIII)*.

Drug-drug interaction studies-GLP agonists

Pharmacodynamic drug-drug interaction (PDDI) studies are conducted for 1/5 MP (Saxenda), and not conducted for 2/5 MPs (Ozempic and Revestive). No information is available in EPAR on PDDI studies for 2/5MPs (Eperzan and Trulicity), *Annex XVIII*.

Pharmacokinetic drug-drug interaction (PKDI) studies are referred to in the clinical section for 2 MPs (Eperzan and Ozempic) and no details on PKDI studies are available for 3 MPs (Saxenda, Revestive and Trulicity), details in *Annex XVIII), data analysis for PD and PK studies in* Annex XVII.

4.3.1.3. Recombinant proteins-Active substance Filgrastim/pegfilgrastim and lipefilgrastim

In all, 12 MPs are approved with the active substance filgrastim (2 new entities Lonquex and Ristempa and 10 biosimilars Accofil, Cegfila, Grasustek, Grastofil, Fulphila, Nivestim, Pelmeg, Pelgraz, Udenyca and Ziextenzo). Ristempa (withdrawn MP) is an informed consent application with reference to Module 4 of Neulasta. Filgrastim analogs are ''immunostimulants and colony stimulating factors''. 7/10 biosimilars have Neulasta as the EU reference product (Cegfila, Grasustek, Fulphila, Pelmeg, Ziextenzo, Pelgraz and Udenyca). 2 biosimilars (Accofil and Nivestim) have Neupogen as the EU reference product. Accofil is a biosimilar product using a duplicate procedure of Grastofil. The active substance Filgrastim is indicated for the treatment of neutropenia.

Pharmacology and pharmacodynamics (PD) studies for Filgrastim products

In vitro and in vivo Primary pharmacodynamic (PD) studies are conducted for all 12 MPs (Annex XX). All 10 biosimilars have conducted comparative in vivo primary PD, which is a regulatory requirement for biosimilar products containing filgrastim as an active substance as per the as per the guideline on biosimilar filgrastims [41] (details in Annex XIX). 11/12 MPs did not conduct secondary PD studies. There is no information available for1 MP (Ristempa). The only justification cited for omission of secondary PD studies is the extensive experience with filgrastim in the clinic. Safety pharmacology (SP) data is available for 2/12 MPs (1 new entity Lonquex and 1 biosimilar Udenyca). 9/10 biosimilars did not conduct SP studies and no information on SP studies are available in the EPAR for Ristempa. SP data for Udenyca is extracted from general toxicology studies (*Annex XXI*). In case of filgrastim analogs, as per the guideline on similar MPs containing recombinant granulocyte-colony stimulating factor [41], SP studies are not required for biosimilar MPs in this product class and the lack of studies is acceptable for 9 biosimilars. Where the studies are conducted, (Udenyca), they are redundant.

Species used in pharmacology: Rats, mice, rabbits and non-human primates are pharmacologically active species for G-CSF (filgrastim). 6/12 MPs (biosimilars: Cegfila, Fulphila, Grasustek, Nivestim, Pelgraz and Pelmeg) used one in vivo specie while 5/12 (1 new entity: Lonquex and 4 biosimilars: Grastofil, Pelgraz, Udenyca and Ziextenzo) used 2 or more species for investigation of the primary PD (Annex XX). As recommended in the guideline [41], non-neutropenic and neutropenic rodent models were used for 6/10 biosimilars (Cegfila, Grasustek, Nivestim, Pelmeg, Udenyca and Ziextenzo). Only neutropenic mice models were used for 4/10 biosimilars (Accofil, Fulphila, Grastofil, Pelgraz) and 1 new entity (Lonquex). In case of Pelmeg, only neutropenic rats were used in comparative in vivo studies but it was acknowledged that this was in line with the principles of 3R. For Ristempa, it is

mentioned PD studies were conducted in a '*variety of species'*, without citing details (*Annex XXI*). 3 MPs also extracted PD data in non-rodent species: Ziextenzo (neutropenic rabbits and Beagle dogs); Udenyca and Lonquex (Cynomolgus monkeys) from combined PD/PK studies and repeat dose toxicity studies respectively (*Annex XXI*).

Pharmacokinetics (PK) studies on filgrastim containing MPs

PK studies are conducted for 9/12 MP (Accofil, Cegfila, Grastofil, Grasustek, Lonquex, Nivestim, Pelmeg, Udenyca and Ziextenzo). No PK studies are conducted for 2 MPs (biosimilars Fulphila and Pelgraz), with a reason that it is a biosimilar and reduced non-clinical program is acceptable. No information is available in the EPAR for Ristempa (reference to Neulasta) on PK studies. Data on absorption (A) is available for 9/12 MPs. No EPAR has information on distribution (D). In vitro metabolism data is available for 1MP (new entity Lonquex). Data on elimination (E) is available for 1 MP (Lonquex). PK parameters for Cmax, tmax AUC and half-life are available only in part from the EPARs.

Species used for PK data: PK data was generated either in healthy rodents in 3 MPs (biosimilars Accofil and Grastofil and new entity Lonquex). Neutropenic rodent models are used for 5 biosimilars (Cegfila, Grasustek, Pelmeg, Udenyca and Ziextenzo). 4/12 MPs used one in vivo specie (biosimilars Accofil, Grasustek, Nivestem and Pelmeg). 5/12 MPs conducted PK studies in more than one specie (4 biosimilars Cegfila, Grastofil, Udenyca, and Ziextenzo; 1 new entity Lonquex). (details in Annex XXII).

Drug-drug interaction studies-filgrastim analogs

Pharmacodynamic drug-drug interaction (PDDI) studies are either not available in EPAR for 1/12 MPs (new entity: Ristempa) or not conducted for 11/12 MPs (1 new entity Lonquex and all 10 biosimilars, (Annex XX). For Lonquex, the information on the lack of drug interaction data is included in SmPC. Pharmacokinetic drug-drug interaction (PKDI) is available only for 1/12 MPs (new entity Lonquex, details in). 4/12 MPs (biosimilars Accofil, Fuphila, Grastofil and Pelgraz) did not conduct PKDI studies. 7/12 MPs (6 biosimilars: Cegfila, Grasustek, Nivestim, Pelmeg, Udenyca and Ziextenzo; 1 new entity) have no details on PKDI studies in the EPARs (details in Annex XX).

4.3.1.4. Recombinant proteins-Parathyroid Hormone (PTH) and analogs

Subgroup with PTH analogs has 3 MPs (1 new entity Natpar and 2 biosimilars Movymia and Terrosa). Natpar is indicated for adjunctive treatment of adult patients with chronic hypo-parathyroidism. Biosimilars Movymia and Terrosa are indicated for the treatment of osteoporosis in postmenopausal women and in men at increased risk of fracture. EU reference product for both biosimilars is Forsteo.

Pharmacology for medicinal products (MPs) with parathyroid hormone as an active substance

In vitro and in vivo primary PD studies are conducted for all 3MPs. However, for the new entity Natpar, the studies on primary PD in rats and monkeys were invalidated and considered only as secondary PD studies by the CHMP as they were carried out to support a separate indication for the

approved product Preotact (approved in the EU in 2006). The lack of primary PD studies was considered acceptable in the absence of clinically relevant toxicities, data from the osteoporosis model for Preotact, clinical data and published literature.

In case of both biosimilars Movymia and Terrosa, in vivo studies are not required as biosimilarity was shown at the in vitro level and therefore, these studies were only considered as supportive. 2/3 MPs (Biosimilars Movymia and Terrosa) have no information in EPAR on secondary PD studies. Safety pharmacology (SP) studies are available for 1/3 MPs (new entity Natpar) and no information is available on SP studies for 2/3 MPs (biosimilars Movymia and Terrosa). (details in (Annex *XXIII*). SP studies are not required for biosimilar MPs and therefore justified, but not discussed in the EPARs.

Species used in pharmacology studies: 2 MPs (biosimilars Movymia and Terrosa) have used SD rats as the species for in vivo primary PD studies (Annex XXV). SP studies for 1 MP (new entity Natpar) are conducted in rats and Beagle dogs. In all cases, pharmacologically relevant species are used. (Annex *XXIII*).

Pharmacokinetics for parathyroid hormone containing MPs

Pharmacokinetics (PK) studies are conducted for all 3 MPs. Data on absorption (A) is available for all 3 MPs (1 new entity Natpar and 2 biosimilars Movymia and Terrosa). No MP conducted studies on distribution (D) and metabolism (M). 1MPs (new entity Natpar) has details on elimination (E), with regards to lacteal excretion and no conventional elimination studies were carried out (Natpar EPAR), (details on PK parameters in *Annex XXIV*).these studies carry little relevance for proteins and the lack thereof is justified.

Species used in PK studies: SD rats were used as a single specie for the biosimilars. PK studies for Natpar are conducted in multiple species i.e. rats, rabbits, dogs and monkeys.

Drug-drug interaction studies-parathyroid hormone analogs

Pharmacodynamic drug-drug interaction (PDDI): No MP has information in the EPARs on PDDI studies. As PTH analogs are unlikely to be involved in any drug-drug interactions related to cytochrome activity and are not expected to bind to plasma proteins, the lack of PDDI studies is justified but not clearly mentioned in the EPARs.

Pharmacokinetic drug-drug interaction (PKDI) studies are not conducted for 2/3 MPs (biosimilars Movymia and Terrosa) and no details on PKDI studies are available for 1MP (new entity Natpar) (details in *Annex XXIV*).

4.3.1.5. Recombinant proteins-Blood factors

Subgroup of recombinant proteins with blood factors has 17MPs (all new entities). These include Jivi, Adynovi, Vihuma, Afstyla, Obizur, Nuwiq, Elocta, Alprolix, Idelvion, Refixia, Ondexxya, Kovaltry, Rixubis, NovoEight, Esperoct, NovoThirteen and Veyvondi. 1 MP (Vihuma) is the same product as

Nuwiq, as it is an informed consent application. Although Alprolix and Elocta are fusion proteins, they have been grouped into this category for a comparison with the other blood factors.

Pharmacodynamics (PD) studies for blood factors

Primary PD studies are submitted for 16/17 MPs (94%). 1 MP Vihuma refers to Module 4 for Nuwiq CTD. Secondary PD studies are conducted for 4/17 MPs (Afstyla, Jivi, NovoThirteen and Veyvondi) and not conducted for 13/17 MPs (Annex XXVIII). For 3/17 MPs, data for secondary PD effects is extracted either from general pharmacology studies (Jivi), repeat dose toxicity studies (RDTS), (Afstyla) and toxicology studies (Veyvondi) in line with ICH S6(R1). Secondary PD data for1 MP (NovoThirteen) is derived from in vitro test systems. Safety pharmacology (SP) studies are conducted for 16 /17 MPs (94%; all except Vihuma), of which 12 are conducted as a part of RDTS (details in *Annex XXVI*).**Species in PD studies:** 12/17 MPs have conducted primary PD investigations in at least 2 in vivo species while 4/17 MPs conducted primary PD studies in 1 in vivo specie. 14/17 MPs had primary PD data in animal models mimicking the disease in humans (knock out models for Haemophilia A, haemophilia B or von Willebrand factor deficient animal models). 2/17 MPs conducted pharmacodynamics studies in normal animals (NovoThirteen: cynomolgus monkeys and New Zealand White rabbits; Ondexxya: mice, rats and rabbits). Details in *Annex XXVI*.

Pharmacokinetics (PK) studies for blood factors

PK studies are conducted for 15/17 MPs and not conducted for 2/17 MPs (Kovaltry and Vihuma) (Annex XXVIII). Data on absorption(A)is available for 15 /17 MPs (88%), distribution (D) for 8/17 MPs (47%), metabolism (M) for 3/17 MPs (18%) and elimination (E) for 7/17 MPs (41%). For Kovaltry, the reason for not conducting Absorption studies is that the route of administration is IV, implying 100% absorption. Vihuma refers to the MA of Neulasta and no studies are conducted, details in *Annex XXVII*. Guideline ICH S6 (R1) is cited for not conducting studies on DME studies for Jivi as they are not relevant for proteins.

Species used for the PK studies on blood factors: The species used for the PK studies for the included either normal animal models or knock out (ko) mice/dog models mimicking the disease condition in humans (*Annex XXVII* for details). Ko mice models were established to investigate the prolongation of elimination half-life in case of factors with FcRn receptor (Alprolix).

Drug-drug interaction studies-Blood Factors

Pharmacodynamic drug-drug interaction (PDDI) studies are conducted for 1/17 MPs (NovoThirteen). Vihuma EPAR has no information on PDDI studies and the remaining 15/17 MPs did not conduct PDDI studies. Pharmacokinetic drug-drug interaction (PKDI) studies are conducted for 1/17 MPs (NovoThirteen) and not conducted for 13/17 MPs. No PKDI information is available for 3/17 MPs (Ondexxya, Veyvondi and Vihuma). For Refixia, it is mentioned that "*no PK interactions with human*

coagulation factor IX known or expected, as nonacog beta pegol is not expected to be metabolised by the drug metabolising CYP450 enzymes nor bind to plasma proteins …" (Refixia EPAR).

4.3.1.6. Recombinant proteins-fusion proteins

The group of fusion proteins includes 6 MPs (4 new entities: Eylea, Nulojix, Strensiq, and Zaltrap and 2 biosimilars: Erelzi and Benepali). Fusion proteins with the Fc region of IgG1 allows prolonging the in-vivo half-life of the molecule.

Pharmacology and pharmacodynamics (PD) studies for fusion proteins

In vivo and in vitro primary PD data is submitted for all 6 MPs (100%). 4 MPs generated data in one in vivo specie (Erelzi, Strensiq, Zaltrap and Benepali) while 2 MPs submitted primary PD data for 2 or more species (Eylea and Nulojix). Secondary PD studies are conducted for 1 MP (Eylea) and not conducted for 5/6 MPs (3 new entities: Strensiq, Zaltrap, Nulojix and 2 biosimilars: Benepali, Erelzi). Safety pharmacology (SP) studies are conducted for 5/6 MPs either as standalone studies (biosimilar Erelzi and new entity Strensiq) or with SP endpoints included in single/repeat dose toxicity studies (new entities Eylea, Nulojix, and Zaltrap), in line with the guideline ICH S6(R1). No SP studies are conducted for 1 MP (biosimilar Benepali), (details in Annex XXIX).

In case of fusion proteins, as they have the potential to bind to the Fc receptors and mediate antibody-dependent cellular cytotoxicity (ADCC) as well as complement-dependent cytotoxicity (CDC), which have to be considered as per the guideline on immunogenicity assessment of therapeutic proteins. This has been conducted for all 3 new entities (Nulojix, Strensiq and Zaltrap) with respect to the binding affinities to Fc receptor subclasses. However, assays to measure complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) have been conducted only for Nulojix and Zaltrap

As per *Guideline on similar…issues* [44], both biosimilars Erelzi and Benepali compared the binding affinity of Fc to relevant receptors (Benepali: FcγRIa-, FcγRIIa-, FcγRIIb-, FcγRIIIa (V-type)-, and FcRn; Erelzi: FcγRI, FcγRII, FcγRIII and neonatal Fc receptor (FcRn) (details in *Annex XXIX*).

Species used: Pharmacological investigations are conducted in disease models mimicking human conditions in all cases and proof of concept for the choice of species is demonstrated (details on all PD studies conducted, and the in vivo models used in *Annex XXIX*). In case of Zaltrap and Eylea, rodent s and non-human primates are used with the justification that the sequence homology between human and rodent vascular endothelial growth factor A (VEGF-A) is 85% while there is 100% homology with the cynomolgus monkeys, justifying the use of species as pharmacologically active species in this sub category. In case of biosimilars Benepali and Erelzi, mouse models of arthritis are used.

Pharmacokinetics (PK) studies for fusion proteins

PK studies are conducted for all 6 MPs (*Annex XXXI*). All 6 MPs (100%) included data on absorption (A). Distribution studies (D) are available for 3/6 MPs (new entities Eylea, Strensiq and Zaltrap) No distribution studies are conducted for 3/6 MPs (2 biosimilars: Benepali, Erelzi; and 1 new entity Nulojix). No MP conducted studies on metabolism (details in *Annex XXX)*. Elimination studies are conducted for 2 MPs (new entities Eylea, Zaltrap) and not conducted for 4 MPs (Benepali, Erelzi, Strensiq and Nulojix).(details in *Annex XXX*).

Species and RoA used for PK studies: rats, mice and rabbits and cynomolgus monkeys are used as species for PK studies in the fusion protein category and the RoA is either intravenous or subcutaneous. Species higher in the phylogeny tree (non-human primates) are used in this subgroup as the Fc element imparts the properties of monoclonal antibodies and cynomolgus monkeys are the most sensitive species to understand the efficacy aspects of fusion proteins.(details in *Annex XXX*).

Drug-drug interaction studies- fusion proteins

Pharmacodynamic drug-drug interaction (PDDI) studies are conducted for 2/6 MPs (new entities Nulojix and Zaltrap) and not conducted for 4MPs (2 biosimilars a Benepali and Erelzi and 2 new entities Strensiq, and Eylea).Eylea cites the following justification for not conducting PDDI studies "…*because topical ocular medications do not reach the posterior segment, drug-drug interactions with VEGF Trap within this ocular compartment are highly unlikely"*. 5/6 MPs (biosimilar Benepali and 4 new entities Eylea Nulojix, Strensiq and Zaltrap) did not conduct PKDI studies and no information is available on PKDI studies for 1 MP (Erelzi). Lack of PKDI studies for Nulojix is justified stating that it *"does not undergo metabolism by the cytochrome P450 enzymes and, thus, it is not expected to have direct PK interactions with molecules that are metabolized by these enzymes" (Annex XXX*). *Data analysis in* Annex XXXI.

4.3.1.7. Recombinant proteins-Interferons

The group of interferons includes 2MPs Besremi and Plegridy. Both are new entities.

Pharmacology and pharmacodynamics (PD) studies for interferons

Both MPs conducted primary pharmacology studies, (Annex XXXIII). In vitro and in vivo primary PD data in male cynomolgus monkeys is available for Besremi and for Plegridy, only in vitro PD was conducted with the aim of determining additional surrogate parameters from in vivo combined PK/PD study, which was considered acceptable.(details in Annex XXXII). The use of non-human primates in this subclass is important as they are the only pharmacologically active species for interferons. While non-clinical proof-of-concept is not provided for Besremi for use in the proposed indication (polycythemia vera), where interferons are used off-label (at the time of assessment), existing clinical data is used to support the use in the indication. Moreover, it was acknowledged that

the mechanism of action of ropeginterferon alfa-2b is not established at the time of the assessment. Secondary PD studies are conducted for 1MP (Plegridy), regarding antiviral and anti-proliferative effects and secondary PD are not conducted for Besremi. No justification is provided and it is not discussed in the EPAR. Safety pharmacology (SP) data is available for both MPs. SP studies are conducted for Plegridy as a part of repeat dose toxicity studies, citing ICH S6(R1) [6].(Annex XXXII).

Pharmacokinetics (PK) studies for interferons

PK studies are conducted for both interferon products (Annex XXXIII) Absorption (A) data is available for both products). Distribution (D) and metabolism (M) data are available only for Plegridy while Elimination (E) data is available for both MPs (Besremi and Plegridy). While metabolism studies in a true sense were not conducted for Plegridy, metabolic stability of the linker between the PEG and interferon beta-1a moiety was performed in vitro (details in Annex *XXXIII*). Elimination studies for Besremi are conducted in cynomolgus monkeys and for Plegridy in rats, only with respect to renal clearance of PEG moiety (non-active species) (Annex *XXXIII*).

Species and RoA for PK studies: Non-human primates are the only relevant species which are pharmacologically active for human interferons. The species used for PK absorption studies for Besremi is Cynomolgus monkeys and Rhesus monkeys for Plegridy (relevant species). Guinea pigs were used for distribution studies for Plegridy.

Drug-drug interaction studies-interferons

Pharmacodynamic drug-drug interaction (PDDI) are not conducted for Besremi and Plegridy. Pharmacokinetic drug-drug interaction (PDDI) PKDI studies are not conducted for Plegridy and no information is available for Besremi in the EPAR (Annex *XXXIII*). Plegridy cites the following justification for not conducting PDDI and PKDI "*there is limited value in the qualitative and quantitative projection of interactions between therapeutic proteins and drug metabolising enzymes from in vitro or non-clinical studies''*. (PK and PD studies summarised in Annex XXXIV).

4.3.1.8. Recombinant proteins-recombinant human Follicular Stimulating hormone (r-hFSH)

The group of FSH analogs has 3 MPs (1 new entity: Rekovelle and 2 biosimilars: Bemfola and Ovaleap). The EU reference product for both biosimilars is Gonal-F.

Pharmacodynamics (PD) studies for r-hFSH) containing MPs.

Primary PD studies are conducted for all 3 MPs (new entity Rekovelle, and biosimilars Bemfola and Ovaleap). Primary PD data was initially not submitted for Rekovelle. In vitro primary PD data was submitted after a scientific advice as the PK and PD of clinical batches of Rekovelle and the comparator Gonal-F® were not comparable in humans. No secondary PD studies are conducted for any of the 3 MPs citing well known profile of follitropins. In vitro and in vivo SP studies are conducted for 2/3 MPs (new entity Rekovelle and biosimilar Ovaleap (Annex XXXV*)*. The omission of SP studies for Bemfola are justified stating that the "*profile of FSH is well known" (Bemfola EPAR).* Biosimilar

FSH analogs have a requirement to conduct in vivo comparative PD studies and they have been conducted for both biosimilars Bemfola and Ovaleap as per the guideline[46].

Species used for Pharmacodynamics: female rats were used as the species for in vivo primary pharmacology studies for Bemfola and Ovaleap and the studies have been performed according to the method published by Steelman and Pohley 1953, which measures the increase in ovary weights in female rats [49], in accordance with the guideline [46].

Pharmacokinetics data for r-hFSH analogues

PK studies are conducted for all 3 MPs. Absorption (A) data is available for all 3 MPs (new entity Rekovelle and biosimilars Bemfola and Ovaleap). Distribution (D) and metabolism (M) studies are not conducted for any of the 3 MPs. Data on Elimination (E) is available for 1/3 MPs (biosimilar Bemfola). Regarding the route of administration (RoA) used for Rekovelle, the CHMP raised an objection as the intended RoA is the subcutaneous while the data in vivo was generated after intravenous administration *(Annex XXXVI).* Both biosimilars used the clinically relevant RoA.

Drug-drug interaction studies-r-hFSH analogs

Pharmacodynamic drug-drug interaction (PDDI) and Pharmacokinetic drug-drug interaction (PKDI) studies are not conducted for any of the 3MPs *(Annex XXXVI).*The justifications for not conducting PDDI studies is *"the existing clinical experience with FSH analogs, as the active substance has been available for a long time".* For Rekovelle, lack of relevant information on drug interaction studies is included in the section 4.5 of SmPC as *"Clinically significant interactions with other MPs have neither been reported during follitropin delta therapy, nor are expected".*(data analysis in Annex XXXVII).

4.3.1.9. Recombinant proteins-Unclassified group

This category has 13 MPs and all are new entities and include Brineura, Jetrea, Kanuma, Lamzede, Mepsevii, Myalepta, Oxervate, Palynziq, Ruconest, Oncaspar, Spectrila, Vimizim and Vpriv. In this group, 8 of the 13 MPs are meant for enzyme replacement therapies for different diseases.

Pharmacodynamics (PD) studies for unclassified proteins category

PD studies are conducted for all 13 MPs in the subgroup 'unclassified recombinant proteins' (Annex XXXIX). In vivo and in vitro primary PD studies are conducted for 10/13 MPs. Only in vitro studies are conducted for 2 MPs (Ruconest and Vimizim) (data analysis in Annex XL). The lack of in vivo studies for Ruconest has been accepted by CHMP as the active substance C1INH is already well established as effective in this disease (*Ruconest EPAR*). Lack of in vivo primary PD studies for Vimizim are justified stating that there is no suitable animal model of the disease. In case of Oncasapar, the primary PD studies submitted are not considered valid as they are conducted in 1980s and do not comply with current standards. However, it was acceptable as large clinical experience exists since Oncaspar is marketed in the US and Germany. In case of Jetrea, as the MP is meant for ocular administration, because of a lack of suitable disease model, vivo studies were carried out on ex-vivo

in eyes donated from rats, guinea pigs, rabbits, porcine and humans. For Lamzede, although a proof of concept for the species was demonstrated in mice, they were dosed at levels higher than humans, not allowing the extrapolation. However, this issue was not taken further. Secondary PD studies are conducted for 2/13 MPs (Jetrea and Myalepta) and not conducted for 11/13 MPs (Annex XXXVIII). For Jetrea, the CHMP invalidated the secondary PD studies as they were conducted in support of a cardiovascular indication (Jetrea EPAR). Safety pharmacology (SP) studies are conducted for all 13 MPs. SP studies for 5/13MPs are conducted as a part of single or repeat dose toxicity studies (Annex XXXVIII, data analysis in Annex XL).

Species used in PD studies: as this group is heterogenous, each MP has conducted in vitro and in vivo proof of concept studies to demonstrate the selection of pharmacologically active species. For example, in case of Lamzede, fibroblast cell lines were investigated for the uptake of velmanase alfa. In these in vitro experiments, mouse and human fibroblasts showed the highest uptake, followed by rats, rabbits and cynomolgus monkey with an intermediate uptake and dog and pig fibroblasts had the lowest uptake. This observation was used for the selection of mice, rats, rabbits and monkeys as the species in non-clinical safety studies (details in Annex XXXVIII).

Pharmacokinetic (PK) studies for unclassified proteins

The PK parameters are partially available for all 13 MPs (Annex XXXIX). Studies on Absorption (A) are available for 11/13 MPs. No absorption studies are conducted for Ruconest and Jetrea. For Ruconest, the intravenous RoA, (100% absorption) is cited as a reason for not conducting absorption studies. PK profile of Jetrea is studied after intraocular and intravenous administration to rabbit and porcine eyes (based on the indication and RoA). Studies on distribution (D) are conducted for 11/13 MPs and not conducted for 2/13 MPs (Jetrea and Spectrila, based on the ocular indication). Metabolism (M) studies are conducted for 1 MP Ruconest and Elimination studies are conducted for 1 MP (Myalepta). For Jetrea, conventional ADME studies are not conducted as the product is meant for ocular use and the product has minimal systemic exposure following (intravitreal) IVT administration'. Details on PK studies and the species used are indicated in *Annex XXXIX,* data analysis in Annex XL.

Drug-drug interaction studies-Unclassified recombinant proteins

Pharmacodynamic drug-drug interaction (PDDI) data is available for 2/13 MPs (Jetrea and Ruconest (Annex XXXIX). Only literature data is cited for Oncaspar and Spectrila. None of the 13 MPs have data on Pharmacokinetic drug-drug interactions (PKDI). 10/13 MPs have not conducted PKDI studies while 3 (Oxervate, Ruconest and Vimizim) have no details on PKDI studies in the EPARs (data analysis in Annex XL; details in Annex XXXIX). Although the assessment of Myalepta states that the 'applicant recognised the potential of metreleptin *to alter the formation of cytochrome P450*', no additional studies were needed and a warning label was included in the product information (data analysis in Annex XL).

Summary of Pharmacodynamics, pharmacokinetics and drug-drug interaction studies for 70 recombinant proteins is detailed in Table 2.

Table 2: Data analysis for pharmacokinetics, pharmacodynamics and drug interactions studies

All recmbinant proteins	**N**	Studies conducted %		Studies not conducted %		No information in EPAR	
		n	%	n	%	n	%
Primary Pharmacodynamics (PD)	**70**	69	99	1	1	0	0
New entities	50	49	98	1	2	0	0
Biosimilars	20	20	100	0	0	0	0
Secondary PD	**70**	19	27	48	69	3	4
New entities	50	15	30	44	88	1	2
Biosimilars	20	4	20	14	70	2	10
Safety pharmacology	**70**	49	70	16	23	5	7
New entities	50	46	92	4	8	0	0
Biosimilars	20	3	15	12	60	5	25
PDDI	**70**	7	10	54	77	9	13
New entities	50	7	14	38	76	5	10
Biosimilars	20	0	0	16	80	4	20
Pharmacokinetics	**70**	66	94	4	6	0	0
New entities	50	48	96	2	4	0	0
Biosimilars	20	18	90	2	10	0	0
PKDI	**70**	4	6	42	60	24	34
New entities	50	4	8	32	64	14	28
Biosimilars	20	0	0	10	50	10	50

As can be observed from the above table, primary pharmacology and pharmacokinetics are important from the aspect of non-clinical program and the data are available for 99% and 94% respectively for all proteins. Particularly low number of MPs have submitted drug-drug interaction studies, where a large proportion of MPs have no information in the EPARs. It is also observed that secondary pharmacological effects have also intentionally not been investigated by more than two third of the total analysed proteins (69%).

4.3.2. Toxicology and Toxicokinetics studies for recombinant proteins

4.3.2.1. Insulin analogues:

3/9MPs (new entities Ryzodeg and Tresiba and biosimilar Semglee conducted single-dose toxicity studies (SDTS) and 6/9 MPs did not conduct SDTS (3 new entities Fiasp, Suliqua and Xultophy and 3 biosimilars Abasaglar, Insulin lispro and Lusduna). 7/9 MPs conducted repeat dose toxicity studies (RDTS) (3 new entities: Ryzodeg, Tresiba and Xultophy and 4 biosimilars: Abasaglar, Insulin lispro Sanofi, Lusduna Semglee). 1MP (new entity Suliqua) did not conduct SDTS and RDTS and referred to MA of the components (Lantus and Lyxumia) in the EPAR. Fiasp refers to NovoRapid dossier (*Annex XLI*). 4/9 MPs conducted toxicokinetic evaluations (4 biosimilars: Abasaglar, Insulin lispro, Lusduna and Semglee). 2 MPs(new entities Suliqua and Tresiba) did not conduct TK studies and no information is available in EPARS from Ryzodeg and Xultophy on TK

studies (details in *Annex XLI*). Fiasp, a new formulation and Suliqua referred to already marketed products for toxicology studies. The longest duration of RDTS is 52 weeks for Tresiba (new entity), while biosimilars insulin lispro and Lusduna have the shortest duration of 4 weeks. As per ICH S6 R (1), for MPs meant for chronic conditions, RDTS of 6 months should be submitted. It also however mentions that RDTS of shorter or longer durations have supported MA, but should be scientifically justified [6]. The recovery period ranges from 14 days for Semglee (biosimilar) to 4 weeks for Lusduna and Tresiba (*Annex XLI*)**. Species used in RDTS and RoA:** In all RDTS, rats are used in 7 studies (as a single species for 5/9 MPs Abasaglar, Insulin lispro Sanofi, Lusduna, Ryzodeg, and Xultophy). All 7MPs where RDTS are conducted, the RoA used is the clinically relevant subcutaneous RoA for the insulin analogues.

4.3.2.2. GLP agonists

3/5 MPs from GLP analogues conducted SDTS (Eperzan, Ozempic and Saxenda). Dedicated SDTS were not conducted for Revestive but data on acute toxicity is available. Justifications for not conducting SDTS for Revestive include the withdrawal of EMA *Note for guidance on single dose toxicity* [50] and the associated document [51]. No information is available on SDTS for 1MP (Trulicity). RDTS are conducted for all 5 MPs (*Annex XLII*). TK data is available for 4/5 MPs (Eperzan, Ozempic and Saxenda and Trulicity). No information on TK studies is available for 1 MP(Revestive) in the EPAR. All GLP agonists conducted 52 week RDTS, in line with ICHS6 (R1) [6] as the MPs are meant for chronic use. **Species used in RDTS and RoA**: All 5 MPs in the GLP analogue category conducted pivotal RDTS in at least 1 rodent(mice/rats) and 1 non-rodent species (cynomolgus monkeys) as required by the ICH guideline(details in *Annex XLI*). In case of Rats mice and cynomolgus monkeys all are pharmacologically active species for GLP analogs, therefore the use of species is justified.

4.3.2.3. Filgrastim (PEG/Lipe)

3/12 MPs (1 new entity: Lonquex and 2 biosimilars: Accofil and Grastofil) conducted SDTS.1MP (Ristempa) refers to Neulasta MAA for toxicology studies and 8/12 MPs did not conduct SDTS. RDTS are conducted for 9/12 MPs and not conducted for 2/12 MPS (biosimilars Cegfila and Pelmeg). In case of biosimilar Udenyca, it submitted comparative RDTS in non-human primates (cynomolgus monkeys), although it is not required by the guideline on biosimilar medicinal products. For Pelmeg, *Guidelines* [30, 41] are cited as justification for omitting RDTS. However, guideline [41] requires at least one repeat dose toxicity study in a relevant specie for a duration of at least 28 days, as has been conducted by the remaining 8 biosimilars in the category.

No data is available on RDTS for Ristempa as it is an informed consent application with reference to Neulasta (details in *Annex XLII).* 9/12 MPs conducted TK studies (1 new entity Lonquex and 8 biosimilars: Accofil, Fulphila, Grastofil, Grasustek, Nivestim, Pelgraz, Udenyca and Ziextenzo).TK

studies are not conducted for 3/13 MPs (Biosimilars: Cegfila and Pelmeg and new entity Ristempa which refers to Neulasta). The duration of RDTS is 4weeks for all biosimilars. Longest duration for new entity Lonquex is 26 weeks (~ 6 months) for rats and 13 weeks in cynomolgus monkeys, in line with the ICH S6(R1). for use in chronic conditions. **Species used for RDTS and RoA:** Rats, mice and cynomolgus monkeys are used as species in toxicology studies. All three are pharmacologically relevant species. 1 MP (new entity Lonquex) has used 1 rodent and 1 non rodent specie for RDTS. Clinically relevant subcutaneous RoA is used for all 9 MPs in RDTS. Accofil and Grastofil have also submitted data using the IV RoA. (*Annex XLI*)

4.3.2.4. Blood factors

14/17MPs (82%, *Annex XLI*) conducted SDTS. 2/17 MPs (Adynovi and Alprolix) did not conduct SDTS with the justification that the product is meant for chronic use. 16/17 MPs conducted RDTS and 1MP (Vihuma) has referred to the Module 4 of Nuwiq for SDTS and RDTS. The duration of RDTS ranged from 5 days for Kovaltry to 52 weeks for Esperoct, although all the MPs are meant for chronic use. This deviation in the duration of RDTS is based on the development of anti-drug antibodies to the test product. The duration for Jivi was restricted to 2 weeks after scientific advice (Jivi EPAR) For Kovaltry and Jivi, the deviation from ICH S6(R1) is justified with the reasoning that the animals would develop neutralizing antibodies and long-term treatment would result in a loss of activity, as has been demonstrated in rabbits (Kovaltry EPAR). No data is submitted for Vihuma (reference to Nuwiq).

TK studies are conducted for 13/17MPs, not conducted for 3/17 MPs (biosimilar Adynovi, Alprolix and Vihuma, with reference to Nuwiq). No information is available on TK studies for 1 MP (Ondexxya). For Alprolix, the EPAR states that TK is not applicable to the product as it is for the treatment of life-threatening disorder (Haemophilia B). However, no other MP has cited this justification. **Species used in RDTS and RoA:** 1/17 MPs (Nuwiq) have submitted data in 1 relevant non-rodent specie (cynomolgus monkeys). However, as the PD and PK studies have been conducted in haemophilia dog model, and considering the well-known profile of human blood factor VIII, use of non-human primates should have been avoided in this case. Remaining 14/17 MPs have conducted RDTS in one rodent and one non-rodent specie (*Annex XLI*). Intravenous (IV) is the clinically relevant RoA and 16 /17 MPs (except Vihuma) have used it as the RoA in RDTS.

4.3.2.5. Parathyroid hormone containing MPs

2/3 MPs (biosimilars: Movymia and Terrosa) in the PTH analogue category conducted SDTS and no SDTS is conducted for Natpar. RDTS are conducted for all 3 MPs. There is no requirement for biosimilars to conduct single and repeat-dose toxicity if invitro comparability has been achieved. Therefore, these studies for Movymia and Terrosa are considered redundant. No details are available on TK studies in the EPARs for all 3 MPs (*Annex XLI)*. The duration of longest RDTS is 4 weeks for

biosimilars (Movymia and Terrosa) and 26 weeks for Natpar, which is in line with the ICH S6(R1). requirements for chronic use.

Species used in RDTS and RoA: 2 MPs (biosimilars Movymia and Terrosa) have used rats as a single specie for RDTS. 1 MP (new entity Natpar) conducted RDTS in 3 species (rats, dogs and cynomolgus monkeys). Initially dog was chosen as the non-rodent specie but was sensitive to the calcaemic effects of Natpar (rhPTH (1-84), resulting in adverse effects on the kidney. Therefore, non-rodent species was changed to the non-human primate (details in *Annex XLI)*. All 3 MPs have conducted RDTS using the clinically relevant subcutaneous RoA.

4.3.2.6. Fusion/chimeric proteins

4/6 MPs (new entities Eylea, Nulojix, Strensiq and Zaltrap) conducted SDTS and 2 biosimilars (Benepali and Erelzi) did not conduct SDTS. All 6 MPs have data on RDTS and toxicokinetics. The duration of longest RDTS is 4 weeks for biosimilars (Benepali and Erelzi) and 6-8m for new entities (*Annex XLI)*.

Species used in RDTS and RoA: All 6MPs conducted pivotal RDTS in cynomolgus monkeys as at least one of the relevant specie. Mice and rats were used as the rodent species in the fusion protein category. 2 MPs (Biosimilars Benepali and Erelzi) conducted RDTS in rodents only. 4 MPs (new entities Eylea, Nulojix, Strensiq and Zaltrap) conducted RDTS in both, rodent and non-rodent species. All 6 MPs submitted RDTS using clinically relevant RoA, in agreement with ICH S6(R1)(*Annex XXXIX*, details in (*Annex XLI*).

4.3.2.7. Interferons as active substances

No MP conducted SDTS as interferons are meant for chronic use and there is little value in conducting SDTS for such products. RDTS is conducted for both MPs Besremi and Plegridy (*Annex XLI*). For Besremi, the pivotal toxicity study in Cynomolgus monkeys did not cover the chronic use in patients, as the study was limited to 4 weeks by the production of neutralizing antibodies. Therefore, the reduced duration of this pivotal toxicity study was accepted by CHMP in scientific advice in light of a valid justification. A reduced duration of RDTS for Plegridy (5weeks) is justified with the same reason as neutralising Abs were observed with the non-PEGylated version. Both MPs conducted TK studies. **Species used in RDTS and RoA**: RDTS were conducted in 1 rodent and 1 non-rodent for Besremi (rats and cynomolgus monkeys) and in Rhesus monkeys only for Plegridy, as it was shown to be pharmacologically responsive to human interferon-beta-1a protein in vitro (Annex XXVIII). Both products conducted RDTS using the clinically relevant subcutaneous or intramuscular RoA. As per the Note 1 in the ICH S6(R1), when non-human primates are the only relevant species (as in case of interferons) and therefore, studies in NHP are sufficient.

4.3.2.8. FSH as the active substance

All 3 MPs (new entity Rekovelle and 2 biosimilars Bemfola and Ovaleap) conducted STDS, RTDS and Toxicokinetics (*Annex XLI*). The duration of RDTS is 4weeks for all 3 MPs. **Species used in RDTS and RoA**: 1 MP (new entity Rekovelle) conducted RDTS in one rodent and 1 non-rodent specie (rats and cynomolgus monkeys). 2 MPs (biosimilars Bemfola and Ovaleap) conducted comparative RDTS in rats with the reference product Gonal-F (*Annex XLI*). All 3 MPs conducted the toxicity studies using the clinically relevant subcutaneous RoA. The guideline on non-clinical and clinical development of similar biological MPs containing recombinant human follicle stimulating hormone (r-hFSH)[46] does not require separate comparative toxicology studies for biosimilar MPs containing FSH. Furthermore, in vivo comparative toxicology studies are not required for biosimilars as per the guideline on similar biological medicinal products [36]. Therefore, these studies are redundant and could have been omitted.

4.3.2.9. Unclassified recombinant protein category

11/13 MPs conducted SDTS. 2 MPs (Lamzede and Myalepta) did not conduct SDTS. For Lamzede, SDTS were not conducted but data on single dose toxicity studies could be extracted from acute toxicity studies. Both Myalepta and Lamzede are enzyme replacement therapies for chronic use and as per ICH S6(R1), SDTS are not mandatory if the information on acute dose response can be achieved from other toxicity studies. All 13 MPs conducted RDTS. TK studies are conducted for 12/13 MPS (except Brineura, where no information is available in the EPAR),(details in *Annex XLI*; data analysis in *Annex XLII*). The duration of longest RDTS ranged from 14 days for Ruconest to 18 months for Brineura, depending on the indication. Ruconest is a product for single use while Brineura is an enzyme replacement therapy for long term use.

Species used in RDTS and RoA: All 13 MPs conducted RDTS in relevant species based on homology to the human proteins *(details in Annex XLI*). For all 13 MPs, clinically relevant RoA was used in the RDTS. (details in *Annex XLI*).

Summary for toxicity studies: Considering all 70 recombinant proteins together, STDS studies are available for 61% of all recombinant proteins (72% for new entities an 35% biosimilars). RDTS data is available for 91% (64/70MPs). 92% (46/50MPs) of new entities and 90% (18/20) of biosimilars have conducted RDTS. It is generally observed that the duration of biosimilars is much shorter than for the new entities across all subgroups analysed, suggesting that a shorter duration of toxicity studies is accepted for biosimilars.

TK data is available for 76% of all recombinant proteins (74% new entities and 80% biosimilars) (Table 3). RDTS data is not available for 4/70 MPs (2 new entities Fiasp and Suliqua, which refer to individual components of the combination), and 2 biosimilars (Cegfila and Pelmeg) have cited the guideline on biosimilar MPs for not conducting RDTS. Another 2 MPs, (Vihuma and Ristempa) are informed

consent applications and have referred to Module 4 of Nuwiq and Neulasta respectively (*Annex XLI*). As per the ICH S6(R1), toxicology studies should be performed in one rodent and one non-rodent species. However, this is the case only for 63% (40/64) of the MPs that conducted RDTS. Of these, RDTS is conducted in at least 1 rodent and 1 non-rodent species for 85% (39/46) of new entities, compared to only 6% (1/18) of biosimilars (as a percentage of studies conducted).

Table 3: Summary for RDTS for recombinant proteins for the studies conducted.

Recombinant proteins	N	SDTS		RDTS				TK	
		Studies conducted		Studies conducted		At least 1 rodent one non-rodent		Studies conducted	
		n	%	N	%	n	%	n	%
All products	70	43	61	64	91	40	63	53	76
New entities	50	36	72	46	92	39	85	37	74
Biosimilars	20	7	35	18	90	1	6	16	80

4.3.3. Genotoxicity and Carcinogenicity for recombinant proteins

Genotoxicity studies: Genotoxicity studies are conducted for 17% of recombinant proteins (12/70MPs). (Details in Table 4). Genotoxicity studies are not conducted for 79%,(55/70) of analysed proteins as proteins are exempted as per ICH S6(R1), and guidelines [6, 36], unless there is a cause for concern. Moreover, ICH S6(R1) also states that administration of large quantities of peptides/ proteins may provide data that are not interpretable [6]. The decision to conduct genotoxicity studies is on a case by case basis if there is a cause for concern (with the presence of an organic linker molecule in a conjugated protein product). Data on genotoxicity is not available in the EPARs of 3 products (4%), insulin analog Lusduna, and GLP agonists Eperzan and Trulicity), where the MA for Lusduna and Eperzan are withdrawn and the term 'genotoxicity' has not been discussed in the non-clinical sections. All 12 MPs where genotoxicity tests are conducted were negative for in vitro genotoxicity. For Besremi and Plegridy, the in vitro genotoxicity tests were carried out as a result of a cause for concern due to structural alert in in silico tests, in line with ICH S6(R1), (details in Annex XLIII).

Table 4: Data analysis- Genotoxicity and Carcinogenicity studies.

All Products		Genotoxicity studies						Carcinogenicity studies					
		Conducted		Not conducted		No informati on EPAR		Conducted		Not conducted		No information in EPAR	
	N	n	%	n	%	n	%	n	%	n	%	n	%
All products	70	12	17	55	79	3	4	8	11	61	87	1	1
New entities	50	12	24	36	72	2	4	8	16	42	84	0	0
Biosimilars	20	0	0	19	95	1	5	0	0	19	95	1	5

Carcinogenicity studies: Data on carcinogenicity studies is available only for 11% (8/70) recombinant proteins. Carcinogenicity studies were not conducted for 87% (61/70) of recombinant proteins analysed and no data on carcinogenicity studies is available for 1 MP (1%, Lusduna, withdrawn MA). (Table 4). Identical nature of the recombinant protein to the endogenous protein is cited as the most common justification for not conducting carcinogenicity studies along with the exemption to conduct carcinogenicity studies as per ICH S6(R1) and EMA guideline on similar biological medicinal products. In case of Zaltrap, as it is an anticancer MP, both ICH S6(R1) and ICH S9 are cited as reasons for omitting carcinogenicity studies. In all, 3 new active insulin analogs, 4 GLP agonists and 1 PTH analog have conducted carcinogenicity studies. In case of Oxervate, ICH S6(R1) was cited for omitting carcinogenicity although the product is a nerve growth factor and carcinogenicity studies are required for growth factors as per ICH S6(R1). To this, the applicant referred to 6-month RDTS and a warning was included in the SmPC.

4.3.4. Reproduction toxicity and Juvenile animal studies-recombinant proteins

4.3.4.1. Insulin analogues

2/9MPs (new entities Ryzodeg and Tresiba) conducted FEED, EFD and PPND studies. No MP conducted JAS.1 MP (Fiasp) referred to the authorized product NovoRapid/Novolog. 2/9 MPs (biosimilars Abasaglar and Insulin lispro) cited exemption from conducting DART studies as per the guideline on biosimilar MPs (*Annex XLV*). 2/9 MPs (Suliqua and Xultophy) referred to individual components of the combination products already authorized and no new studies were conducted for the combination products. 2 biosimilars (Lusduna and Semglee), where no justification is given are also exempt from conducting DART studies

4.3.4.2. GLP agonists

4/5 MPs (new entities: Eperzan, Ozempic, Revestive and Saxenda, *Annex XLVI*) conducted FEED studies. No information on FEED is available for Trulicity. All 5 MPs conducted EFD studies and 4/5MPs (Eperzan, Ozempic, Revestive and Saxenda) conducted PPND studies. No information on PPND studies is available for Trulicity. 2/5 MPs (Ozempic and Revestive) submitted data on JAS. 1 MP (Saxenda) did not conduct JAS and no information on JAS is available for 2/5MPs (Eperzan and Trulicity), details in *Annex XLV*. Only Revestive is indicated for paediatric population (JAS are available to support paediatric indication and Eperzan is currently withdrawn from the market). The PIP for all 4 marketed products is not yet completed.

4.3.4.3. Filgrastim as active substance

None of the 12 products conducted FEED studies. 2/12 MPs (1 new entity Lonquex and one biosimilar Ziextenzo) conducted EFD studies. No information on EFD studies is available for 2 MPs (1 biosimilar: Grasustek (reference to Neulasta) and 1 new entity Ristempa). The Neulasta EPAR has details on FEED, EFD and PPND studies and no information on JAS. None of the 12 MPs conducted

PPND or JAS. For Lonquex, lack of FEED and PPND studies was acceptable as the effects on fertility and development are also expected from the concomitant cytotoxic chemotherapy. CHMP also stated that additional studies will be required in case of approval of another indication. Biosimilars are exempted from conducting DART studies but Ziextenzo submitted EFD studies. As 10 of the 12 MPs in this group are biosimilars, DART studies are not required. Besides, lack of DART studies in this subgroup is considered acceptable as animal studies with G-CSF and derivatives do not indicate harmful effects with respect to fertility. List of justifications is indicated in *Annex XLV*.

4.3.4.4. Blood factors

1/17 MPs (Novoeight, only fertility studies) conducted FEED studies. 16/17 MPs did not conduct FEED studies.4/17 MPs (Adynovi, Afstyla, Esperoct and Novoeight) did not conduct EFD studies. 13/17 MPs have no information in EPARs regarding EFD studies (*Annex XLV* and *Annex XLVI*). None of the 17MPs conducted PPND studies. 1/17 MP conducted JAS (Jivi) and 16/17 MPs did not conduct JAS. All MPs in the group have provided appropriate justifications for not conducting DART studies, which are either based on the indication (Hemophilia A affects male population -Jivi and Kovaltry),no adverse effects observed in RDTS-(Novoeight and NovoThirteen) or the lack of information on effects on fertility are indicated in appropriate SmPC sections-Alprolix, Idelvion and Obizur). For Kovaltry, JAS is not conducted due to lack of adverse effects on organ systems undergoing postnatal development. (*Annex XLV)*. In general, Adverse effects on fertility, postnatal development and reproduction as well as teratogenic effects are not expected for blood factors in humans and the lack of studies is therefore justified.

4.3.4.5. Parathyroid hormone containing MPs

Biosimilar MPs Movymia and Terrosa have not conducted developmental and reproductive toxicity studies in accordance with the guideline for biosimilar medicinal products. In case of new entity Natpar, FEED, EFD and PPND studies are conducted in rats. It is mentioned that in an agreed paediatric investigation plan, a juvenile study is planned to study the impact of PTH in children.

4.3.4.6. Fusion/chimeric proteins

4/6 MPs (new entities Eylea, Nulojix, Strensiq and Zaltrap) conducted studies on FEED and EFD. 2/6 MPs (biosimilars Benepali and Erelzi) did not conduct FEED and EFD studies, citing the biosimilarity guideline. 2/6 MPs (new entities Nulojix and Strensiq) conducted PPND studies. 3/6 MPs (1 new entity Eylea and 2 biosimilars (Benepali and Erelzi) did not conduct PPND studies. The indication and the patient population are cited as the reason for omitting PPND studies for Eylea a pregnancy warning is included to section 4.3 of the SmPC. 3/6 MPs (new entities Eylea, Strensiq, Nulojix and Zaltrap) conducted JAS in juvenile cynomolgus species *(Annex XLV)*. 2/6 MPs (1 biosimilar Benepali) did not conduct JAS. For Nulojix, all studies were conducted for abatacept, which is a potent version

of the active substance belatacept. The lack of DART and JAS studies for Erelzi and Benepali are justified as they are biosimilars. *(Annex XLV* and *Annex XLVI*).

4.3.4.7. Interferons

No FEED, PPND and JAS studies were conducted for Besremi and Plegridy (*Annex XLV*). For Besremi, the justification cited in the EPAR for not conducting the reproductive toxicology studies is the mechanism of action (MoA) of interferons, '*DART studies with ropeginterferon alfa-2b are not considered to add value to understanding of its safety and toxicity profile'* (Annex XLV). For Plegridy, the applicant did not conduct the DART studies citing the abortifacient activity of interferon beta-1a in rhesus monkeys, based on the EPAR from Avonex (Avonex EPAR), which was acceptable to CHMP. No information is available on EFD studies. Both MPs are indicated for the treatment of adults and therefore, JAS are not required. The PIP for both MPs is not yet completed.

4.3.4.8. Recombinant proteins-FSH analogues

FEED studies are conducted for 1/3 MPs (new entity Rekovelle) and not conducted for 2/3 MPs (biosimilars Bemfola and Ovaleap). For Rekovelle, a reduction in female fertility rates was observed in FEED studies, but no concerns were raised as Rekovelle is indicated for women undergoing assisted reproductive technologies (decision based on indication) and adapting a risk-based approach, Rekovelle is contraindicated for pregnant and lactating women in the SmPC. For Bemfola, the justification for omitting DART studies is the' *well-known MoA of FSH' (Annex XLV)*. Furthermore, biosimilar products are exempt from conducting DART studies as per the guideline on biosimilar medicinal products [30]. Rekovelle belongs to the pharma-therapeutic group 'Sex hormones and modulators of the genital system' and therefore as the MP has a class waiver, there is no obligation to submit a PIP and no JAS are required.

4.3.4.9. Unclassified recombinant proteins

FEED studies are conducted for 9/13 products and not conducted for 3/13 MPS (Jetrea, Oncaspar and Ruconest) *(Annex XLV)*. For Brineura, the FEED studies were conducted as a part of RDTS. The EPAR for Spectrila mentioned only literature data (indicated for leukemia).

EFD studies are conducted for 9/13 MPs (Kanuma, Lamzede, Mepsevii, Myalepta, Oxervate, Palynziq, Ruconest, Vimizim and Vpriv) and not conducted for 3/13 MPs (Brineura, Jetrea and Oncaspar). EFD studies for Oxervate were conducted only in rabbits and the timing of starting the male fertility studies was not as recommended in the ICH S5(R2) guideline [16]. Although this was raised as a concern by CHMP, as the safety profile of NGF is well known, no additional studies were required. For Jetrea, the indication (ocular use) was cited as a reason for not conducting developmental and reproductive toxicity studies.

PPND studies are conducted for 6/13 MPs (Kanuma, Lamzede, Myalepta, Palynziq, Vimizim and Vpriv). No PPND studies are conducted for 6/13 MPs (Brineura, Jetrea, Mepsevii, Oncaspar, Oxervate and Ruconest). For Mepsevii, a developmental and PPND study in rats was recommended to further

characterise the potential reproductive toxicity effect of Mepsevii in line with the ICH S6(R1).
JAS are conducted for 5/13 MPs (Brineura, Kanuma, Lamzede, Mepsevii and Vimizim). All 5 MPs are indicated for patients of all age groups and therefore JAS is necessary to support paediatric indication. JAS are not conducted for 5/13 MPs (Jetrea, Oncaspar, Oxervate, Ruconest and Spectrila). Of these, Jetrea has a full waiver and no obligation to submit PIP and therefore, lack of JAS is justified. Oncaspar (indicated for neoplastic diseases) has a class waiver and no obligation for PIP justifying the lack of JAS and clinical data is also present for Oncaspar for use in paediatric indication. Oxervate and Vpriv have product specific waivers, justifying the lack of JAS. In case of Ruconest, EC decision on PIP is pending for paediatric indication (data not shown). PIP was not completed and deferred to a later stage for Palynziq (at submission). Spectrila has cited only literature data with respect to developmental and reproductive toxicity (indicated for leukemia). No information is available in EPAR from3 MPs Myalepta, Palynziq and Vpriv on JAS studies.
The summary of developmental and reproductive toxicity studies for all 70 recombinant proteins are indicated in Table 5.
Table 5: Summary for developmental and reproductive toxicity studies, and juvenile animal studies (accounting for studies conducted).

All Recombinant proteins	N	FEED		EFD		PPND		JAS	
		n	%	n	%	n	%	n	%
	70	22	31	23	33	15	21	12	17
New entities	50	22	44	22	44	15	30	12	24
Biosimilars	20	0	0	1	5	0	0	0	0

4.3.5. Non-clinical studies on immunogenicity, human tissue cross reactivity (TCR), local tolerance and environmental risk assessment (ERA) for recombinant proteins

The details of studies related to immunogenicity, tissue ross reactivity (TCR), local tolerance and ERA are enlisted in *Annex XLVII*.

1. **Insulin analogues:** Immunogenicity studies are conducted for 8/9 MPs (89%; 4 new entities and 4 biosimilars). There is no data in any of the EPARs in this category for human TCR studies. Local tolerance studies are conducted for all 9 MPs and no MP conducted ERA. In line with the guideline [35]. Other justifications for omitting ERA are indicated in *Annex XLVII*.

2 GLP agonists: Immunogenicity studies are conducted for 3/5 MPs (Eperzan, Ozempic and Revestive). In case of Revestive, because of the presence of anti-drug antibodies, even though the PK was not affected by increased ADAs in mice and monkeys, CHMP required the applicant to conduct further assessment of antibody and safety data in the on-going long-term clinical study and to report the respective case reports the periodic safety update report (PSUR). No information is available regarding TCR studies. Local tolerance studies are conducted for 3/5 MPs (Ozempic, Revestive and

Saxenda). No MP submitted data for ERA in the GLP category. Details on local tolerance studies and justifications on the non-submission of ERA are indicated in *Annex XLVII*.

3 Active substance Filgrastim (Peg/lipe): 6/12 MPs conducted immunogenicity studies (1 new entity (Lonquex) and 5 biosimilars Grasustek, Nivestim, Pelgraz, Udenyca and Ziextenzo). EPARs for 3 MPs (Accofil, Cegfila and Grastofil) have no information on immunogenicity studies. Immunogenicity studies are not conducted for Ristempa. No information is available for any of the MPs regarding TCR studies. Local tolerance studies are conducted for 9/12 MPs (1 new entity: Lonquex and 7 biosimilars: Accofil, Fulphila, Grastofil, Grasustek, Nivestim, Pelgraz, Udenyca, and Ziextenzo). No local tolerance studies are submitted for 2 MPs (Cegfila, and Pelmeg). In this product class, local tolerance studies are required I at least one specie as per the product-class specific guideline [11]. In case of both Cegfila and Pelmeg, the EPAR section referring to local tolerance mentions "*The applicant did not submit pharmacodynamic drug interaction studies (see non-clinical discussion)"*, which appears to be a misprint and no details can be extracted. No information is available for Ristempa as it refers to the data on Neulasta MAA. None of the 12 MPs in this category conducted ERA.
The product specific guideline on biosimilar filgrastim analogs [41],recommends the analysis of immunogenic responses and therefore, the lack of immunogenicity studies for 3 biosimilars(Accofil, Cegfila and Grastofil) in this product class is not in line with the guidance and also not discussed. Details and justifications *Annex XLVII*.

4 Parathyroid hormones: All 3 MPs (Movymia, Natpar and Terrosa) have submitted data on the immunogenic potential and local tolerance. Data on ERA is not submitted by any of the products and no MP has information on TCR studies.

5 Blood factors: 16/17 products conducted immunogenicity studies. 2 MPs (Adynovi and Jivi) have submitted TCR studies and no information is available on TCR studies for 15/17 MPs. Local tolerance is conducted for 16/17 MPs (except Vihuma which refers to Nuwiq MA). 1MP (Adynovi) has conducted phase 1 ERA with respect to the bioaccumulation potential of PEG moiety, which is a requirement in cases where recombinant proteins have been modified for increased stability, as per the guideline on ERA [35] details in (*Annex XLVII*)

6 Fusion proteins: All 6 MPs in the fusion protein category conducted immunogenicity and local tolerance studies (*Annex XLVII*). For local tolerance studies, Cynomolgus monkeys were used as at least one species for 4 products (Benepali, Erelzi, Eylea and Zaltrap). Human TCR studies are conducted for 1 MP (new entity Zaltrap). No TCR studies are conducted for Erelzi and no information is available on TCR studies for 5/6 MPs (biosimilar Benepali, and new entities Eylea, Nulojix and Strensiq). No MP conducted ERA. (*Annex XLVII*).

7 **Interferons:** Immunogenicity and local tolerance studies are conducted for both MPs (Besremi and Plegridy). The species used for local tolerance studies are Cynomolgus monkeys for Besremi and Rhesus monkeys for Plegridy. No information is available in the EPARs on human TCR studies for Besremi and Plegridy. ERA is conducted only for Plegridy for PEG moiety. (*Annex XLVII*).

8 **Recombinant proteins with FSH as active substance:** Immunogenicity data and local tolerance data is available for all 3 MPs (new entity Rekovelle and 2 biosimilars Bemfola and Ovaleap). TCR studies are not conducted for the new entity Rekovelle and no information is available in the EPARS for Bemfola and Ovaleap. ERA is not conducted any MP citing that endogenous nature of the proteins *(Annex XLVIII).*

9 **Unclassified recombinant proteins:** Immunogenicity and local tolerance studies are conducted for 10/13 MPs each (77%). No information on immunogenicity studies is available for 3/13 MPs (Brineura, Mepsevii and Vimizim). Local tolerance studies are not conducted for 3/13 MPs (Brineura, Mepsevii and Vimizim). None of the EPARs have information regarding TCR studies. ERA is not conducted for any of the MPs (*Annex XLVIII*). Local tolerance studies were not conducted for Brineura as the catheter-mediated drug delivery was evaluated after single administration in Cynomolgus monkeys and dogs. For Mepsevii and Vimizim, observations were made from single and repeat dose toxicity studies. End points for local tolerance were incorporated in other studies for 6/13 MPs (Kanuma, Lamzede, Mepsevii, Oxervate, Palynziq and Vpriv) (*Annex XLVII*).

Immunogenicity, human TCR, local tolerance and ERA for 70 recombinant proteins

As per the ICH S6(R1), testing for immunogenicity (presence of ADAs) is not always necessary for recombinant proteins as most human proteins induce a rapid and robust anti-drug antibody response in preclinical studies and the presence of circulating drug as drug-antibody complex interferes with the detection of antibodies. However, with the immunogenicity studies conducted for 81% of the recombinant proteins analysed, it appears to be important for the non-clinical program from the perspective of decreased availability of the medicinal product. TCR studies are available for 7% of total proteins analysed. Local tolerance studies are available for 87% of the proteins.

Table 6: Summary on studies conducted for Immunogenicity, human tissue cross reactivity (TCR), local tolerance and ERA (excluding studies not available/no information in EPAR).

All recombinant proteins	N	Immunogenicity		TCR		Local tolerance		ERA	
		n	%	n	%	n	%	n	%
All products	70	57	81	5	7	61	87	3	4
New entities	50	42	84	5	10	43	86	3	6
Biosimilar	20	15	75	0	0	18	90	0	0

Recombinant proteins are readily biodegradable and do not pose any additional risk to the environment, unless the structure is chemically altered to increase the stability. Therefore, only 3% have submitted ERA citing the EMA guideline on the environmental risk assessment.

Data not analysed: Impurities, metabolites and other toxicity studies were not included in the analysis as in most cases, the sections were not there in the EPARs or the studies were not conducted.

5. Summary

5.1. ATMPS

5.1.1. PD parameters relevant for ATMPs

Primary PD studies in ATMPs should enable the understanding of the mode of action (MoA) of the ATMP in a relevant in vitro or in vivo animal model as per the Guideline [7]. Primary PD data is available for 100% of ATMPs analysed in the present work, providing the proof of concept for the functionality of ATMP. Secondary PD studies were conducted for 20% of ATMPs. (no information for Luxturna). Safety pharmacology studies were conducted for 10% of the analysed ATMPs (1 CBMP Alofisel). In other cases, the limitations of the animal models with respect to the use of syngeneic systems or xenotransplantation are recognised and the justifications presented by the applicants were acceptable to the regulatory authorities. However, aspects related to drug interactions are not sufficiently discussed except in cases where patients are immunosuppressed before therapy or during preconditioning.

5.1.2. PK parameters relevant for ATMPs

Biodistribution studies are conducted for 90% of ATMPs (except 1 CBMP Provenge). For CBMPs, homing, persistence and kinetics of cell behaviour are only partially discussed in the EPARs. Justifications have included the lack of information on the pathways of homing, migration and elimination of eASCs cells (Alofisel). In such cases, risk of tumorigenicity is included in the RMP as important potential risk to be followed up with a post-authorisation safety study (PASS). Homing and persistence are available only in part, due to lack of model systems and the limitation of quantitation methods in case of systemically administered cells. In case of Provenge, as clinical data for the product is existing, it supersedes the non-clinical requirements. In general, lack of drug interactions studies is considered acceptable based on the product, mode of action and application. Pharmacokinetic drug interaction data is only partially available for ATMPs. PK parameters specific to GTMPs i.e. Vector persistence studies have been conducted for 100% (8/8) GTMPs. Vector clearance studies have been conducted for 43% (3/8 GTMPs) and the risk of germline transmission has been investigated for 15% (1 GTMP Glybera). The aspect of vector shedding to investigate the dissemination of the virus through secretions and excreta is conducted for 15% of GTMPs (1 GTMP

Imlygic). The studies are not conducted for 57 % (4/8 GTMPs). In all cases, the lack of data on specific aspects related to risks have been added to the SmPC and the risk management plan for GTMPs.

5.1.3. Toxicology studies

Single dose studies have been submitted for 50% ATMPs and 30% have submitted repeat dose toxicity studies (RDTS). Lack of model systems for investigation or 'patient specific product' are cited as justifications for omitting studies. In case of single use ATMPs (3MPs), no RDTS are required. 1CBMP (Provenge) has only submitted a discussion on the risk-based approach for omitting the studies.

5.1.4. Genotoxicity studies (conventional)

Conventional genotoxicity data are available only for 10% ATMPs. Exemption of genotoxicity studies was in part agreed upon during scientific advice or hemotoxic parameters relevant to ATMPS are either addressed in the biodistribution studies or the lack of information is included in the SmPC and risk management plan.

Vector specific considerations include in vivo/in vitro integration site analysis have been conducted for 63% (5/8) GTMPs. This is largely guided by the prior knowledge on the kind of viral vector used (integrating or non-integrating vectors). The cases where integration site analysis have been conducted (Glybera, Kymriah, Strimvelis, Zalmoxis and Zynteglo) are also guided by transcriptional factors guiding the expression of a transgene.

The risk of insertional mutagenesis has been analysed for 25%GTMPs (2/8, Zynteglo and Zalmoxis). The lack of studies on insertional mutagenesis is attributed to the lack of suitable animal models and in other cases, follow-up measures were indicated to analyse the potential of insertional mutagenesis in post authorisation studies.

5.1.5. Tumorigenicity:

The risk of tumorigenicity has been addressed for 50% (1 CBMP Alofisel). As per the overarching guideline on GTMPs [7], standard rodent carcinogenicity studies are not required for GTMPs. The decision to conduct carcinogenicity studies should be guided by the weight of evidence approach in the ICH S6(R1) guideline. For all GTMPs using viral vectors, the weight of evidence supports the concern for carcinogenic potential based either on the mechanistic pathway, literature evidence and the use of immunosuppressants during therapy. Therefore, no conventional carcinogenicity studies are warranted. In all cases where there is an identified risk, it is included in the product labeling and risk management plan. Therefore, lack of conventional carcinogenicity studies in vivo is justified for all GTMPs, depending on the intended use, type of product and the indication

5.1.6. Other toxicity studies

5.1.6.1. Immunogenicity: studies are conducted for 40% of ATMPs. Immunogenicity has not been conducted for 40% of ATMPs and no information is available for 20% ATMPs regarding immunogenicity. In cases where the patients will be immunocompromised before treatment with the

ATMP, the lack of immunogenicity studies is justified, as for Zynteglo. For autologous CBMPs, immunogenicity is generally not expected but should be discussed. Allogeneic ATMPs Alofisel and Zalmoxis, immunogenicity studies are conducted as there is an associated risk of immune response from allogeneic cells as well as secreted substances. This is in line with the guideline on human cell based medicinal products [8]. Immunotoxicity is conducted for 20% ATMPs (Imlygic and Luxturna). GTMPs and allogenic CBMPs have the potential to trigger immune responses and this aspect has to be carefully considered but not appropriately addressed in the EPARs. Human TCR is investigated for 20% ATMPs (Provenge and Kymriah).

5.1.6.2. Other toxicity studies: ATMP specific studies related to the product type and the method of production of viral cells have been conducted for 50% of ATMPs. These have addressed issues such as the use of antiviral medication on the replication of the oncolytic virus for Imlygic. In case of Luxturna, the risks of toxicity due to the presence of solvents and excipients used in the production for Luxturna are addressed. A case by case basis approach is followed based on the product as recommended by relevant guidelines.

5.1.7. Reproductive toxicity

20% ATMPs (GTMPs Glybera and Imlygic) have conducted FEED studies and 10% (1GTMP Imlygic) conducted EFD study. No GTMP submitted PPND or juvenile animal studies. Embryofoetal development (EFD) studies were conducted for 1 GTMP Glybera). Studies on reproductive toxicity are required for human CBMPs only if there is a risk of interaction with the DNA. Lack of reproductive toxicity studies is therefore justified for CBMPs. For GTMPs, reproductive toxicity studies have to be conducted based on the vector used, indication and from the evidence in biodistribution studies [7]. Only if the vector DNA is detected in the gonads, further breeding studies are required. In all cases, the lack of reproduction toxicity studies is justified, based on the 'kind of product', 'limited risk of biodistribution' and prior clinical experience. the lack of information on the effects on fertility and development are indicated in appropriate sections of the SmPC, applying a risk-based approach.

5.1.8. Local tolerance

Local tolerance studies are conducted for 40% of ATMPs. The overarching guideline for GTMPs and CBMPs allows the flexibility to conduct local tolerance studies and they are not necessary if the proposed clinical formulation and route of administration have been examined in other animal studies. Local tolerance studies have been included as endpoints in other PK/PD studies and no ATMP has conducted standalone studies, in line with the guidelines on GTMPs and human CBMPs.

5.1.9. Drug-drug interactions (DDI)

10% ATMP (1 GTMP Glybera) have details on pharmacodynamic drug-drug interactions (PDDI). The remaining 9 ATMPs (2 CTMPS and 7 GTMPs) have not conducted PDDI studies. With regards to pharmacokinetic drug-drug interactions (PKDI), 40% of ATMPS have not conducted PKDI studies and no information is available in the EPARs for 60% of the ATMPs. As the decision to conduct DDI studies

is based on the product type, indication and the mode of action of the ATMP, no interpretations can be made regarding the omission of these studies. However, they are not discussed in the EPARs.

5.1.10. Environmental risk assessment (ERA)

Of particular importance for ATMPs is the analysis on ERA, which is available for 80% of ATMPs (100% GTMPs and 0% CBMPs) as it is a regulatory requirement. Cell-based MPs have not submitted ERA as no genetically modified organisms are used.

5.2. Recombinant proteins

The classification of recombinant protein group with 70 MPs into product class-based categories assists with the comparison of biosimilars and new entities within a product class. The data on defining a suitable species for PD/PK and toxicology is one of the most important requirements of the non-clinical studies for biotech-based pharmaceuticals.

5.2.1. Pharmacodynamics studies for recombinant proteins

Primary pharmacodynamics (PD) studies are available from 99% (69/70) MPs indicating the importance of primary PD studies in the non-clinical studies. The remaining 1% (1MP Vihuma) has referred to the dossier of another product and not carried out any additional studies. 6% have conducted in vitro primary PD studies only while 3% have conducted in vivo PD studies only. Where only in vitro data is provided, appropriate justifications in the EPARs have been provided in all cases with respect to the lack of suitable model system or in case of Vpriv, where the safety and efficacy of the enzyme C1INH is already established in the disease condition.

Secondary PD studies are conducted for 27% (19/70) of all recombinant proteins analysed in the present work. Safety pharmacology (SP) studies are available for 70% (49/70MPs) of which 92%, (46/50) are new entities and 15%, (3/20) are biosimilars. In 53% (26/49 MPs), SP endpoints are included in other toxicity studies, in line with recommendations of ICH S6(R1).

5.2.2. The choice of animal species and models for in vivo PD studies

For new entities, the choice of in vivo animal species is dependent on the proposed indication, duration of use and most importantly, pharmacodynamic activity. In vitro binding and affinity studies as well as the homology of proteins between species has been shown to support the choice of pharmacologically active species. In most subgroups analysed (except interferons), multiple species were recognised as pharmacologically relevant species.

The use of surrogate models, transgenic models or normal rodents/ non-rodent species in pharmacology studies is also dependent on the category of recombinant proteins reviewed, and in accordance with product class specific guidelines. Transgenic models are used in case of blood factors (Hem A, Hem B knockout mouse and dog models as rodent and non-rodent species), as the functionality can only be demonstrated in animal models mimicking the lack of specific blood factors in knock out animal models. The choice of animal models used in the evaluation process of PD effects is by and large justified. Only in 1 case (Natpar), primary PD studies were invalidated by the CHMP as

the studies were not relevant to the proposed indication. But in this case, no additional studies were required as the product profile is well characterised.

5.2.3. Pharmacokinetics (PK)

PK studies for biotech derived pharmaceuticals primarily provide information about the absorption profile as DME pattern of the recombinant protein is expected to be similar to the endogenous proteins. However, PK studies provide important information regarding the differences in drug kinetics between species. PK studies are conducted for 94% (66/70) (96% (48/50) for new entities and 90% (18/20) for biosimilars). PK studies are not conducted for 4% (3/70) and no information is available on 1% (1/70) of recombinant proteins analysed. In cases where PK studies are not conducted, the MP is either a biosimilar (Fulphila, Pelgraz) or an informed consent application (Vihuma). No information is detailed for Ristempa (reference to Module 4 Neulasta) Data on absorption is available for 90% (63/70MPs), distribution for 40% (28/70MPs), Metabolism for 14% (10/70MPs) and Excretion is available for 30%(21/70MPs). Where absorption data is not available, either the EPAR refers an already authorized product or the RoA is IV (100% absorption) or ocular.

5.3.4. Drug interaction studies

Pharmacodynamic drug interaction (PDDI) studies: PDDI studies are conducted for 10% of MPs (7/70 recombinant proteins, 0 biosimilars) and not conducted for 77% (54/70MPs) recombinant proteins (54/70 MPs). No information is available for 13% (9/70 MPs). The lack of PDDI data, where studies are not conducted, is reflected in SmPC for a few MPs. For MPs where no information is available in the EPARs, no conclusion can be derived if the studies were conducted or if the information is just missing in the EPARs.

Pharmacokinetic Drug Interaction studies (PKDI): PKDI studies are conducted for 6% (4 MPs) of recombinant proteins; intentionally not conducted for 60% (42/70 MPs). No information is available for 34% (24/70 MPs) of analysed recombinant proteins. The lack of drug interaction studies is partly reflected in the appropriate sections of the SmPC. In one case, the potential of drug- interactions is recognised and the applicant is recommended to update the product information (Myalepta).

5.3.5. Toxicity and toxicokinetic studies:

61% (43/70) of all recombinant proteins have conducted single dose toxicity studies of which 72% (36/50) are new entities and 35% are biosimilars (7/20). RDTS are conducted for 91% (64/ 70) of recombinant proteins. Toxicokinetic studies are conducted for 76% (53/70MPs) recombinant proteins (71% new entities and 80% biosimilars). 81% of new entities and 6% of biosimilars have conducted RDTS in at least 1 rodent and 1 non rodent species as per the ICH S6(R1). Generally, biosimilars are not required to submit separate SDTS and RDTS studies if the in vitro biosimilarity exercise has been accomplished and it has been commented in several EPARs. Therefore, especially in case of biosimilars, there remains a scope to reduce the in vivo toxicity studies wherever possible,

in line with the principles of 3R[37]. Deviations in the duration of RDTS for new entities justified partly on the basis of a lack of a suitable animal model (due to generation of neutralising antibodies for interferons Besremi and Plegridy) or in case of the intended duration of use (Ondexxya, meant for single use). The choice of animal species used in toxicology studies is largely justified. In a few cases, the objections to the choice of animal model is resolved as in case of PTH analog Natpar, where the chosen dog model was overly sensitive to the effects of the active substance and therefore, the non-rodent animal model was changed to non-human primate.

5.3.6. Genotoxicity and carcinogenicity

Genotoxicity data are available for 17% (12/70) of recombinant proteins (24% new entities (12/50) MPs and 0% for biosimilars) and carcinogenicity studies are conducted for 11% of recombinant proteins (15% new entities (8/50 MPs) and 0% biosimilars). Biosimilars are exempt from conducting genotoxicity and carcinogenicity studies and therefore the lack of studies is well-justified. New entities where genotoxicity studies are conducted, cause for concern due to structural alert has been identified. From this analysis, it can be stated that the recombinant proteins analysed in the present work adhere to the ICH S6(R1) and the EMA guideline on biosimilar proteins.

5.3.7. Reproductive toxicity and Juvenile animal studies

FEED, EFD, PPND and JAS are conducted for 31% (22/70), 33% (23/70), 21% (15/70) and 17% (12/70) of recombinant proteins respectively. No biosimilars have submitted FEED, EFD, PPND or JAS and is well justified and in accordance with the guideline on biosimilar proteins. Where fertility studies are not conducted, or where maternal toxicity or secretion of the MP is expected in milk but no confirmatory studies are conducted, details are included in appropriate sections 4.3 (contra-indications) for Zynteglo and Trulicity, 4.6 (pregnancy and lactation) for Alprolix, Eylea, Obizur and Oncaspar; and 5.2 (pharmacokinetic properties) for Obizur in the SmPC, addressing the lack of data on developmental and reproductive toxicity. For 1MP (Mepsevii), it was recognised the applicant was asked to conduct additional FEED and PPND studies to comply with the requirements of ICH S6 (R1).

5.3.8. Immunogenicity studies

immunogenicity studies are conducted for 81% (57/70MPs) of the recombinant proteins. 84%of new entities and 75% of biosimilars have conducted immunogenicity studies. Immunogenicity studies inform on the potential for drug neutralisation and resulting reduction of efficacy through the generation of antidrug antibodies. As a large percentage of MPs analysed in this study have conducted immunogenicity, they are important from the perspective of the non-clinical program.

5.3.9. Tissue cross reactivity (TCR) studies

Human TCR studies are conducted for 7% (5/70) MPs. The information on TCR studies is not available for 93% (65/70) MPs. The only categories of recombinant proteins where the TCR studies are carried out include the blood factors and fusion proteins. As TCR studies provide supplementary information

with respect to preclinical toxicity and are not predictive of human immunogenic response, they seem to carry little significance in the non-clinical assessment for recombinant proteins analysed.

5.3.10. Local tolerance studies

Local tolerance studies are conducted for 86% (60/70) of recombinant proteins. 86% of new entities (43/50 MPs) and 85% (17/20MPs) of biosimilars have conducted local tolerance studies. Local tolerance endpoints are integrated into repeat dose toxicity studies, developmental toxicity studies or as a part of PD evaluation for 53%(32/60MPs) of the conducted studies as per ICH S6(R1). In most cases, local tolerance studies are conducted using the clinically relevant RoA along with other RoA to mimic accidental exposure. Rabbits, rats and mice were largely used as the species in local tolerance and are acceptable as the ICH S6(R1) does not mention the specific use of pharmacologically active species for local tolerance studies.

5.3.11. Environmental risk assessment (ERA)

ERA is not conducted for 97% (67/70) of the recombinant proteins analysed, as most recombinant proteins are readily biodegradable truncated versions or modified versions of the endogenous proteins and are not considered an environmental risk. As per the guidelines on ERA [35], the requirement for ERA arises only when a biotech-derived product is modified to an organic linker molecule such as PEG moiety to increase the stability. ERA is conducted for 3 MPs Adynovi, Plegridy and Oncaspar, which have an attached PEG moiety. Therefore, studies on Phase 1 ERA are provided. In short, the guidelines have been adhered to for all recombinant proteins and justifications for not conducting ERA.

6. Conclusion

EPARs provide comprehensive information on the studies submitted for the process of evaluation of a dossier submitted for marketing authorization and provide valuable insights into the studies that are critical from the point of view of the assessors. EPARs can also be used as a reference for the design of a non-clinical program for future applications for marketing authorisations.

In the present work, the non-clinical requirements of advanced therapy medicinal products (ATMPs) and recombinant proteins have been analysed for the MPs authorised in the EU through centralised procedure in the last decade.

The aim of the non-clinical program for ATMPs is to provide basic studies for the demonstration of a positive benefit risk balance, so as to enable moving into the clinical phase. Flexibility in the design of studies on a case by case basis, and a risk-based approach is adopted for the non-clinical program. For the ATMPs analysed in the present work, the studies conducted are often limited due to unavailability of suitable animal model systems representative of the human conditions. Therefore, several gaps in knowledge are accepted adopting a risk-based approach and implementation of post-

authorisation measures and risk mitigation practices to better understand the safety and efficacy. In case of novel recombinant proteins, a comprehensive non-clinical program is expected for the assessment of efficacy and safety relevant aspects. On the other hand, the design of non-clinical program for biosimilars is often abridged, guided by guidelines on biosimilar medicinal products and product class specific guidelines.

EPARs are a secondary source of information and represent an abridged version of all the studies conducted on the part of the applicant to demonstrate the efficacy and safety of the potential medicinal product. It is observed that not all EPARs elaborate all the sections of the studies and often lack justifications for omission of studies from the point of the assessors. Several issues are often not addressed, but this may be a personal preference of the assessor. Therefore, in cases where the EPARs do not carry sufficient information on subsections, it does not necessarily imply that the applicant did not conduct the studies. In conclusion, while the data analysis provides information on the trends and the adherence/deviations to the guidelines specific to product class regarding non-clinical aspects, any conclusions derived on the basis of EPAR data alone should be dealt with caution.

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8. Annexes

Annex I Classification of 80 MPs based on pharma therapeutic group (EMA classification) and self-annotated groups, approved between 01.12.2009-31.12.2019.

Pharma therapeutic group assigned by EMA	No. of MPs	Self annotated groups	No. of MPs
Immunostimulants	13	ATMPs	10
Antihemorrhagics	13	Insulin Analogs	9
Other alimentary tract and metabolism products	9	GLP agonists	5
Drugs used in diabetes	10	Filgrastim Analogs	12
Antineoplastic agents	7	Blood factors	17
Opthalmologicals	3	Parathyroid hormone/analogs	3
Sex hormones and modulators of the genital system	3	Fusion proteins/ chimeric proteins	6
immunosuppresants	3	Interferons	2
Calcium homeostasis	3	FSH analogs	3
Other haematological agents	2	Unclassified recombinant proteins	13
Immunostimulants, Colony stimulating factors	2		
Drugs used in diabetes, other blood glucose lowering drugs excl. insulins	2		
Blood factors	2		
Vitamin K and other hemostatics, Blood factors	1		
Not yet assigned	1		
Lipid modifying agents	1		
Insulins and analogues for injection, fast acting	1		
Immunosuppressant, TNF- alpha inhibitors	1		
Enzymes	1		
All other therapeutic products	1		
Other immunostimulants	1		

MP: medicinal product; FSH: follicular stimulating hormone. GLP: glucagon like peptide; ATMP: advanced therapy medicinal products.

Annex II: Description of ATMPs.

ATMPs	EMA classification of the ATMP	Description
Alofisel	Somatic cell therapy	Alofisel is a suspension for injection containing expanded human allogeneic mesenchymal adult stem cells extracted from adipose tissue (expanded adipose stem cells eASC)
Provenge	Somatic cell therapy	Autologous peripheral blood mononuclear cells activated with PAP-GM-CSF
Glybera	Gene therapy	replication-deficient adeno-associated viral vector designed to deliver and express the human LPL gene variant LPLS447X
Imlygic	Gene therapy	Oncolytic immunotherapy vector derived from HSV-1. Talimogene laherparepvec has been modified to replicate within tumours and to produce the immune stimulatory protein human GM-CSF.
Kymriah	Gene therapy	CTL019 (murine) HIV-1 vector is a replication-defective, recombinant third-generation self-inactivating lentiviral vector derived from the HIV-1 lentiviral genome. It encodes a CAR against human CD19 expressed under the control of the human elongation factor 1α (EF-1α) promoter
Luxturna	Gene therapy	AAV gene therapy vector with a CM enhancer and chicken beta actin promoter driving expression of normal human retinal pigment epithelium 65 kDa protein (hRPE65) gene
Strimvelis	Gene therapy	autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence
Yescarta	Gene therapy	autologous T-cells genetically modified ex vivo by retroviral transduction using an MSCV-based retroviral vector to express a CAR comprising an anti-CD19 single chain variable fragment (scFv) linked to CD28 and CD3-zeta co-stimulatory domains.
Zynteglo	Gene therapy	autologous CD34+ cell-enriched population with haematopoietic stem cells transduced with lentiviral vector encoding the βA-T87Q-globin gene
Zalmoxis	Somatic cell therapy (it is classified as somatic cell therapy but is genetically modified with a retrovirus. Therefore, for the purpose of this thesis, it will be considered as gene therapy product)	allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2)

PAP: prostatic acid phosphatase

Annex III: Classification of 80 Biological products into subgroups (self-annotated).

Medicine Name	Indication (self annotated)	Active substance	Biosimilar	Date of MA in EU	ATC code	Conditional approval/ orphan designation	Additional monitoring	Pharmatherapeutic group (EMA)
ATMPs								
Alofisel	treatment of complex perianal fistulas in adult patients with non-active/mildly active luminal Crohn's disease	darvadstrocel	No	23.03.2018	L04	O	+	Immunosuppressants
Provenge (withdrawn)	indicated for treatment of asymptomatic or minimally symptomatic metastatic prostate cancer in male adults not indicated for chemotherapy.	autologous peripheral-blood mononuclear cells (PBMCs) including a minimum autologous CD54+ cells activated with prostatic acid phosphatase granulocyte-macrophage colony-stimulating factor	No	06.09.2013	L03AX17		+	Other immunostimulants
Glybera (withdrawn)	adult patients diagnosed with familial lipoprotein lipase deficiency (LPLD)	alipogene tiparvovec	No	25.10.2012	C10AX10			
Imlygic	treatment of adults with unresectable melanoma that is regionally or distantly metastatic	talimogene laherparepvec	No	16.12.2015	L01XX51		+	Antineoplastic agents
Kymriah	Paediatric and young adult patients up to 25 years of age with B-cell acute lymphoblastic leukaemia, Adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) after two or more lines of systemic therapy	tisagenlecleucel	No	22.08.2018	L01	O	+	
Luxturna	Luxturna is indicated for the treatment of adult and paediatric patients with vision loss due to inherited retinal dystrophy caused by confirmed biallelic *RPE65* mutations	voretigene neparvovec	No	22.11.2018	-	O	+	Not yet assigned
Strimvelis	treatment of patients with severe combined immunodeficiency due to	autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with	No	26.05.2016	L03	O	+	Immunostimulants

Medicine Name	Indication (self annotated)	Active substance	Biosimilar	Date of MA in EU	ATC code	Conditional approval/ orphan designation	Additional monitoring	Pharmatherapeutic group (EMA)
	adenosine deaminase deficiency (ADA-SCID)	retroviral vector that encodes for the human ADA cDNA sequence						
Yescarta	treatment of adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) and primary mediastinal large B-cell lymphoma (PMBCL)	axicabtagene ciloleucel	No	23.08.2018	L01X	O	+	Antineoplastic agents
Zalmoxis (withdrawn)	Zalmoxis is indicated as adjunctive treatment in haploidentical haematopoietic stem cell transplantation (HSCT) of adult patients with high-risk haematological malignancies.	Allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2)	No	18.08.2016	L01	C		Antineoplastic agents
Zynteglo	treatment of patients 12 years and older with transfusion-dependent β thalassaemia (TDT) for whom haematopoietic stem cell (HSC) transplantation is appropriate	Autologous CD34+ cells encoding βA-T87Q-globin gene	No	29.05.2019	B06A	AA	+	Other hematological agents
Insulin analogs								
Abasaglar (Absaria)	Treatment of diabetes mellitus in adults, adolescents and children aged 2 years and above	Insulin glargine	Yes	09.09.2014	A10AE04			Drugs used in diabetes
Fiasp (new formulation)	Treatment of diabetes mellitus in adults, adolescents and children aged 1 year and above	insulin aspart	No	09.01.2017	A10AB05		+	Insulins and analogues for injection, fast acting
Insulin lispro Sanofi	treatment of adults and children with diabetes mellitus	insulin lispro	Yes	18.07.2017	A10AB04		+	Drugs used in diabetes
Lusduna (withdrawn MA)	Treatment of diabetes mellitus in adults, adolescents and children aged 2 years and above	insulin glargine	Yes	03.01.2017	A10AE04			
Ryzodeg	Treatment of diabetes mellitus in adults, adolescents and children from the age of 2 years	Insulin aspart / insulin degludec	No	21.01.2013	A10AD06			Drugs used in diabetes

Medicine Name	Indication (self annotated)	Active substance	Biosimilar	Date of MA in EU	ATC code	Conditional approval/ orphan designation	Additional monitoring	Pharmatherapeutic group (EMA)
Semglee	Treatment of diabetes mellitus in adults, adolescents and children aged 2 years and above	insulin glargine	Yes	23.03.2018	A10AE04		+	
Suliqua	treatment of adults with type 2 diabetes mellitus	insulin glargine / lixisenatide	No	11.01.2017	A10AE		+	
Tresiba	Treatment of diabetes mellitus in adults.	Insulin degludec	No	20.01.2013	A10AE06			
Xultophy	treatment of adults with type-2 diabetes mellitus	insulin degludec / liraglutide	No	18.09.2014	A10			
GLP agonists								
Eperzan (withdrawn)	indicated for the treatment of type 2 diabetes mellitus in adults	albiglutide	No	20.03.2014	A10BJ04			Drugs used in diabetes
Ozempic	Treatment of adults with insufficiently controlled type 2 diabetes mellitus	semaglutide	No	08.02.2018	A10BJ06		+	Drugs used in diabetes, other blood glucose lowering drugs excl. insulins
Revestive	treatment of patients aged 1 year and above with Short Bowel Syndrome (SBS)	teduglutide	No	30.08.2012	A16AX08	O	+	Other alimentary tract and metabolism products
Saxenda	weight management in adult patients	liraglutide	No	23.3.2015	A10BJ02			Drugs used in diabetes
Trulicity	treatment of adults with insufficiently controlled type 2 diabetes mellitus	Dulaglutide	No	21.11.2014	A10BJ05		+	Drugs used in diabetes, other blood glucose lowering drugs excl. insulins
Active substance Filgrastim (Peg/Lipe)								
Accofil	Reduction in the duration of adult and child neutropenia	filgrastim	Yes	17.09.2014	L03AA02			Immunostimulants
Cegfila) Pegfilgrastim Mundipharma	Reduction in the duration of adult neutropenia	pegfilgrastim	Yes	19.12.2019	L03AA13			
Fulphila (Withdrawn)	Reduction in the duration of adult and child neutropenia	pegfilgrastim	Yes	20.11.2018	L03AA13		+	
Grastofil	Reduction in the duration of adult and child neutropenia	filgrastim	Yes	17.10.2013	L03AA02			

Medicine Name	Indication (self annotated)	Active substance	Biosimilar	Date of MA in EU	ATC code	Conditional approval/ orphan designation	Additional monitoring	Pharmatherapeutic group (EMA)
Grasustek	Reduction in the duration of adult neutropenia	pegfilgrastim	Yes	20.06.2019	L03AA13		+	
Lonquex	Reduction in the duration of adult neutropenia	lipegfilgrastim	No	25.07.2013	L03AA14			Immunostimulants, Colony stimulating factors
Nivestim	Reduction in the duration of adult and child neutropenia	filgrastim	Yes	07.06.2010	L03AA02			Immunostimulants
Pelgraz	Reduction in the duration of adult neutropenia	pegfilgrastim	Yes	21.09.2018	L03AA13		+	Immunostimulants, Colony stimulating factors
Pelmeg	Reduction in the duration of adult neutropenia	pegfilgrastim	Yes	20.11.2018	L03AA13		+	Immunostimulants
Ristempa (Withdrawn) (informed consent application)	Reduction in the duration of adult neutropenia	pegfilgrastim	No	13.04.2015	L03AA13		+	
Udenyca	Reduction in the duration of adult neutropenia	pegfilgrastim	Yes	21.09.2018	L03AA13			
Ziextenzo	Reduction in the duration of adult neutropenia	pegfilgrastim	Yes	22.11.2018	L03AA13		+	
Blood factors								
Adynovi	Treatment and prophylaxis of bleeding in patients 12 years and above with haemophilia A	rurioctocog alfa pegol	No	8.1.2018	B02BD02		+	Antihemorrhagics
Afstyla	Treatment and prophylaxis of bleeding in patients with haemophilia A, all age groups	lonoctocog alfa	No	4.1.2017	B02BD02		+	
Alprolix	Treatment and prophylaxis of bleeding in patients with haemophilia B	eftrenonacog alfa	No	12.5.2016	B02BD04	O	+	Vitamin K and other hemostatics, Blood coagulation factors
Elocta	Treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency), all age groups	efmoroctocog alfa	No	19.11.2015	B02BD02		+	Antihemorrhagics
Esperoct	Treatment and prophylaxis of bleeding in patients 12 years and above with haemophilia A	turoctocog alfa pegol	No	20.6.2019	B02BD02		+	
Idelvion	Treatment and prophylaxis of bleeding in patients with	albutrepenonacog alfa	No	11.5.2016	B02BD02	O	+	Blood coagulation factors

Medicine Name	Indication (self annotated)	Active substance	Biosimilar	Date of MA in EU	ATC code	Conditional approval/ orphan designation	Additional monitoring	Pharmatherapeutic group (EMA)
	haemophilia B (congenital factor IX deficiency)							
Jivi	Treatment and prophylaxis of bleeding in previously treated patients ≥ 12 years of age with haemophilia A	Damoctocog alfa pegol	No	22.11.2018	B02BD02		+	Antihemorrhagics
Kovaltry	Treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency), all age groups	octocog alfa	No	18.2.2016	B02BD02		+	
NovoEight	Treatment and prophylaxis of bleeding in patients with haemophilia A, all age groups	turoctocog alfa	No	13.11.2013	B02BD02			
NovoThirteen	prophylactic treatment of bleeding in adult and paediatric patients 6 years and above with congenital factor-XIII-A-subunit deficiency	catridecacog	No	3.9.2012	B02BD11			
Nuwiq	Treatment and prophylaxis of bleeding in patients with haemophilia A, all age groups	simoctocog alfa	No	22.7.2014	B02BD02		+	Blood coagulation factors
Obizur	Treatment of bleeding in adult patients with acquired haemophilia caused by antibodies to Factor VIII.	susoctocog alfa	No	11.11.2015	B02	EC	+	Antihemorrhagics
Ondexxya	For adult patients treated with a direct factor Xa (FXa) inhibitor	andexanet alfa	No	26.4.2019	V03AB	CM	+	all other therapeutic products
Refixia	Treatment and prophylaxis of bleeding in patients 12 years and above with haemophilia B	nonacog beta pegol	No	2.6.2017	B02BD04		+	Antihemorrhagics
Rixubis	Treatment and prophylaxis of bleeding in patients with haemophilia B	nonacog gamma	No	19.12.2014	B02BD04			
Veyvondi	Adults with von Willebrand Disease (VWD)	vonicog alfa	No	31.8.2018	B02BD10		+	
Vihuma	Treatment and prophylaxis of bleeding in patients with haemophilia A	simoctocog alfa	No, Reference to Nuwig	13.2.2017	B02BD02		+	
Parathyroid Hormone/analogs								

Medicine Name	Indication (self annotated)	Active substance	Biosimilar	Date of MA in EU	ATC code	Conditional approval/ orphan designation	Additional monitoring	Pharmatherapeutic group (EMA)
Movymia	Treatment of osteoporosis in postmenopausal women and in men at increased risk of fracture.	teriparatide	Yes	11.01.2017	H05AA02		+	Calcium homeostasis
Natpar	adjunctive treatment of adult patients with chronic hypoparathyroidism	parathyroid hormone	No	24.04.2017	H05AA03	CM, O	+	
Terrosa	Treatment of osteoporosis in postmenopausal women and in men at increased risk of fracture	teriparatide	Yes	04.01.2017	H05AA02		+	
Fusion proteins/chimeric proteins								
Benepali	Adult RA, JIA children above 2y, adult psoriatic arthritis Adult spondyloarthritis Adult psoriasis Paediatric plaque psoriasis children above 6y	etanercept	Yes	13.01.2016	L04AB01		+	Immunosuppressants
Erelzi	Adult RA, JIA in children above 2y, adult psoriatic arthritis Adult spondyloarthritis Adult psoriasis Paediatric plaque psoriasis children above 6y	etanercept	Yes	23.06.2017	L04AB01		+	Immunosuppressants, TNF- alpha inhibitors
Eylea	Adult AMD	aflibercept	No	21.11.2012	S01LA05			Ophthalmologicals
Nulojix	prophylaxis of graft rejection in adults receiving a renal transplant	belatacept	No	17.06.2011	L04AA28			Immunosuppressants
Strensiq	long-term ERT therapy in patients with paediatric-onset hypophosphatasia	asfotase alfa	No	28.08.2015	A16AB	EC, O	+	Other alimentary tract and metabolism products
Zaltrap	Treatment of adult patients with metastatic colorectal cancer	aflibercept	No	01.02.2013	L01XX44			Antineoplastic agents
Interferons								
Besremi	Monotherapy in adults for the treatment of PV	ropeginterferon alfa-2b	No	15.2.2019	L03AB15		+	Immunostimulants
Plegridy	Treatment of adult patients with relapsing remitting MS	peginterferon beta-1a	No	18.7.2014	L03AB13			
FSH analogs								

Medicine Name	Indication (self annotated)	Active substance	Biosimilar	Date of MA in EU	ATC code	Conditional approval/ orphan designation	Additional monitoring	Pharmatherapeutic group (EMA)
Bemfola	Adult women: anovulation (including PCOD), women undergoing assisted reproduction. Adult men: stimulation of spermatogenesis in men	follitropin alfa	Yes	26.03.2014	G03GA05			Sex hormones and modulators of the genital system
Rekovelle	Controlled ovarian stimulation for the development of multiple follicles in women undergoing ART such as an in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) cycle	follitropin delta	No	12.12.2016	G03GA10		+	
Ovaleap	Adult women: anovulation (including PCOD), women undergoing ART. Adult men: stimulation of spermatogenesis in men	follitropin alfa	Yes	27.09.2013	G03GA05			
Unclassified Recombinant Proteins								
Brineura	treatment of neuronal ceroid lipofuscinosis type 2 (CLN2) disease, also known as tripeptidyl peptidase 1 (TPP1) deficiency	cerliponase alfa	No	30.05.2017	A16AB	AA, EC, O	+	Other alimentary tract and metabolism products
Jetrea	adults for the treatment of vitreomacular traction (VMT)	ocriplasmin	No	13.03.2013	S01XA22			Ophthalmologicals
Kanuma	ERT in patients of all ages with lysosomal acid lipase (LAL) deficiency.	sebelipase alfa	No	28.08.2015	A16	AA, O	+	Other alimentary tract and metabolism products
Lamzede	Treatment of non-neurological manifestations in patients with mild to moderate alpha-mannosidosis	velmanase alfa	No	23.03.2018	A16AB15	EC, O	+	
Mepsevii	treatment of non-neurological manifestations of Mucopolysaccharidosis VII	vestronidase alfa	No	23.08.2018	A16AB18	EC, O	+	Enzymes

Medicine Name	Indication (self annotated)	Active substance	Biosimilar	Date of MA in EU	ATC code	Conditional approval/ orphan designation	Additional monitoring	Pharmatherapeutic group (EMA)
Myalepta	replacement therapy to treat the complications of leptin deficiency in lipodystrophy (LD) patients 2 y and above for generalised LD and 12 y and above for partial LD	metreleptin	No	29.07.2018	A16AA	EC, O	+	Other alimentary tract and metabolism products
Oxervate	Treatment of adults with moderate or severe neurotrophic keratitis	cenegermin	No	06.07.2017	S01	AA, O	+	Ophthalmologicals
Palynziq	treatment of patients with phenylketonuria (PKU) aged 16 years and older	pegvaliase	No	03.05.2019	A16AB19	O	+	Other alimentary tract and metabolism products
Ruconest	treatment of acute angioedema attacks in adults with hereditary angioedema	conestat alfa	No	28.10.2010	B06AC04			Other haematological agents
Oncaspar	combination therapy in acute lymphoblastic leukaemia, all age groups	pegaspargase	No	14.01.2016	L01XX24		+	Antineoplastic agents
Spectrila	combination therapy in acute lymphoblastic leukaemia, all age groups	asparaginase	No	14.01.2016	L01XX02		+	
Vimizim	Treatment of mucopolysaccharidosis, type IVA, all age groups	elosulfase alfa	No	27.04.2014	A16AB12	O	+	Other alimentary tract and metabolism products
Vpriv	long-term ERT in patients with type-1 Gaucher disease, all age groups	velaglucerase alfa	No	26.08.2010	A16AB10	AA, O		

AA: accelerated assessment; O: orphan designation; EC; exceptional circumstances; CM: Conditional Marketing authorisation, +: subjected to additional monitoring.

PV: polycythaemia vera; MS: Multiple Sclerosis; JIA: Juvenile idiopathic arthritis; RA: rheumatoid Arthritis; AMD: age related macular degeneration; ART: assisted reproductive technologies; PCOD: polycystic ovarian disease; ERT: enzyme-replacement therapy

Annex IV: Primary PD, secondary PD, safety pharmacology and Pharmacodynamic drug-drug interaction studies for ATMPs

Medicinal Product	Primary PD	Primary PD in vivo model	Secondary PD	Safety Pharmacology	PDDI	Tests to show species relevance
Alofisel	In vitro, In vivo	Colitic mice after agreement in SA as no model exists for anal fistulas	N	Yes, Male and female athymic nude rats (CNS)	N*	Y
Provenge	In vivo	Rodent models using species-specific variations of the human cell therapy product have been used	Immunohistochemistry in human tissues to identify the spectrum of expression of PAP protein	Yes, as part of the pharmacodynamic studies	N	Yes
Glybera (EXP)	In vitro, In vivo	LPL deficient mice and LPL deficient cats	N, as per guideline for gene therapy medicinal products, EMEA/CHMP/GTWP/587488/2007 and guideline (EMEA/CHMP/GTWP/12459/2006, Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products [15]. Specificity of Glybera	na	Y A pharmacodynamic study was conducted to support use with immunosuppressive treatment	Y, studies in LPL deficient mice, cats, with disease conditions mimicking human condition. Purpose bred LPL -/- mice, 'rescued' with Advl human LPL soon after birth, to determine active dose of AMT-010 and using plasma TG as the primary proof of activity, supported by measurement of human LPL in plasma and tissue samples
Imlygic	In vitro, In vivo	B16F10- muNectin1 Melanoma Syngeneic Tumour Model in Female C57BL/6 Mice, BALB/c mice bearing A20 – induced tumours	N	N	N Mode of action	Y Anti-tumour efficacy of Imlygic in immunosuppressed mice
Kymriah	In vitro, In vivo	Mice, leukaemia in NOD/Shi-scid IL-2Rγ null (NOG) mice	N	N	N	The in vitro and in vivo non-clinical studies were performed using tumour cells from patients with ALL, and not from patients with diffuse large B-cell lymphoma (DLBCL)
Luxturna	In vitro, In vivo	RPE65-/- mice or Briard (RPE65-/-) dogs and monkeys	na	N not deemed to be necessary	na	Y
Strimvelis	In vitro, In vivo	substitute human to small animal xenotransplantation models has been employed (syngeneic mouse lymphoma mode)	N specific, unique and ubiquitous role of the ADA enzyme, this is unlikely to cause any off-target activity	N, not feasible, patient derived product, Steps taken during the manufacturing to ensure vector levels below LOQ. Impact on S, E to be considered low. No transduction of non-target cells in the non-	N	Not feasable or relevantY

Medicinal Product	Primary PD	Primary PD in vivo model	Secondary PD	Safety Pharmacology	PDDI	Tests to show species relevance
				clinical biodistribution study		
Yescarta	In vitro, In vivo	Murine Model of Lymphoma and Anti- murine CD19 CAR T Cells	N	N	N Lack of appropriate models	Y In vitro demonstration of specific activity of Yescarta against its target antigen CD19. A murine surrogate CD19 CAR T cells were established for in vivo evaluation in a syngeneic mouse lymphoma model.
Zalmoxis	In vitro, in vivo	humanized NOD/SCID mouse to evaluate cell engraftment and the efficacy of the suicide system in a xenograft model of GVHD, and humanized NOD/SCID mice subcutaneously transplanted with 50 mm2 of full thickness human skin	N Not required based on product type	N Not required based on product type	N	Yes
Zynteglo	In vitro, In vivo	β-thalassaemic (Hbbth1/th1) mouse model, NSG mice	Y (evaluated in pivotal pharmacology studies)	N, no evidence of adverse effects on the circulatory, respiratory, neurologic or digestive systems observed in in vivo studies conducted. lack of stand-alone safety pharmacology studies is considered acceptable	N	Ye

Y: study conducted; N: study not conducted; S: Safety; E; Efficacy; LOQ: Limit of quantitation; LPL: Lipoprotein lipase; SCID: severe combined immune deficient; RPE: retinal pigment epithelium; MoA: Mode of action; SA: scientific advice

*Literature evidence provided

.

Annex V: Pharmacokinetics with relevant parameters for CBMPs

ABMP	Biodistribution Species (RoA)	Homing/ Migration	Persistennce	Kinetics	PKDI	Justification for no studies
Alofisel	Y female athymic nude rats (rnu/rnu) with eASC##	Y	Y	N	na	##This data will be supplemented with long-term follow-up of tumour formation in patients administered Alofisel under a Post Approval Safety Study (as described in the RMP)
Provenge	N	N	N	N	N	final product consists of activated PBMCs, no conventional ADME studies are submitted as per CHMP guideline on human CBMP (EMA/CHMP/410869/2006). While data on distribution and trafficking of cells would reveal if the cells distribute to the prostate or non-prostate tissues expressing PAP antigen or to their draining lymph node. In view of the available clinical efficacy and safety data, it does not add to the relevant clinical information

Y: study conducted; N: study not conducted; na: no information available in EPAR.

##This data will be supplemented with long-term follow-up of tumour formation in patients administered Alofisel under a Post Approval Safety Study (as described in the RMP)

Annex VI: Pharmacokinetics with relevant parameters for GTMPs.

	Biodistribution			Germline transmission	Vector shedding	PKDI	Justifications for no studies/comments in EPAR
	Biodistribution (number of studies)	Vector Persistence	Clearance				
Glybera (Withdrawn)	Y cats, mice and rabbits (IM) (at least 3, not mentioned)	Y	Y. 10 weeks as per the applicant but not complete by day 180	Y, only females, vector DNA detected in gonads	N Shedding only from patients in clinical trials	na	EMEA/CHMP/GTWP/587488/2007, (EMEA/CHMP/GTWP/12459/2006
Imlygic	Y (4x) naïve or tumour-bearing BALB/c mice (SC, IT, IV)	Y	Y	na	Y In BALB/c mice,28-42 days after vector administration using **plaque assa**y	N, Mode of action	biodistribution studies are along with viral shedding, and replication of Imlygic
Kymriah	Y (1x) NOG mice (engraftment)	Y	N viral clearance studies are not considered feasible	N	N	N	Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors, EMEA/273974/2005 indicates that the risk of germline transmission associated with the administration of genetically modified human cells is considered to be low and, as animal testing of human cells may be difficult or not meaningful, non-clinical germline transmission studies of human genetically modified cells are not recommended
Luxturna	Y*** RPE65-/- mice and RPE65-/- dogs (sub-retinally)	Y	Y	N	N	na	no dissemination of to the gonads in either dogs or monkeys and published literature. There is existing literature evidence that r AAV2 (and other rAAV subtypes) can be detected in the semen of animals dosed systemically, but virus does not persist, vector sequences are not detected in spermatocytes. The risk is reduced comparing this information derived from intravenous dosing, with subretinal dosing,
Strimvelis	Y (2x) NSG mouse pre-conditioned with busulfan and dosed with transduced cells (single IV dose (2	N	na	N discussion related to risk of germline transmission; vector DNA detected in gonads	N,	na	possibility of vector shedding after Strimvelis administration is extremely low as the number of infectious vector particles was below the LLQ, RVs are known to be inactivated by human serum via the classical complement cascade. Strimvelis is for single use, limiting the possibility of vector shedding. No evidence of replication competent retrovirus (RCR) was detected in clinical trials.

	Biodistribution			Germline transmission	Vector shedding	PKDI	Justifications for no studies/comments in EPAR
	Biodistribution (number of studies)	Vector Persistence	Clearance				
	x105cells) of CD34+ cells derived from human UCB transduced with either mock or functional vector (IV)						
Yescarta	Y (1x) syngeneic mouse lymphoma model (surrogate) (infusion)	Y syngeneic mouse lymphoma model up to 209 days	N	N	N	N, lack of appropriate models, Addressed in clinical studies	based on the type of product, the expression pattern of the target antigen and the lack of a relevant animal model. The risk of inadvertent germline transmission of the CD19 CAR construct has not been addressed; Guideline (EMEA/273974/2005)indicates that the risk of germline transmission associated with the administration of genetically modified human cells is considered to be low and, as animal testing of human cells may be difficult or not meaningful, non-clinical germline transmission studies of human genetically modified cells are not recommended
Zynteglo	Y(2x) LVV-transduced β-thalassaemic mouse BMCs, human healthy donor HSCs were also evaluated in vivo in NSG mice	Y 4-6m study (Only peripheral blood and bone marrow)	na	N	na		na
Zalmoxis	Y	Y	N	N	N	N, type of product, not expected	No viral clearance studies are conducted as the finished product is a cell therapy product which cannot undergo any elimination/inactivation virus step during the manufacture process. No direct in vivo administration of the retroviral vector is performed and shedding is limited to viral particles eventually associated to transduced T cells at time of patient administration

Non-clinical Studies of ATMPs (Gene Therapy, Somatic Cell Therapy), and Recombinant Proteins other than Monoclonal Antibodies

Y: study conducted; N: study not conducted; na: no information available in EPAR.

NSG: NOD scid gamma mouse; NOD: Non-obese diabetic; LVV: lentiviral vector; IT: intratumorally; eASC: ex vivo expanded autologous stem cells; LLQ: Lower limit of quantitation.
RV: retroviruses; MoA: mode of action; eASC- expanded adipose stem cells

*** as a part of general toxicity studies
for ATMPs, relevant biodistribution studies are reported with the species used and the RoA in brackets.

Annex VII: Toxicology studies for ATMPS

Medicine Name	**SDTS/species/RoA**	**NOAEL**	**RDTS/ Species**	**Duration-longest Pivotal RDTS**	**Recovery period- pivotal RDTS/RoA**	**Other toxicity studies**
CBMPs						
Alofisel	Y rats[&,] /IV, SC	Y	Y athymic nude rats (Crl:NIH-Foxn1rnu)	14d	6m IV, **perianal**	immunogenicity (Immunogenic potential of eASC Natural Killer cells and eASC crosstalk T cell recognition of eASC Studies in Support of the Choice of Animal Model
Provenge	N					No, risk-based approach discussion on unwanted immunogenicity, treatment failure, disease transmission, and toxicity as potential risks associated with the manufacture and administration of sipuleucel-T. The risk profiling based on risk-risk factor relationships is adequately justified the extent of non-clinical data based on the risks listed above. RMP
GTMPs						
Glybera (Withdrawn)	Y Mice, / IM, IV EMEA/CHMP/GTWP/587488/20007		N, single use (CPMP/BWP/3088/99)-		, **IM**	A theoretical assessment of baculoviral DNA impurity-associated toxicity
Imlygic	N	Y (PFU/dose)	Y Balb/c mice	12w	1d,28d, 56d, 84d , **intratumorally**, IV, SC-	Effect of antiviral drug acyclovir, used to treat HSV infection, on replication of Imlygic 8oncolytic virus) Tolerability and Anti-Tumour Effects on Human Colorectal Carcinoma tumours in SCID mice
Kymriah	N patient specific product, which is not appropriate to administer to immune competent animals	-	N single IV infusion	-		Toxicity from the use of solvents and impurities, in vitro toxicity studies, in situ hybridisation and tissue cross reactivity studies
Luxturna	Y Briard (RPE65-/-) dogs, normal RPE65 status (dogs, monkeys)	na	Y Briard (RPE65-/-) dogs normal RPE65	7m	Y **subretinally**	na

			status (dogs, monkeys)			
Strimvelis	N patient specific product, which is not appropriate to administer to immune competent animals*			-	-	Not applicable
Yescarta	N engineered human T-cells, no representative in vitro assays, ex vivo models, or in vivo models -			-	-	Not performed
Zynteglo	Y 5x C57BL/6 Hbbth1/th1 β-thalassaemic mice, NSG mice, CD45.1+ (Ly5.1+)C57BL/6 mice	NA	N, Single injection	-	**IV**	No immunotoxicity conducted
Zalmoxis	Y, as a part of PD studies	na	N Extrapolation of repeat dose infusion from mouse to human is limited	-	**-**	No

Y: Studies conducted; N: studies not conducted; an: no information in EPAR, IV: Intravenously; PFU: plaque forming units; STDS: single dose toxicity studies; RTDS: repeat dose toxicity studies; d:days; w: weeks; m: months; SC: subcutaneously; IM: intramuscularly; CBMP: cell based medicinal product; GTMP: Gene therapy medicinal product; RoA: route of administration

*

Annex VIII: Analysis for the risk of Tumorigenicity (CBMPs)/insertional oncogenicity (GTMPs)

ATMP	Study conducted	Comments/ Justifications
Alofisel	yes	in vitro in vivo
Provenge	No	nature of the product (anti-cancer therapy). No such effects are expected to be associated with Provenge. (autologous)
Glybera (Withdrawn)	No	The applicant argued that no study design is relevant to assess insertional mutagenesis and the risk of oncogenicity. CAT and CHMP agreed that the data do not substantiate a concern for tumorigenicity. Available evidence suggests no or low risk.
Imlygic	no	Because wild-type HSV-1 does not integrate into the host genome, the risk of insertional mutagenesis with talimogene laherparepvec is negligible (SmPC section 5.3)
Kymriah	no	No alternative adequate animal models are available (SmPC, section 5.3). Insertion of lentiviral vector sequences has the potential to dysregulate the gene expression in the host and carries a theoretical risk of insertional oncogenesis from the activation of genes regulating cell growth or secondary malignancies. These are classified as a potential risk (included in the Risk Management Plan).
Luxturna	no	AAV integration requires the cells to be dividing and as a consequence, the risk of insertional mutagenesis seems limited
Strimvelis	no	lack of suitable model for long term cell engraftment. cannot be determined at the time of assessment. Due to these known risks, the applicant was advised in 2007 to produce data from animal studies and/or discuss the applicability of all available animal models for insertional oncogenesis with the view of monitoring post-treatment for 12 months (the latency period for hematopoietic malignancies can be >6 months). The applicant discussed why it was not possible to generate suitable experimental conditions to allow for long term analysis of the in vivo carcinogenicity potential of the test item
Yescarta	No	The clinical experience with administration of human T cells that were transduced with γ-retroviral vectors to either express the KTE-C19 CAR construct itself or other transgenes did so far not reveal cases of insertional oncogenesis. These data imply a very low likelihood for T cell transformation induced by γ-retroviral insertional mutagenesis
Zalmoxis	Yes	In vitro evaluation of clonality of the transduced cell population and the profile of integration sites assess any break out of replication competent retroviruses (RCR) which have been linked to leukomogenesis in immunosuppressed non-human primate
Zynteglo	Yes	Literature evidence cited In vitro analysis of clonality, integration profile of retroviral vector, results do not suggest oncogenic risk

Annex IX: Vector specific considerations: integration site analysis for GTMPs

GTMP	Integration site analysis	Justifications for not conducting/comments
Glybera (Withdrawn)	Yes	No risky integration hotspots or clonal skewing
Imlygic	No	HSV-1 virus, non-integrative, literature evidence provided,
Kymriah	Yes	Lentiviral integration analysis
Luxturna	No	some integration events were acknowledged at random sites, where the concentration of the virus is greatest i.e. retinal cells, which are post-mitotic in patients that treated with Luxturna.
Strimvelis	Yes	integrations are found predominantly in and around the transcriptional start site of genes and insertions have been found in genes associated with cell cycle control, cell signalling and near known oncogenes such as LMO-2.
Yescarta	No	Literature evidence
Zynteglo	Yes	The issue of the integration and the potential effect upon treatment of patients with BB305 LVV-transduced CD34+ cells will also be further followed in the long-term registry for this GTMP with integrative LVV.
Zalmoxis	Y In vitro	lower risk category and therefore, in the light of recommendations reported in the EMA document related to inadvertent germ line transmission of gene therapy vectors (EMA/273974/2005, 2007), no specific studies have been performed to evaluate germ line transmission of SFCMM-3 M in vitro assessment of characterisation of the insertional pattern and literature citing preclinical and clinical evidence submitted

Annex X: Toxicological parameters relevant for ATMPs

ATMPs	Toxicology				Justifications for no studies/comments in EPAR
	GTMP/CTMP	Immortalization /tumorigenic growth potential	Immune response/ ADA	Germline transmission	
Alofisel	Cell Therapy	Y$	Y	NA	**$**: Ectopic tissue formation and tumorigenicity included in RMP as important potential risk, will be followed up with the agreed post-authorisation safety study (PASS).
Glybera (Withdrawn)	Gene Therapy	N§	na	Y	**§**: not conducted due to lack of suitable study design to assess insertional mutagenesis and the risk of oncogenicity, Possibility of genomic integration has not been satisfactorily assessed
Provenge	Cell therapy	N	Y	N	Conventional reproductive and development toxicity studies were not considered relevant given the nature and the intended clinical use of this autologous cell therapy product.
Imlygic	Gene Therapy	N§§	N	na§§§	**§§**: carcinogenic potential for an HSV-1-based therapy only through literature review. **§§§**: No viral DNA detected in testes and ovaries in BALB/c mice Because wild-type HSV-1 does not integrate into the host genome, the risk of insertional mutagenesis with talimogene laherparepvec is negligible (SmPC section 5.3)
Kymriah	Gene Therapy	N	na	N#	**#**: The Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors, EMEA/273974/2005 [29]. indicates that the risk of germline transmission associated with the administration of GM human cells is considered to be low and, as animal testing of human cells may be difficult or not meaningful, non-clinical germline transmission studies of human GM cells are not recommended
Luxturna	Gene Therapy	N	Y	N	Long term follow up in studies in the literature with rAAV vectors have not identified specific concerns. No dissemination of voretigene neparvovec to the gonads in either dogs or monkeys
Strimvelis	Cell therapy/tissue-engineered MP	N	N	N	From a non-clinical perspective, the carcinogenic potential due to insertional mutagenesis and the potential for clonal expansion could not be established at the time of assessment. Accordingly, the SmPC and RMP have been updated. The applicant has agreed to a long-term follow up of patients in PASS (15 years) to monitoring of potential mutagenicity.
Yescarta	Gene Therapy	Y	N	N	addressed in a SA procedure The Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors, EMEA/273974/2005 [29]. indicates that risk of germline transmission with GM human cells is low and, and animal testing of human cells is difficult/not meaningful, non-clinical germline transmission studies are not recommended
Zynteglo	Gene Therapy	Y	Not anticipated	N	immunogenicity with Zynteglo not expected as patients will be immunosuppressed during treatment
Zalmoxis	Gene therapy	Y	Y	N	Germline transmission of the integrated transgene is not an expected event because of the provirus is integrated in the final human cell host and mobilization of the virus is unlikely to occur

Y: study conducted; N: study not conducted; na: no information in EPAR.SA: Scientific advice; NA: not applicable as it is a cell-based product; rAAV: recombinant adenoviral vector; HSV: Herpes simplex virus; RMP: risk management plan, SmPC: summary of product characteristics; ADA: anti-drug antibodies.

Annex XI Reproductive toxicity studies for ATMPS

Medicine Name	FEED	Species	EFD	Species	PPND	Species	JAS	Species	Justifications/comments in EPAR
ATMPs									
CBMP									
Alofisel	N	-	N	-	N	-	N	-	preclinical biodistribution studies indicated no migration and integration of eASC into reproductive organs following administration of eASC via different routes.
Provenge	N	-	N	-	N	-	N	-	Not relevant, autologous product, based on intended clinical use
GTMP									
Glybera (Withdrawn)	Y	Pregnant mice	na		na		na		no effects of female reproduction or fetal development were detected. It would nevertheless be prudent to avoid dosing during pregnancy.
Imlygic	Y	mice	Y	mice	N	-	N	-	Negligible amounts (< 0.001% of maternal blood levels) of talimogene laherparepvec DNA were found in foetal blood (SmPC section 5.3), A warning on the risk of placental transfer in pregnant women has been included in section 4.6 of the SmPC
Kymriah	N	-	N	-	N	-	N	-	Guideline EMEA/273974/2005 indicates that the risk of germline transmission associated with the administration of genetically modified human cells is considered to be low and, as animal testing of human cells may be difficult or not meaningful, non-clinical germline transmission studies of human genetically modified cells are not recommended.
Luxturna	N	-	N	-	N	-	N	-	1) there is no dissemination of Luxturna to the gonads in either dogs or monkeys and 2) reference to published literature.
Strimvelis	N	-	N	-	N	-	N, not considered necessary	-	Patients will be pretreated with busulfan, which has gonadotoxic effects in humans and animals. This was confirmed in biodistribution study in mice. Some relevant toxicity endpoints, including histopathology of testes & ovary, were evaluated in pilot and definitive biodistribution study and the presence of the transgene was reported. No JAS deemed necessary.
Yescarta	N	-	N	-	N	-	N	-	acceptable based on the type of product, the expression pattern of the target antigen and the lack of a relevant animal model.
Zynteglo	N	-	N	-	N	-	N	-	Zynteglo in contraindicated in pregnant women, reflected in section 4.3 of the SmPC, because of myeloablative conditioning.
Zalmoxis	N	-	N	-	N	-	N	-	previously treated with highly aggressive myeloablative treatments that are associated with sterility, thus excluding any possible vertical transmission of vector related sequence to the progeny

Y: studies conducted; N: studies not conducted; na: no information available in EPAR.

Annex XII: Human tissue cross reactivity, Local tolerance and Environmental risk assessment (ERA) for ATMPs.

Medicine Name	**Biosimilar**	**Human TCR**	**Local tolerance/RoA**	**Species used**	**Local tolerance as a part of other studies**	**ERA**	**Justification for no ERA**
Alofisel	N	na	Y		As a part of repeat dose toxicity	N	(EMEA/CHMP/BWP/473191/2006)
Glybera (Withdrawn)	N	na	Y	wild type mice	in general toxicity studies	Y	
Provenge	N	Y	N			N	(EMEA/CHMP/SWP/4447/00). Provenge is not expected to pose a risk for the environmental due to the specific nature of its constituents and adequate measures will be in place for the correct disposal
Imlygic	N	na	Y/intratumoral	BALB/c nude mice and the SCID mice		Y	
Kymriah	N	Y, human membrane surface protein array	N	-	-	Y	
Luxturna	N	na	N Addressed in general toxicity studies in dogs and monkeys	-		Y	
Strimvelis	N	na	N/ not considered necessary due to well-established clinical therapy			Y	
Yescarta	N	na	N	-	-	Y	
Zalmoxis		na	N			Y	Y
Zynteglo	N	na	Y		as a part of in vivo studies	Y	

Y: studies conducted; N: studies not conducted; na: no information available in EPAR; RoA: route of administration; ERA: environmental risk assessment

Annex XIII: Primary PD, Secondary PD and Safety Pharmacology Study Summaries for Insulin Analogues.

Medicine Name/INN	**Biosimilar**	**Primary PD**		**Secondary PD**	**Safety Pharmacology**	
		In vitro	*In vivo*	*In vitro*	*In vitro*	*In vivo*
Abasaglar (Absaria)	Yes	Rank orders of affinity to and potency as autophosphorylation of Lantus and insulin reference compounds at the IGF-1, IR-A and IR-B receptors. Lipogenic potency in mouse adipocytes.	N	Y, *Mitogenic response in human osteosarcoma SAOS-2 cells and in rat H4IIe hepatoma cells not required for biosimilar applications.	N not required for biosimilar applications.	
Fiasp (insulin aspart)	No	Not submitted, **reference to older formulation NovoRapid**	N	N	N cited as a part of development of NovoRapid.	
Insulin lispro Sanofi (insulin lispro)	Yes	(IR-A and IR-B binding, binding kinetics and activation, metabolic responses, IGF-1R binding and activation.	N	mitogenic activities	N as per guideline on similar biological medicinal products containing recombinant human insulin and insulin analogues [16]	
Lusduna (insulin glargine) Withdrawn MA	Yes	binding to IR using Biacorex: CHO cells expressing human IR1. Activation of IR by MK-1293 and Lantus: ELISA. Functional assays of IR activation by MK-1293 and Lantus. Binding of Lusduna and Lantus to IGF1R); Activation of IGF1R by Lusduna and Lantus; rat H-4-II-E hepatoma cells: inhibition of glucose production	**rats** treated with STZ and **dogs** treated with somatostatin models of human T1DM: Efficacy of Lusduna and Lantus.	Mitogenic potential of Lusduna and Lantus: tritium-labelled thymidine uptake assay using Saos-2/B10 cells.	na	
Ryzodeg Insulin degludec /insulin aspart (IDegAsp) is a coformulation	No	Comparison of insulin degludec activity to human insulin. Binding studies to IGF-1R. IR signal transduction.	**Rats and Pigs***: metabolic effects of insulin degludec *In vivo*, euglycemic clamp studies. *To select appropriate formulation for early clinical trials.	Mitogenic response in a number of cell lines. Metabolic effects of IR signalling in cell lines and primary liver cells.	67 different assays of standard receptors and transporters, including the hERG potassium channel.	Rats and dogs: CNS, CV and respiratory effects

Medicine Name/INN	**Biosimilar**	**Primary PD**		**Secondary PD**	**Safety Pharmacology**	
		In vitro	*In vivo*	*In vitro*	*In vitro*	*In vivo*
Semglee (insulin glargine)	Yes	Receptor binding, receptor activation (autophosphorylation in CHO cells and HepG2 cells). Adipogenesis (3T3-L1cells) Off-target binding to the IGF-1 receptor (IGF1R) and consecutive mitogenesis. * (all studies non-comparative to the reference Lantus). Biacore: IGF-1 receptor binding affinity	Mice: dose dependent decrease of glucose	Saos-2 cell line: Mitogenic bioassay	na	
Suliqua (Insulin glargine / lixisenatide)	No	Binding and activation of the IGF-1R, IR and GLP-1 R. Cell apoptosis and cell proliferation. *In vitro* studies in 1.1B4 cells, simultaneously expressing mRNA of GLP-1R, IGF1R and IR, and rat thyroid c-cell line RTC6-23, shown to co-express active GLP-1R and IR	**db/db mice**: effect of Insulin glargine/ lixisenatide on glucose homeostasis. **Beagle dogs**: monotherapy vs. combination	N	N	CV SP study in the anesthetised dog model with insulin glargine/lixisenatide IV co-administration
Tresiba (Insulin degludec)	No	Binding studies (rat, dog, pig, human cells). IR signal transduction (no mention of the type of cells). Metabolic effects of IR signalling in cell lines and primary liver cells.	**Rats and pigs**: Metabolic effects of Tresiba with euglycemic clamp studies were performed (to select formulation for clinical trials).	Mitogenic response was studied in a number of cell lines.	67 assays of standard receptors and transporters, including the hERG potassium channel.	Rats and dogs: CNS, cardiovascular and respiratory effects.
Xultophy	No	N	**Male Wistar rats: (comparative)** and individual effects of IDeg and liraglutide.	N (reference to components of the combination)		

Y: study conducted; N: study not conducted; na: no information available in EPAR.
STZ: streptozotocin; hERG: human ether-a-go-go

Annex XIV: Details on Pharmacokinetics, Pharmacodynamic drug-drug interactions and pharmacokinetic drug-drug interactions for insulin analogues

Medicine name/INN	A	D	M	E	Methods	Cmax	tmax/t 1/2	AUC/ AUEC	PDDI	PKDI
Abasaglar (Absaria)	Y	N	N	N	na				N	na
Fiasp	Y Pigs, SC (comparative; Fiasp and NovoRapid)	na	na	na	na	nd	nd	nd	Y	na
Insulin lispro Sanofi	Y As a part of TK Rats,	na	na	na	na				N (EMEA/CHMP/BMWP/ 32775/2005 Rev. 1)	-
Lusduna (withdrawn)	Y SD rats and Beagle dogs	Y SD rats: comparative distribution; Beagle dogs: injection site deposition	Y In vivo (rats); In vitro spiking into fresh rat, dog, and human plasma	na	na	Y	Y	Y	na	na
Ryzodeg	Y Rats and pigs	N	N	N	na	na	Y	na	N Generally, not observed	Y
Semglee	Y As part of RDTS Rats, rabbits	N	N	N	na				na	na-
Suliqua	Y Dogs	na	na	na	na	nd			N Considered acceptable	na
Tresiba	Y Rats, Dogs, Human data	Y Rats, Dogs, Human data	Y Rats, Dogs, Human data	Y	ELISA, RIA	Y	Y	Y	-N	Y
Xultophy (combination product)	Y as a part of general toxicity in rats, and pigs (SC)	N, only for components	N, only for components	N	na	Y	Y	Y	na	na

Y: study conducted; N: study not conducted; na: no information available in EPAR; nd: not discussed in detail in the EPAR. A: Absorption; D: Distribution; M: Metabolism; E: Elimination; PDDI: pharmacodynamic drug-drug interactions; PKDI: Pharmacokinetic drug-drug interactions.

Annex XV: Data analysis on Pharmacodynamics and pharmacokinetic studies for insulin analogues

Insulin analogs		**Studies conducted**		**Studies not conducted/ reference to other MA**		**No information in EPAR**	
PD	**N**	n	%	n	%	n	%
Primary PD	**9**	9	100	0	0	0	0
New entities	5	5	100	0	0	0	0
Biosimilars	4	4	100	0	0	0	0
Secondary PD	**9**	6	67	3	33	0	0
New entities	5	4	80	1	20	0	0
Biosimilars	4	2	50	2	50	0	0
Safety pharmacology	**9**	3	33	4	44	2	22
New entities	5	3	60	2	40	0	0
Biosimilars	4	0	0	2	50	2	50
PDDI	**9**	1	11	5	56	3	33
New entities	5	1	20	3	60	1	20
Biosimilars	4	0	0	2	50	2	50
PK	**9**	9	100	0	0	0	0
New entities	5	5	100	0	0	0	0
Biosimilars	4	4	100	0	0	0	0
PKDI	**9**	2	22	0	0	7	78
New entities	5	2	40	0	0	3	60
Biosimilars	4	0	0	0	0	4	100

Annex XVI: Primary PD, Secondary PD and Safety Pharmacology Studies for GLP agonists.

Medicine name	**Primary PD**		**Secondary PD**	**Safety Pharmacology**		**PDDI**
	In vitro	*In vivo*	*In vitro and /or vivo*	*In vitro*	*In vivo*	
Eperzan (Withdrawn MA)	HEK293-hGLP-1R: interaction of albiglutide with the human GLP-1R Increased beta cell mass reduced food consumption and body weight	na	Y gastric emptying	na	cynomolgus monkey: CV and respiratory effects; Qualitative neurobehavioral assessment as a part of repeat dose toxicity studies	na
Ozempic (semaglutide)	BHK cell membranes, stably expressing the human GLP-1R, Ex vivo stimulation of insulin secretion: rat isolated perfused pancreas,	**Normal male rats**: in vivo potency; diabetic db/db mice: dose dependent reduction of glucose beta-cells; **Göttingen minipig**: duration of action of GLP-1R agonists, hyper-glycaemic clamp study; DIO aged female rats: sub-chronic efficacy on body weight; **DIO mice**: effects on hypothalamic appetite signal; **young, growing pig**s.: effect on lowering of food intake; **SD rat, C57BL, LP-1R KO) mice**: neuronal interaction in brain. **ApoE- and LDL-R Ko mouse models**: development of atherosclerosis.	Y	hERG channel: HEK 293 cells	SD Rats: CNS Irwin study male conscious unrestrained cynomolgus monkeys: CV effects- arterial BP and ECG parameters, cardiac electro-physiology Rats: renal function And studies from RDTS	N Was agreed
Revestive (Teduglutide)	cAMP accumulation in HEK-293 cells expressing rat or human GLP-2 receptor.	Intestinal weight increases in mouse, rat, ferret, piglet, dog and monkey. Dose-response relationship in mice. Rat model of hypoplasia; Rat model of short bowel syndrome	Panel of GPCRs Also supported with literature data.	hERG channel in HEK cells.	Purkinje Fibres: Beagle dogs CV and respiratory safety: Beagle dogs CNS safety: Rats.	N
Saxenda (Liraglutide)	cAMP binding assay	Activation of Brain Regions in rats.	Y panel of 75 receptors and channels.	Evaluated in CNS, respiratory system and CV system.	Mice, rats and monkeys: CNS, Respiratory and CV system.	Y

Medicine name	Primary PD		Secondary PD	Safety Pharmacology		PDDI
	In vitro	*In vivo*	*In vitro and /or vivo*	*In vitro*	*In vivo*	
Trulicity (Deduglutide)	GLP1-R binding, cAMP production in HEK cells expressing human GLP-1R in rat, human and monkey cells. Insulin secretion in INS-1 832/3 rat insulinoma cell line, human and monkey cells.	2 *In vivo* models: IVGTT and the SGI model for insulin secretion in rats and monkeys.	Not conducted, considered acceptable for the nature of the product	CV only, QTc prolongation non-GLP hERG channel study	QTc prolongation in monkeys (at doses higher than that proposed clinically).	na

na: no information in EPAR

BHK: Baby hamster kidney; CV: cardiovascular; CNS: Central nervous system; DIO: diet-induced obese; GLP: glucagon-like peptide; GPCR: G protein coupled receptors; HEK: human embryonic kidney cells; hERG: human ether-a-go-go; INS: insulin; Ko: Knock-Out; R: receptor; LDL: low density lipoprotein; QTc: QT Interval.

Annex XVII: Pharmacodynamics and pharmacokinetics studies for GLP agonists

GLP analogs	N	Studies conducted		Studies not conducted/ reference to other MA		No information in EPAR	
PD	5	n	%	n	%	n	%
primary PD	5	5	100	0	0	0	0
Secondary PD	5	4	80	1	20	0	0
Safety pharmacology	5	5	100	0	0	0	0
PDDI	5	1	20	2	40	2	40
PK	5	5	100	0	0	0	0
PKDI	5	0	0	2	40	3	60

Annex XVIII: Pharmacokinetics, pharmacodynamic drug-drug interactions and pharmacokinetic drug-drug interactions for GLP agonists.

Medicine name	A/Species	D/Species	M/Species	E/Species	Methods	Cmax	tmax/ t1/2	AUC/AUEC	PKDI
Eperzan	Y Mice and monkeys	N not considered informative for a recombinant protein				na	Y	na	N, evaluated in clinical section
Ozempic	Y Mice, rats, rabbits, monkeys	Y Mice, rats, rabbits, monkeys	Y Mice, rats, rabbits, monkeys	Y Mice, rats, rabbits, monkeys	LC-MS/MS (mouse, rat, monkey) and ELISA (mouse, rabbit, monkey)	Y	Y	Y	N, evaluated in clinical section
Revestive	Y Wistar rats	Y Wistar rats	N*	Y Wistar rats	ELISA	Y	Y	Y	na
Saxenda	Y Rats, pigs, monkeys, human data	Y Rats, pigs, monkeys, human data	Y Rats, pigs, monkeys, human data	Y Rats, pigs, monkeys, human data	RIA, ELISA	Y	Y	Y	na
Trulicity	Y mice, rats, rabbits and monkeys	N	N	N**	ELISA	N	Y#	Y#	na

Y: study conducted; N: study not conducted; na: no information in EPAR.

RIA: radioimmuno assay; LC-MS: light chromatography-mass spectrometry; ELISA: enzyme linked immune sorbent assay.GLP-2: glucagon like 2 peptide.

*Native GLP-2 peptide is rapidly cleaved by the serine protease DPP-IV resulting in a peptide with a significantly lower activity. It is expected that the teduglutide will be cleaved into small peptides, and undergo the physiological metabolism of peptides and amino acids.
** Although absorption via the gut in neonates is considered to be minimal, breast feeding has been contraindicated; the wording within Section 4.6 of the SmPC is considered to be acceptable and is similar to that used for other members of this pharmacological class
#: only briefly discussed.

Annex XIX: Primary PD, Secondary PD and Safety Pharmacology Study Summaries for Filgrastim Analogues.

Medicine name/INN	**Primary PD**		**Secondary PD**	**Safety Pharmacology**	
	In vitro	*In vivo*	*In vitro*	*In vitro*	*In vivo*
Accofil	Potency assay in a murine myeloblastic cell line.	1) A bioassay for G-CSF in mice, 2) restoration of neutrophil blood cell counts by Neukine vs. Neupogen in **neutropenic female BALB/c mice** and 3) a comparative effect study of Neukine with Neupogen when administered SCto **swiss albino mice with induced neutropenia.** Data from a general 28 Day rat study was also used to demonstrate pharmacological changes	N	N	N (EMEA/CHMP/BMWP/31329/2005)
Cegfila (Pegfilgrastim Mundipharma)	*In vitro* comparative studies: cell proliferation assay on M-NFS-60 murine myoblastic cell line and RBA to G-CSFR by SPR.	**Normal and Chemotherapy-induced neutropenic rats**	N	N	N (EMEA/CHMP/BMWP/31329/2005)
Fulphila	Cell proliferation assay, binding to target receptor by the SPR method. SPR: RBA to G-CSF receptor(comparative).	**Chemically-induced neutropenic rats**.	N	N	N (EMEA/CHMP/BMWP/31329/2005)
Grastofil (filgrastim)	In vitro, a potency assay was conducted in a murine myeloblastic cell line	Mice: a bioassay for GCSF, restoration of NBCs by or Neukine vs. Neupogen (Filgrastim) in neutropenic female BALB/c mice and comparative effect study of Neukine with Neupogen Relative Potency: Swiss albino mice **Neutropenic female BALB/c mice**: restoration of NBCs Wistar rats: comparative study on increase in NBC	N	N	N (EMEA/CHMP/BMWP/31329/2005)
Grasustek	Biosimilarity: *In vitro* cell-based models (M/G-NFS-60 cells, a murine myeloblastic cell line), RBA: SPR; The RBA of pegfilgrastim to the G-CSF receptor: SPR using Biacore 3000 and T200 instruments.	*In vivo* PD studies in **normal and neutropenic rats**. Comparative efficacy Grasustek/Neulasta in terms of ANC in **normal/neutropenic rats** after SC injection.	N Experience with filgrastims	N	N (EMEA/CHMP/BMWP/31329/2005)

Medicine name/INN	**Primary PD**		**Secondary PD**	**Safety Pharmacology**	
	In vitro	*In vivo*	*In vitro*	*In vitro*	*In vivo*
Lonquex	Binding interaction between hGCSF-R and either filgrastim (the unPEGylated precursor of lipegfilgrastim), lipegfilgrastim or pegfilgrastim was tested *In vitro*.	**Neutropenic rat model of CYP-induced neutropenia**. A single combined PD study on CD rats (comparative), SC administration on hematological parameters. A single combined PD/PK study in Cynomolgus monkeys	N	N	CNS effects in rats CV and respiratory effects in Beagle dogs.
Nivestim	*In vitro* bioassay and RBA. Cell-based potency assay for ability of G-CSF to stimulate cell proliferation. Receptor-binding properties (comparative).	**CYP-induced neutropenic rats**. Efficacy in healthy animals. PD response in ANC in neutropenic rat model.	N	N	N (EMEA/CHMP/BMWP/31329/2005)
Pelgraz	Biological potency: cell proliferation assay in murine myeloblastic cell line. RBA: SPR to confirm the binding affinity as well as to probe the receptor kinetics. RBA using human granulocytes: flow cytometry	**Neutropenic rodent model**. Comparative *In vivo* Efficacy SC administration to neutropenic swiss albino mice, A study evaluating the restoration of neutrophil blood cell counts by Pelgraz vs. Neulasta EU and Neulasta US in **neutropenic female Balb/C mice**.	N	N	N (EMEA/CHMP/BMWP/31329/2005)
Pelmeg	Biosimilarity studies: *In vitro* (M-NFS-60 cells (murine myeloblastic cell line) and RBA by SPR;	PK/PD studies in **normal and neutropenic rats**.	N	N	N (EMEA/CHMP/BMWP/31329/2005)
Ristempa (Withdrawn)	Not listed in detail in EPAR, reference to Module 4 of Neulasta	**'Variety of species', unclear description in EPAR**	na	na	na
Udenyca	comparability b/w Neulasta (EU) and Neulasta (US) evaluated to establish bridging from Neulasta (US) comparator used in non-clinical i*n vivo* and clinical studies to the EEA-authorized reference product. Non-clinical i*n vivo* pharmacology and toxicology studies compared Udenyca against Neulasta (US). G-CSF-induced proliferation of NFS-60 myeloid leukemia cells; Binding affinity to rhuman G-CSF receptor by SPR;	*In vivo* PD study in rat model of **cyclophosphamide (CYP)-induced neutropenia** [non-GLP]. PD effects of Udenyca and Neulasta (US) evaluated in a **SD rat model of CYP-induced neutropenia**. *In vivo* PD from the 4-week toxicity study in cynomolgus monkeys.	N	No standalone studies, only as part of general toxicity studies	

Medicine name/INN	Primary PD		Secondary PD	Safety Pharmacology	
	In vitro	*In vivo*	*In vitro*	*In vitro*	*In vivo*
Ziextenzo	Proliferative effect on NFS-60 cells.	Biosimilarity: in *vivo* studies in rats, rabbits and dogs SC Comparative PD: **normal/neutropenic rats; non-neutropenic rabbits** and dogs. **Neutropenic/non-neutropenic rats**: ANC or PD parameters derived from ANC Male and female SD rats administered SC to cover intended clinical regimen, Beagles, rabbits and rats: PD studies.	N	N	N (EMEA/CHMP/ BMWP/31329/ 2005)

rhG-CSF: recombinant human G-CSF receptor; RBA: receptor binding affinity; KD: binding constant; CYP: cytochrome P; ANC: Absolute neutrophil counts. NBC: neutrophil blood cell counts; AUEC: area under the effective curve.

Annex XX: Data analysis on Pharmacodynamics and pharmacokinetics studies for MPs with filgrastim as an active substance

Active substance Filgrastim		Studies conducted		Studies not conducted/ reference to other MA		No information in EPAR	
PD	**N**	n	%	n	%	n	%
Primary PD	**12**	12	100	0	0	0	0
New entities	2	2	100	0	0	0	0
Biosimilars	10	10	100	0	0	0	0
Secondary PD	**12**	0	0	11	92	1	8
New entities	2	0	0	1	50	1	50
Biosimilars	10	0	0	10	100	0	0
Safety pharmacology	**12**	2	17	9	75	1	8
New entities	2	1	50	0	0	1	50
Biosimilars	10	1	10	9	90	0	0
PDDI	**12**	0	0	11	92	1	8
New entities	2	0	0	1	50	1	50
Biosimilars	10	0	0	10	100	0	0
PK	**12**	9	75	3	25	0	0
New entities	2	1	50	1	50	0	0
Biosimilars	10	8	80	2	20	0	0
PKDI	**12**	1	8	5	42	6	50
New entities	2	1	50	0	0	1	50
Biosimilars	10	0	0	5	50	5	50

Annex XXI: Summary of pharmacodynamic studies on medicinal products with filgrastim as an active substance.

Medicinal Product	Primary pharmacology studies	Primary PD	Primary PD in vivo study species	Secondary PD studies	Safety pharmacology
Accofil (copy of Grastofil as stated in eCTD)	Yes, comparative	In vitro, In vivo	Swiss Albino mice; **neutropenic female BALB/c mice**	N	N
Cegfila (Pegfilgrastim Mundipharma)	Yes, comparative	In vitro, In vivo	**Normal and chemotherapy induced Neutropenic rats**	N, only literature citations	N
Fulphila (Withdrawn)	Yes, comparative	In vitro, In vivo	**Chemically-induced neutropenic rats**	N	N
Grastofil	Yes, comparative	In vitro, In vivo	**neutropenic female BALB/c mice**	N Acceptable based on clinical experience with filgrastims	N
Grasustek	Yes, comparative	In vitro, In vivo	**Normal and neutropenic rats**	N	N
Lonquex	Yes	In vitro, In vivo	**Neutropenic rat model**; Cynomolgus monkeys	N	Rats and Beagle dogs
Nivestim	Yes, comparative	In vitro, In vivo	**Normal and cyclophosphamide induced neutropenic rats**.	N	N
Pelgraz	Yes, comparative	In vitro, In vivo	**Neutropenic swiss albino mice; female Balb/C mice**	N	N
Pelmeg	Yes, comparative	In vitro, In vivo	**Normal and neutropenic rats**	N	N
Ristempa (Withdrawn)	Yes, comparative	In vitro, In vivo	No description on species	na	na
Udenyca	Yes, comparative	In vitro, In vivo	**Normal and cyclophosphamide -induced neutropenic rats**, cynomolgus monkeys	N	No standalone studies, as part of tox studies
Ziextenzo	Yes, comparative	In vitro, In vivo	**Normal, neutropenic and non-neutropenic rats**, rabbits and dogs, SD Rats, Beagle dogs	N	N

Y: study conducted; N:
study not conducted; na: no information in EPAR. Bold font: required models for in vivo PD studies

Annex XXII: Pharmacokinetics, pharmacodynamic drug-drug interactions and pharmacokinetic drug-drug interactions for filgrastim containing MPs.

Medicine name	A	D	M	E	Methods	Cmax	tmax	AUC/ AUEC	PDDI	PKDI
Accofil*** (copy of Grastofil)	Y Wistar rats	N	N	N		Y	Y	Y	N	N
Cegfila (Pegfilgrastim Mundipharma)	Y Normal/neutropenic rats, healthy volunteers	N	N	N		Y	Y	Y	N	na
Fulphila	N	N	N	N		N	N	N	N	N
Grastofil***	Y*** Wistar rats	N	N	N	na	na	na	na	N	N
Grasustek	Y, Non-neutropenic & neutropenic rats	N	N	N	ELISA	Y	na	Y	N	N
Lonquex	Y CD rats, male SD Rats, male Cynomolgus monkeys	na	Y (Invitro)	Y male rats		Y	Y	Y	N, addressed in SmPC	Y, in vitro
Nivestim**	Y Male and Female SD Rats, as a part of RDTS	na	na	na		Y	Y	Y	N	na
Pelgraz	N	N	N	N		N	N	N	N	N
Pelmeg***	Y normal/neutropenic rats	na	na	na		Y	Y	Y	N	na
Ristempa (Withdrawn)	na	na	na	na		na	na	na	na	na
Udenyca*	Y Neutropenic SD rats, Monkeys	N	N	N	ELISA	Y	Y	Y	N	na
Ziextenzo	Y naïve and neutropenic rats, rabbits	na	na	na		Y	Y	Y	N	na

Y: study conducted; N: study not conducted; na: no information in EPAR.
* PK data in monkeys generated as a part of general toxicology studies
** PK data as a part of repeat dose toxicity studies
***PK data generated from toxicokinetic data

Annex XXIII: Primary PD, Secondary PD and Safety Pharmacology Study summaries for parathyroid Hormone Products

Medicine name	Pharmacology studies	Primary PD	Primary PD in vivo study species	Secondary PD studies	Safety pharmacology programme	Pharmacodynamic drug-drug interactions
Movymia	Y, comparative*	In vitro, In vivo	SD rats	na	na	na
Natpar	Y	*Rats and monkeys but in reference to other indication. Only literature evidence considered by the assessors as new data not submitted for the proposed indication**	na	Primary PD considered as secondary PD studies in this case	Rats and Beagle dogs	na
Terrosa	Y, comparative*	In vitro, In vivo	SD rats	na	na	na

*EU reference product Forsteo for both biosimilars Movymia and Terrosa.
**studies taken in part from previously authorised product Preotact (approved in the EU in 2006 and withdrawn in 2014). Lack of primary PD data for proposed indication acceptable in the. absence of clinical relevance of the observed toxicities in non-clinical studies, available nonclinical data in osteoporosis models, published literature on hypocalcaemic animal models and clinical data.

Annex XXIV: Summary of PK and drug interaction data for MPs with PTH analogues as an active substance.

Medicine name	A/Species/RoA	D	M	E	Methods	Cmax	tmax	AUC/ AUEC	Pharmacokinetic drug-drug interactions
Movymia	Y Female SD rats, SC	N (EMEA/CHMP/BMWP/42832)			ELISA	Y	na	Y	N (EMEA/CHMP/BMWP/42832
Natpar	Y rats, rabbits, dogs and monkeys, SC	N	N	Y (only lacteal excretion)*	nd				na
Terrosa	Y SD rats, SC	N (EMEA/CHMP/BMWP/42832)			nd	Y	na	Y	N (EMEA/CHMP/BMWP/42832

Y: study conducted; N: study not conducted; na: no information in EPAR. nd: EPAR mentions that methods were provided in MAA, but they have not been detailed in the EPAR.

*PTH (1-84) is rapidly cleared from plasma in the liver by nonspecific peptidases as its primary clearance pathway and to a lesser extent in the kidney; SC: subcutaneously.

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Annex XXV: Data analysis on pharmacodynamics (PD) and pharmacokinetics (PK) studies for parathyroid hormone (PTH) analogues.

PTH as active substance		Studies conducted		Studies not conducted		No information in EPAR	
PD	**N**	n	%	n	%	n	%
Primary PD	**3**	3	100	0	0	0	0
New entities	1	1	100	0	0	0	0
Biosimilars	2	2	100	0	0	0	0
Secondary PD	**3**	0	0	1	33	2	67
New entities	1	0	0	1	100	0	0
Biosimilars	2	0	0	0	0	2	100
Safety pharmacology	**3**	1	33	0	0	2	67
New entities	1	1	100	0	0	0	0
Biosimilars	2	0	0	0	0	2	100
PDDI	**3**	0	0	0	0	3	100
New entities	1	0	0	0	0	1	100
Biosimilars	2	0	0	0	0	2	100
PK	**3**	3	100	0	0	0	0
New entities	1	1	100	0	0	0	0
Biosimilars	2	2	100	0	0	0	0
PKDI	**3**	0	0	2	67	1	33
New entities	1	0	0	0	0	1	100
Biosimilars	2	0	0	2	100	0	0

PD: pharmacodynamics; PDDI: pharmacodynamic drug-drug interactions; PKDI: pharmacokinetic drug-drug interactions

Annex XXVI: Summaries of primary PD, secondary PD and safety pharmacology and pharmacological drug-drug interaction studies for blood factors.

Medicine name	Pharmacology studies	Primary PD	Primary PD in vivo study species	Secondary PD studies	SP	PDDI
Adynovi	Y, comparative	In vitro, In vivo	E17 *FVIII Ko* mice (exon 17 ko mice; strain B6;129S- F8tm2Kaz/J):	N	CV and respiratory SP in rabbits and Cynomolgus monkeys no specific CNS SP study was provided	N
Afstyla	Y, comparative	In vitro, In vivo	E17 *FVIII Ko* mice (exon 17 ko mice; strain B6;129S- F8tm2Kaz/J)	No dedicated studies, as a part of RDTS	Monkeys, Rats (CNS) and Beagle dogs (CV, respiratory), as a part of RDTS	N
Alprolix	Y, comparative	In vitro, In vivo	HemB mice, HemB dogs	N	No dedicated studies, SP parameters on CV, CNS or respiratory system in rats or cynomolgus monkeys, part if RDTS	N
Elocta	Y, comparative	In vitro, In vivo	HemA mice, HemA dogs	N	Monkeys (CV only), no separate studies, part of RDTS	N
Esperoct	Y, comparative	In vitro, In vivo	F8 Ko mice and HemA dogs	N	male cynomolgus monkeys (CV, CNS), part of RDTS	N
Idelvion	Y, comparative	In vitro, In vivo	HemB mice, HemB dogs	N	Rats, Cynomolgus monkey. Part of SDTS, RDTS	N
Jivi	Y	In vitro, in vivo No dedicated studies, data from general pharmacology	C57BL/6J; BALB/cJ mice, Hem A Mice	No dedicated studies, data from general pharmacology	CNS and renal function in Rats, part of RDTS	N
Kovaltry	Y, comparative	In vitro, In vivo	Hem A mice	N	Beagle dogs (CV), conscious unrestrained rats (Respiratory)	N
NovoEight	Y	In vitro, In vivo	F8 Ko mice, CB57BL mice, HemA dogs	N	Cynomolgus monkeys, part of toxicity studies	N
NovoThirteen	Y	In vitro, In vivo	Cynomolgus monkeys, NZW rabbits	in vitro test systems	in vitro (HUVEC cells, Endothelial cells, Whole blood and in vivo studies (CV model in rabbit, extra-corporal circulation model in cynomolgus monkey). Independent and endpoints incorporated RDTS.	Y
Nuwiq	Y, comparative	In vitro, In vivo	Dog model of Hem A, Cynomolgus monkeys	N	Dogs, cynomolgus monkeys, No standalone studies, a part of primary PD and RDTS	N
Obizur	Y	In vitro, In vivo	E16 *FVIII Ko mice*, Hem A Dog Model	N	Hem A Dogs, cynomolgus monkeys, part of pharmacology and toxicity studies	N
Ondexxya	Y, comparative	In vitro, In vivo	Mice, rats, and rabbits	N*	SD rats, Cynomolgus monkeys, part of RDTS	N
Refixia	Y, comparative	In vitro, In vivo	F9-Ko mice, Hem B dog	N	Cynomolgus monkeys, part of RDTS	N

Medicine name	Pharmacology studies	Primary PD	Primary PD in vivo study species	Secondary PD studies	SP	PDDI
Rixubis	Y, comparative	In vitro, In vivo	F9 knock-out mice	N	rabbits and monkeys	N
Veyvondi	Y	In vitro, In vivo	von Willebrand factor deficient (VWd) mice and VWd dogs.	No standalone studies, Secondary PD effects in SP and toxicology studies.	Spontaneously hypertensive rats, Guinea pigs, rabbit stasis model: anaphylactoid potential of rVWF. Single-dose CV and respiratory SP study in anesthetized dogs.	N
Vihuma	N	N	N	N	N	

Y: study conducted; N: study not conducted; na: no information in EPAR,

PD: pharmacodynamic; RDTS: Repeat dose toxicity studies; PD: pharmacodynamics; PDDI: pharmacodynamic drug-drug interactions

E16 *FVIII Ko* mice: strain E 16 mice produced by targeted disruption of exon 16 in the factor VIII gene and backcrossed into a C57BL/6 background), CV: cardiovascular; CNS: Central Nervous System; Hem A: Hemophilia A; Hema B: Hemophilia B; Ko: knock out; rFVIII: recombinant human Factor VIII; rVWF: recombinant human von Willebrand factor; RDTS: repeat dose toxicity studies; SP: Safety Pharmacology; VWd: von Willebrand factor deficient.

Annex XXVII: Pharmacokinetics, pharmacodynamic drug-drug interactions and pharmacokinetic drug-drug interactions for blood factors.

Medicine name	A Species	D Species	M Species	E Species	Placental Transfer	Methods	RoA	Cmax	Tmax /t1/2	AUC/ AUEC	PKDI
Adynovi	Y E17 FVIII knock out (ko) mice, normal rats and Cynomolgus monkeys	Y Rats	Y Rats	Y Rats	na	FVIII: Antigen ELISA & PEG-factor FVIII ELISA	IV	Y	Y	Y	N
Afstyla	Y monkeys, FVIII ko mice	N	N	N	na	ChS and OSCA	IV	Y	Y	Y	N
Alprolix	Y Normal mice, FIX-ko HemB mice, FcRn ko mice, hFcRn transgenic mice, Sprague Dawley rats, HemB dogs, and cynomolgus monkeys.	na	na	Y FcRn ko mice	Pregnant Female Factor IX Deficient (HemB) Mice	ELISA, one stage aPTT assay	IV	na	Y	na	N
Elocta	Y Hem A mice, FcRn-Ko and hFcRn-Tg mice, normal Sprague Dawley rats, cynomolgus monkeys	Y Mice	N	N	Y	ELISA, Autoradiography,	IV	Y	Y	Y	N
Esperoct	Y Wistar rats, Rovett nude rats, cynomolgus monkeys	Y wistar rats	N	Y wistar rats		ELISA, OSCA, radiolabelling /autoradiography	IV	Y	Y	Y	N
Idelvion	Y Monkeys and in Hem B dogs	Y* Rats	Y Rats	Y* Rats	na	radiochemical analysis (QRA) and HPLC	IV	Y **	Y	Y**	N
Jivi	Y Rats and Rabbits	N	N	N	na	ELISA, LC-MS	IV	Y	Y	Y	N
Kovaltry	N$	N	N	N		ChS	IV	Y	Y	Y	N
NovoEight	Y male FVIII ko mice, Hem A dogs, Cynomolgus monkeys, SD rats	Y male C57Bl/6 mice, male vWF KO mice	N	N		ELISA, COA, Radiolabeling	IV	Y	Y	Y	N
NovoThirteen	Y Juvenile and mature Cynomolgus monkeys, Rats	Y Cynomolgus monkeys	N***	**Y** Cynomolgus monkeys	na	Autoradiography	IV	Y	Y	Y	Y
Nuwiq	Y Hem A dog model,	N	N	Y	na	na	IV	Y	Y	na	N
Obizur	Y Dogs with severe Hem A, cynomolgus monkey	N	N	N	na	OSCA and/or ChS	IV	Y	Y	Y	N

Ondexxya	Y Rats and monkeys (both rhesus and cynomolgus	N	N	N	na	ELISA	IV	na	na	na	na
Refixia	Y F9 KO mice, Hem B dogs, minipig as second non-rodent species	Y	Y F9 KO mice, and rats	Y F9-Ko mice and rats	na	ELISA, LOCI	IV	Y	Y	Y	N
Rixubis	Y F9 KO mice, SD rats, cynomolgus monkeys	N	N	N		OSCA, ChS, ELISA	IV	Y	Y	Y	N
Veyvondi	Y Rats and Cynomolgus monkeys, VWd mice and VWd dogs mimicking condition in humans	Y VWd mouse model	na	na	Y (ex vivo)	PET	IV	na	na	na	na
Vihuma	reference to Module 4 of Nuwiq										

Y: study conducted; N: study not conducted; na: no information in EPAR.

IV: intravenously;
ELISA: Enzyme linked immunosorbent assay
LOCI: Luminescent Oxygen Channelling Immunoassay
ChS: chromogenic assay
OSCA: One-stage clotting assay
* although mentioned in EPAR sections that D, E studies not conducted, comparative tissue distribution and elimination studies in rats are mentioned on page 22/177 of the EPA
**Only mentioned in EPAR, no details available
*** The metabolism of rFXIII was expected to follow the same catabolic routes as its endogenous counterpart and the CHMP considered the lack of studies acceptable.
na: not mentioned in EPAR
$ not conducted as RoA is IV

Annex XXVIII: Data analysis for Pharmacology and pharmacokinetics studies for blood factors

Blood factors	N	Studies conducted		Studies not conducted		No information in EPAR	
PD	17	N	%	n	%	n	%
primary PD	17	16	94	1	6	0	0
Secondary PD	17	4	24	13	76	0	0
Safety pharmacology	17	16	94	1	6	0	0
PDDI	17	1	6	16	94	0	0
PK	17	16	94	1	6	0	0
PKDI	17	1	6	14	82	2	12

PD: pharmacodynamics; PDDI: pharmacodynamic drug-drug interactions; PKDI: pharmacokinetic drug-drug interactions
Basic PK for Kovaltry

Annex XXIX: Summaries for Primary PD, secondary PD and Safety Pharmacology studies for Fusion Proteins.

Product/ INN	**Primary PD**		**Secondary PD**	**Safety Pharmacology**		**PDDI**
	In vitro	*In vivo*	*In vitro*	*In vitro*	*In vivo*	
Benepali EU Reference: Enbrel	MoA: relative binding of Benepali and Enbrel to TNF-α or LT-α3; Inhibitory activity: reporter gene assay; relative binding potency: FRET-based assays. Panel of assays submitted included FcγRIa-, FcγRIIa-, FcγRIIb-, FcγRIIIa (V-type)-, and FcRn-binding	(BALB/c) model of collagen antibody-induced arthritis (CAIA)	N (EMEA/CHMP/BMWP/42832/2005 Rev. 1)		N (EMEA/CHMP/BMWP/42832/2005 Rev. 1)	N (EMEA/CHMP/BMWP/42832/2005 Rev. 1)
Erelzi EU reference Enbrel	Binding and functional neutralization of TNFα and LTα in a reporter gene assay; Binding to TNFα using SPR; Binding to human Fc receptors (FcγRI, FcγRII, FcγRIII and neonatal Fc receptor (FcRn):SPR; Binding to C1q using ELISA, TNFα neutralization and inhibition of TNFα-mediated apoptosis, Initiation of ADCC- and CDC-mediated depletion of trans-membrane TNFα-expressing target cells in cell-based assays, Binding to tmTNFα on stably tmTNF-transfected human cell lines, Caspase induction in tmTNF-transfected cell lines.	Tg197 strain mouse model: in life Tg197 arthritic pathology and histopathology of the underlying lesions in the synovium and the arthritic joints	N, as per EMEA/CHMP/BMWP/42832/2005 Rev. 1		Not required for biosimilars but included as ECG and blood pressure measurements in the RDTS in monkeys.	N
Eylea	Binding of VEGF Trap to human VEGF-A165 and to human PIGF-2. VEGF Trap completely blocks VEGF-mediated phosphorylation of VEGFR-2 on HUVEC; Binding to VEGFA from human, mouse, rat and rabbit with sub-picomolar KD values.	Efficacy study of VEGF Trap in a laser induced-CNV model in Cynomolgus monkeys further supports the presumption of high affinity of VEGF Trap to monkey VEGF.	SCID mice SC implanted with mouse B16F1 melanoma, human A673 rhabdomyosarcoma, or mouse MMT mammary carcinoma.	N*	CNS SP from the RDTS in cynomolgus monkeys. In vivo effect of VEGF trap in Cynomolgus monkeys: absence of ECG abnormalities. NZW rabbits: effects of VEGF Trap on venous and arterial thrombus formation in after electrolytic injury. As per ICH S6(R1), respiratory effects examined as part of the toxicity studies.	N

Product/ INN	Primary PD		Secondary PD	Safety Pharmacology		PDDI
	In vitro	*In vivo*	*In vitro*	*In vitro*	*In vivo*	
Nulojix	In vitro binding studies; In vitro functional analysis: target saturation in human whole blood and allo-response assay, **Fc Receptor-mediated functions**	mouse, rat, rabbit and **monkey**: In vivo functional studies Effect of CD80/CD86 blockade on host pathogen defence.	N	N	Monkeys: as a part of single and RDTS (CV, CNS/PNS and respiratory systems)	Y
Strensiq	Affinity and retention in bone tissue PPi-induced reduction of calcium concentrations: MC3T3-E1 cell cultures	murine Ko model (designated Akp2-/-) of human HPP, hind paw bone mineralisation-associated defects; Prophylactic treatment of Akp2-/-mice; reduced bone mineralisation defects.	N		Rats: acute reactions Rats: respiratory study juvenile monkeys: ECG	N
Zaltrap	The equilibrium dissociation constants, KD for interaction of aflibercept to 9 VEGF family related ligands from human (monkey), mouse, rat and rabbit determined by SPR.	SCID mice: Effects on tumour blood vessel density. SCID mice: Levels of VEGF-aflibercept complex, levels of unbound aflibercept (free aflibercept) circulating in blood and effects of aflibercept on the tumour burden	N	N	No dedicated SP studies, included as a part of general and RDTS in mice, rats, rabbits or monkeys. SD rats: respiratory parameters. C57BL/6 male mice: renal function. NZW rabbits: venous and arterial thrombus formation.CV: effects on CNS and ECG parameters from pivotal monkey RDTS studies	Y

Y: study conducted; N: study not conducted; na: no information in EPAR.

CV: cardiovascular; CNS: Central Nervous System; hERG: NZW; New Zealand White; VEGF: Vascular endothelial growth factor; CNV: choroidal neo-vascularisation; SCID: Severe combined immunodeficiency; SP: safety pharmacology; HUVEC: Human umbilical vein endothelial cells; Tg197: Human TNFα transgenic mouse model of polyarthritis; RDTS: repeat dose toxicity studies; Ko: knockout; ADCC: antibody dependent cell cytotoxicity; CDC: complement dependent cytotoxicity; FcγRIa-, FcγRIIa-, FcγRIIb-, FcγRIIIa: Fcγ receptor subtypes; SPR: surface plasmon resonance; ECG: electrocardiography; CNS/PNS: central/peripheral nervous system; SC: subcutaneously.

* considered irrelevant for high molecular weight proteins

Annex XXX: Pharmacokinetics, pharmacodynamic drug-drug interactions and pharmacokinetic drug-drug interactions for fusion proteins.

Medicine name	A Species/ RoA	D Species/RoA	M Species	E Species/RoA	Methods	Cmax	tmax/ t1/2	AUC/ AUEC	PKDI
Benepali	Y SD rats SC	N CHMP guideline on similar biological medicinal products containing monoclonal antibodies (EMA/CHMP/BMWP/403543/2010)				Y	Y	Y	CHMP guideline on similar biological medicinal products containing monoclonal antibodies (EMA/CHMP/BMWP/403543/2010) N
Elrezi	Y rabbits and monkeys SC	N			ELISA	Y	Y	Y	na
Eylea	Y mice, rats, and cynomolgus monkeys, IV and SC	Y rats	N*	Y functionally-nephrectomised and sham-operated female rats and serial VEGF	ELISA, radiolabeled [125I]-VEGF Trap	Y	Y	Y	N
Nulojix	Y mice, rats, rabbits and monkeys; IV	N			ELISA	Y	na	na	N
Strensiq	Y mice, rats, rabbits, and monkeys/ IV, SC	Y juvenile mice/IV	N	N	serum assays, radiolabeling	Y	Y	Y	N
Zaltrap	Y mice, rats and cynomolgus monkeys; IV, SC	Y female rats	N	Y functionally-nephrectomised and sham-operated female rats	radiolabeling	Y	Y	Y	N

Y: study conducted; N: study not conducted; na: no information in EPAR.

IV: intravenously; SC subcutaneously, ELISA: enzyme-linked immune sorbent assay; VEGF: vascular endothelial growth factor.
* The expected metabolism of VEGF Trap is expected to be to small peptides and amino acids, so further classical biotransformation studies are not required.

Annex XXXI: Data analysis on pharmacology and pharmacokinetics studies for fusion proteins

Fusion proteins		Studies conducted		Studies not conducted/ reference to other MA		No information in EPAR	
Pharmacology	**N**	n	%	n	%	n	%
Primary PD	**6**	6	100	0	0	0	0
New entities	4	4	100	0	0	0	0
Biosimilars	2	2	100	0	0	0	0
Secondary PD	**6**	1	17	5	83	0	0
New entities	4	1	25	3	75	0	0
Biosimilars	2	0	0	2	100	0	0
Safety pharmacology	**6**	5	83	1	17	0	0
New entities	4	4	100	0	0	0	0
Biosimilars	2	1	50	1	50	0	0
PDDI	**6**	2	33	4	67	0	0
New entities	4	2	50	2	50	0	0
Biosimilars	2	0	0	2	100	0	0
Pharmacokinetics	**6**	6	100	0	0	0	0
New entities	4	4	100	0	0	0	0
Biosimilars	2	2	100	0	0	0	0
PKDI	**6**	0	0	5	83	1	17
New entities	4	0	0	3	75	1	25
Biosimilars	2	0	0	2	100	0	0

PD: pharmacodynamics; PDDI: pharmacodynamic drug-drug interactions; PKDI: pharmacokinetic drug-drug interactions

Annex XXXII: Summaries for pharmacodynamics studies for Interferons

Medicine name	Primary pharmacology studies	Primary PD	Primary PD in vivo study species	Secondary PD studies	Safety pharmacology programme
Besremi	Y, compared to peginterferon alfa-2a Serum activities of 2',5'-oligoadenylate synthetase (OAS)	In vivo	Male Cynomologus Monkeys	N	in vitro Cloned hERG Potassium Channels in HEK 293 cells, rats, Cynomolgus monkeys
Plegridy	Y comparative	In vitro, in vivo (from PK and Toxicity studies)	Rhesus monkeys	In vitro: antiviral and antiproliferative activity	SP parameters were monitored in the RDTS,

Annex XXXIII: Pharmacokinetics, pharmacodynamic drug-drug interactions and pharmacokinetic drug-drug interactions for Interferons.

Medicine name	A Species	D Species	M Species	E Species	Methods	Cmax	tmax/ t1/2	AUC/ AUEC	PDDI	PKDI
Besremi	Y cynomolgus monkey	na	na	Y cynomolgus monkey	na	Y	Y	Y	N	na
Plegridy	Y Rhesus monkeys	Y Guniea pigs	N human and monkey serum in vitro** & literature evidence on catabolism	Y Rats*	ELISA, radioactivity (125I)	Y	Y	Y	N***	N***

Y: study conducted; N: study not conducted; na: no information in EPAR.

ELISA: enzyme-linked immune sorbent assay; RDTS: repeat dose toxicity studies; HEK: Human embryonic kidney; hERG: human Ether a go go

* mice and rats are not pharmacologically responsive to human interferon beta-1a, but used as a pre-clinical model for PK studies to evaluate changes in renal clearance and enzymatic proteolysis due to PEGylation, single dose structure activity relationship study.

**evaluation of metabolic stability of mPEG and interferon beta-1a moiety
*** not carried out as there is limited value in the qualitative and quantitative projection of interactions between therapeutic proteins and drug metabolising enzymes from in vitro or non-clinical studies.

Annex XXXIV: Primary PD, secondary PD and Safety pharmacology for interferons.

Interferons	N	Studies conducted		Studies not conducted		No information in EPAR	
PD	2	n	%	n	%	n	%
Primary PD	2	2	100	0	0	0	0
Secondary PD	2	1	50	1	50	0	0
Safety pharmacology	2	2	100	0	0	0	0
PDDI	2	0	0	2	100	0	0
PK	2	2	100	0	0	0	0
PKDI	2	0	0	1	50	1	50

Annex XXXV: Pharmacodynamics studies for recombinant human Follicular stimulating hormone (r-hFSH) analogues.

Medicine name	Primary PD	Primary PD in vivo study species	Secondary PD studies	Safety pharmacology programme
Bemfola	Receptor affinity in HEK293 cell line, Steelman-Pohley assay in vivo to determine potency of three batches each for drug substance and drug product	Female rats	N, as the profile of FSH is well known	N, No pharmacological effects on other organ systems except the gonads would be anticipated with Bemfola
Ovaleap*	In vitro binding assays, Steelman-Pohley assay in vivo to determine potency vs Gonal F	Immature female rats	N, as the profile of FSH is well known	rats (CNS), Beagle dogs (CV), female wistar rats (respiratory)
Rekovelle*	In vitro, not submitted initially but after SA	NA	N, as secondary effects are not anticipated, citation of clinical evidence with FSH	hERG test in stably transfected HEK293 cells, telemetered Cynomolgus primates

*Gonal-F is here taken as reference comparator; NA: not applicable, as stated in EPAR; SA: Scientific advice.

Annex XXXVI: Pharmacokinetics, pharmacodynamic drug-drug interactions(PDDI) and pharmacokinetic drug-drug interactions(PKDI) for recombinant human Follicular stimulating hormone (r-hFSH) analogues.

Medicine name	A Species/RoA	D Species	M Species	E Species/RoA	Methods	Cmax	tmax/t1/2	AUC/AUEC	PDDI	PKDI
Bemfola	Y Female rats/ IV, SC	N	N	Y Rats/IV, SC	ELISA	Y	Y	Y	N	N
Ovaleap	Y Rats/SC	N	N	N	ELISA	Y	Y	Y	N	N
Rekovelle	Y Rats/ IV**	N	N	N*	ELISA	Y	Y	Y	N	N

Y: studies conducted; N: studies not conducted; na: no information available in EPAR; ELISA: Enzyme linked immune-sorbent assay.
RoA: Route of Administration; IV: intravenously; SC subcutaneously; ELISA: enzyme-linked immune sorbent assay
* Literature data cited on elimination of gonadotropins
** issue raised by assessors on RoA as intended RoA is SC

Annex XXXVII: Primary PD, Secondary PD, Safety pharmacology, Pharmacokinetics and drug interaction studies for r-hFSH containing MPs

FSH analogs		**Studies conducted**		**Studies not conducted**	
PD	**N**	n	%	n	%
primary PD	**3**	3	100	0	0
New entities	**1**	1	100	0	0
Biosimilars	**2**	2	100	0	0
Secondary PD	**3**	0	0	3	100
New entities	**1**	0	0	1	100
Biosimilars	**2**	0	0	2	100
Safety pharmacology	**3**	2	67	1	33
New entities	**1**	1	100	0	0
Biosimilars	**2**	1	50	1	50
PDDI	**3**	0	0	3	100
New entities	**1**	0	0	1	100
Biosimilars	**2**	0	0	2	100
Pharmacokinetics	**3**	3	100	0	0
New entities	**1**	1	100	0	0
Biosimilars	**2**	2	100	0	0
PKDI	**3**	0	0	3	100
Nnew entities	**1**	0	0	1	100
Biosimilars	**2**	0	0	2	100

PD: pharmacodynamics; PDDI: pharmacodynamic drug-drug interactions; PKDI: pharmacokinetic drug-drug interactions

Annex XXXVIII: Pharmacodynamics (PD), secondary pharmacodynamics and Safety pharmacology for unclassified recombinant proteins.

Medicine Name	**Primary PD**	**Primary PD in vivo study species**	**Use of species/model justified**	**Secondary PD studies**	**Safety pharmacology**
Brineura	In vitro, In vivo	TPP1 KO mice and juvenile TPP1-null longhaired dachshund dogs	yes	N	No standalone studies, CV and CNS function were integrated into one SDTS in Cynomolgus monkeys and repetitive administration in TPP1-null and WT dachshund dogs, part of RDTS
Jetrea	In vitro, ex vivo No suitable model	Rats, Guinea pigs, Cats, rabbits and human donor eyes; Porcine eyes ex vivo: induction of PVD	No appropriate model for VMA. Therefore, a number of in vitro, in vivo and ex vivo publications are submitted with PD profile of ocriplasmin evaluated in vivo and ex vivo in rat, guinea pig, rabbit, cat, porcine and human eyes, and the scope of the studies is considered to be extensive	submitted but considered invalid by CHMP as studies performed for separate indication	Beagle dogs: in compliance to ICH S7A. covering CV, respiratory and haematological endpoints. Cats and rabbits: effect of ocriplasmin on the vitreoretinal interface
Kanuma	In vitro, In vivo	"Yoshida" rat model. This homozygous LAL-deficient rat analogous to human disease.	yes	N Nature of the product, recombinant form of the endogenous human lysosomal acid lipase (hLAL)	CNS, Respiratory, CV: rats Cynomolgus monkey: CV effects
Lamzede	In vitro, In vivo	α-mannosidosis KO mice (Tg+/α-mKO mice) (mouse models of α-mannosidosis),	Yes, nonclinical pharmacology of recombinant GUS, including recombinant mouse (rm-GUS) and multiple recombinant human versions of GUS (rh-GUS	N	CNS, respiration and CV functions in juvenile cynomolgus monkeys as a part of RDTS

Medicine Name	Primary PD	Primary PD in vivo study species	Use of species/model justified	Secondary PD studies	Safety pharmacology
Mepsevii	In vitro, In vivo	MPS VII mice: rmGUS and different versions of rhGUS (immunologically tolerant to human GUS) in mouse model of the human disease.	Yes	N	SD rats: respiratory effects. SD rats: Central nervous system (CNS), GLP study. Cynomolgus monkeys: RDTS
Myalepta	In vitro, In vivo	Leptin-deficient, euleptinemic and hyperleptinemic animals, and in dyslipidemic/ atherosclerotic models	Yes	Y effect of metreleptin on wound healing in female ob/ob and db/db mice. SD rats: metabolic and behavioural effects of metreleptin.	CD-1 mice: CNS Irwin test, anti-convulsant and proconvulsant tests, influence of hexobarbital. SD rats: CNS grip strength, rotarod performance, locomotor activity, tail flip latency. SD rats and beagle dogs: CV effects. SD rats: renal function. SD rats, Dunkin Hartley Guinea pigs
Oncaspar	In vitro, in vivo	dogs with lymphosarcoma	No, old studies since 1980s not compliant with present day standards**	N, Acceptable as extensive clinical experience is available on the product	Lymphoma bearing dogs: ECG parameters As a part of pharmacology and RDTS
Oxervate	In vitro, in vivo	Rat models, rabbits	Yes, but final evaluation of efficacy will need to rely on the clinical data	N, justified on the basis that rhNGF binds only the TrkA and p75 receptors and ocular administration results in very low systemic exposure to NGF	Irwin SP study: CNS; Considering the RoA, cardiac SP were not deemed appropriate. This was confirmed in a CHMP Scientific Advice.
Palynziq	In vitro, in vivo	ENU2 mouse model.	Yes, rodent model of PKU, ENU2 mice exhibit HPA similar to PKU patients	N	Male and female SD rats: CNS effects, Cynomolgus monkeys: CV effects
Ruconest	In vitro***			N	Anesthetised dogs: effect of rhC1INH on CV (arterial BP, heart rate, PR and QT interval and QRS complex duration) and respiratory systems
Spectrila	In vitro, In vivo	Murine model of leukaemia; Effects on human leukaemia xenografts in SCID mice	Yes	N, Not necessary as the chemical structure and in vitro activity of recombinant ASNase is very similar to that of the ASNase Medac	Beagle dogs: CV and respiratory parameters. Rats: Effects on diuresis and saluresis. Mice: Effects on spontaneous motility, hexobarbital sleeping time, Additional literature data. observed effects hyperglycaemia (and hypoinsulinemia),

Medicine Name	Primary PD	Primary PD in vivo study species	Use of species/model justified	Secondary PD studies	Safety pharmacology
					acute hypersensitivity reactions (and possibly pancreatitis) and disturbances in hepatic function and clotting are included in the SmPC section 4.8
Vimizim	In vitro	N, due to lack of appropriate animal model	No	N	Rats: CNS parameters, respiratory parameters; Cynomolgus monkeys: CV parameters
Vpriv	In vivo	A mouse model of Gaucher disease (D409V/null Mouse),	Yes	N	cardiovascular safety was evaluated in a 6-month rhesus monkey toxicity study

Y: study conducted; N: study not conducted; na: no information in EPAR.

MPS: Mucopolysaccharidosis; SCID: severe combined immune deficiency; rhGUS: recombinant human beta-glucuronidase; rmGUS: recombinant mouse beta-glucuronidase rhNGF: recombinant human nerve growth factor; HPA: hyperphenylalaninaemia; PKU: phenylketourea; VMA:
** considered acceptable as large clinical experience exists since Oncaspar is marketed in the US and in Germany since 1994. Therefore, no additional non-clinical studies are needed
*** lack of in vivo studies accepted as C1INH is already well established as effective in this disease

Annex XXXIX:
Pharmacokinetics, pharmacodynamic drug-drug interactions and pharmacokinetic drug-drug interactions for unclassified proteins
.

Medicine name	A Species/RoA	D Species/RoA	M Species	E Species/RoA	Cmax	tmax/ t1/2	AUC/ AUEC	PDDI	PKDI
Brineura	Y Cynomolgus monkeys, TPP1-null and WT dachshund dog/IV	Y Cynomolgus monkeys, TPP1-null & WT dachshund dog, Beagle dogs	N	N	Y	Y	Y	N	N no interference with co-administered small molecule drugs is expected for the unmodified protein
Jetrea**	N	N	N	N	N	N	N	Y	N
Kanuma	Y SD rat, Cynomolgus monkey and pregnant New Zealand White rabbit /IV	Y SD rat, Cynomolgus monkey and pregnant New Zealand White rabbit /IV	N	N	Y	Y	Y	N nature of the product	N
Lamzede	Y Mice, Rats, Juvenile rats, Pregnant rabbits, Monkeys	Y Monkeys; Rats, Tg+/α-mKO mice	N	N	Y	Y	Y	N	N
Mepsevii	Y SD rats/ IV	Y MPS VII mice	N	N	Y	Y	Y	N ERT, will not interfere	N; recombinant human enzyme, not metabolized by cytochrome P450
Myalepta	Y Mice, dogs and Merino wethers sheep	Y Mice and dogs	N	Y male CDN1 mice/IV	Y	Y	Y	N$	N§
Oxervate	Y intact and abraded corneas in rat/ocular administration to, rabbits/ IV	Y albino rats/topical administration	N	N	Y	Y	Y	N	na
Palynziq	Y homozygote (-/-) male ENU2 mice/IV, SC; homozygote (-/-) male BTBRPahenu2 (ENU2) mice, monkeys/ SC	Y ENU2 mice/IV, SC, cynomolgus monkeys	N	N	Y	Y	Y	N	N
Ruconest	N	Y rats and dogs/IV	Y male Wistar rats	N				Y	na
Oncaspar	Y	Y (systemic distribution) rats, dogs	N	N	Y	Y	Y	N	N#

Medicine name	A Species/RoA	D Species/RoA	M Species	E Species/RoA	Cmax	tmax/ t1/2	AUC/ AUEC	PDDI	PKDI
	mice; rats; rabbits; dogs; monkeys IM, IV, IP							Summary of literature data#	
Spectrila	Y female mice (strain: NOD/SCID), Beagle dog/IV	N Literature data	Literature data	Literature data	Y	Y	Y	N##, literature data	N
Vimizim	Y Rats and monkeys/ IV	Y mice	N N	N N	Y	Y	Y	N	na
Vpriv	Y Rats, dogs, rhesus monkeys /IV	Y bolus IV injections	na	Y Rat	Y	Y	Y	N	N

Y: study conducted; N: study not conducted; na: no information in EPAR; wt: wild type
IV: intravenously; SC: subcutaneously; ERT: Enzyme replacement therapy

$: applicant acknowledged the established risk of hypoglycemia in patients treated with metreleptin who are on high doses of insulin or insulin secretagogues
§: No PK interaction studies were submitted, but the applicant recognised the potential of metreleptin to alter the formation of cytochrome P450 and recommended to include the following information "that when starting or stopping therapy with metreleptin, patients taking medicinal products which are individually adjusted and metabolised via CYP450 (e.g., theophylline, warfarin, phenprocoumon, phenytoin, ciclosporin) should be monitored as doses may need to be altered to maintain therapeutic effect".
acceptable given the significant clinical experience with Oncaspar
##Pharmacodynamic interactions with other chemotherapeutic agents has been described based data from the literature.

Annex XL: data analysis on Primary PD, secondary PD and safety pharmacology for unclassified category.

Unclassified recombinant proteins	N	Studies conducted		Studies not conducted		No information in EPAR	
PD	13	n	%	n	%	n	%
primary PD	13	13	100	0	0	0	0
Secondary PD	13	2	15	11	85	0	0
Safety pharmacology	13	13	100	0	0	0	0
PDDI	13	2	15	11	85	0	0
Pharmacokinetics	13	13	100	0	0	0	0
PKDI	13	0	0	10	77	3	23

PD: pharmacodynamics; PDDI: pharmacodynamic drug-drug interactions; PKDI: pharmacokinetic drug-drug interactions

Annex XLI: Toxicology and toxicokinetics data on 70 recombinant proteins.

Medicine Name	SDTS/species/RoA	NOAEL	RDTS/ Species	Duration-longest Pivotal RDTS	Recovery period-pivotal RDTS	TK	Species	RoA reflecting clinical route
Insulin analogs								
Abasaglar (Absaria)	N	Y	Y Rats	6m	na	Y[3]	rats	Y **SC**
Fiasp (new formulation)	N Reference to NovoRapid/Novolog	na	N Reference to NovoRapid/Novolog -	-	-	N Ref to Novorapid	-	-
Insulin lispro Sanofi	N	na	Y SD rats	4w	na	Y[3]	SD rats	Y **SC**
Lusduna (withdrawn MA)	N	na	Y Rats	1m	4w	Y	rats	Y **SC**
Ryzodeg (insulin aspart + insulin degludec)	Y rats, dogs	na	Y rats[,$]	13w		na	-	Y, **SC**
Semglee	Y mice, rats, NZW rabbits		Y rats 2x, rabbits1x	90d	14d (rats)	Y[3]	rats, rabbits	Y, **SC**
Suliqua (combination product Insulin glargine (Lantus/Optisulin+ Lixisenatide (Lyxumia)	N Only reference to component marketing authorizations	na	N Only reference to component marketing authorizations -	-	-	N## Reference to components	-	-
Tresiba	Y rats, dogs	Y	Y Wistar rats 2x, SD rats 1x, dogs 2x	52w /SD rats; 26w dogs	4w (dogs)	N	-	Y, **SC**
Xultophy (insulin degludec (Tresiba) + Victoza (liraglutide)	N	na	Y rats	13w	na	na	na	Y, **SC**
GLP agonists								
Eperzan (withdrawn)	Y Mice; cynomolgus monkey	Y	Y Mouse; cynomolgus monkeys	52w	na	na	rats, cynomolgus monkeys	Y, **SC**
Ozempic	Y mouse, rats	Y	Y mice, rats; cynomolgus monkeys	52w	na	Y	mouse, rats, rabbits, monkeys and minipigs	Y, **SC**
Revestive	N[#]	na	Y CD-1 mice; cynomolgus monkeys	52w	several weeks	na	na	Y, **SC**

Saxenda	Y mice and rats (standard design), monkeys (MTD)	Y	Y mice; rats; and cynomolgus monkeys	52w	na	Y[2,3]	rats	Y, **SC**
Trulicity	na	na	Y rats, monkeys	na	na	Y[3]	na	Y, **SC**
Filgrastim analogs								
Accofil	Y Rats and mice	na	Y Wistar rats, Swiss albino Mice and SD rats	4w (2x)	4w	Y	Wistar rats	Y; **SC**, IV
Cegfila-Pegfilgrastim Mundipharma	N	na	N	-	-	N	-	-
Fulphila	N	Y	Y SD rats	4w	2w	Y	SD rats	Y, **SC**
Grastofil	Y Rats and mice	Y	Y Wistar rats, Swiss albino Mice and SD rats	4w	4w	Y	Wistar rats	Y; **SC**, IV
Grasustek	N	na	Y CD rats	4w	4w	Y[3]	CD rats	Y, **SC**
Lonquex	Y[ß] Rats	na	Y CD rats 3x, cynomolgus monkeys 2x	26w rats, 13w monkeys	8w	Y[3]	rats, rabbits and monkeys	Y, **SC**
Nivestim	N	na	Y SD rats	4w	na	Y[3]	SD rats	Y, **SC**
Pelgraz	N	na	Y Wistar rats	4w	2w	Y[3]	wistar rats	Y, **SC**
Pelmeg	N	na	N	-	-	N, not required	-	-
Ristempa (Withdrawn)	Neulasta ref	na	Neulasta	-	-	Neulasta ref	-	-
Udenyca	N	na	Y cynomolgus monkeys	4w	4w	Y[3]	Cynomolgus monkeys	Y, **SC**
Ziextenzo	N	na	Y Wistar rats, SD rats 2x	4w	8w	Y[3]	Wistar rats, SD rats 2x	Y, **SC**
Blood factors								
Adynovi	N	Y	Y rats, cynomolgus monkeys	4w	2w	N Extrapolation from monkey SP studies	-	Y, **IV**
Afstyla	Y rats and monkeys	Y	Y CD rats 2x, cynomolgus monkeys 2x	6d, 4w;6d, 4w	14d; 14d	Y[3]	CD rats, Cynomolgus monkeys	Y, **IV**

Alprolix	N	na	Y SD rats 1x, cynomolgus monkeys 2x	27w		Not applicable	-	Y, **IV**
Elocta	Y cynomolgus monkeys	Y	Y SD rats 2x; cynomolgus monkeys, 3x	4w;4w,4w	1m;1m	Y[3]	rats, monkeys	Y, **IV**
Idelvion	Y rats 2x, monkeys 1x	Y	Y CD Rats; cynomolgus monkeys	4w;4w	2w; na	Y	CD Rats; Cynomolgus Monkeys	Y, **IV**
Jivi	Y male rats and rabbits	Y	Y rats; male rabbits	2w; 2w[§]	na	Y[3]	rats and rabbits	Y, **IV**
NovoThirteen	Y cynomolgus monkeys	Y	Y rats 2x; monkeys, 4x	27w	na	Y[3]	cynomolgus monkeys	Y, **IV**
Nuwiq	Y Rats	Y	Y cynomolgus monkeys	4w	2w	Y[3]	dogs, cynomolgus monkeys	Y, **IV**
Obizur	Y dogs, monkeys	Y	Y cynomolgus monkeys	90d	na	Y[3]	cynomolgus monkeys	Y, **IV**
Ondexxya	Y Monkeys	Y	Y rats 1x; monkeys, 2x	2w Single use application	na	na	na	Y, **IV**
Refixia	Y Rats	Y	Y Rowett nude rats; cynomolgus monkeys	26w,4w	26w,4w	Y[3]	Rowett nude rats, cynomolgus monkeys	Y, **IV**
Veyvondi	Y ADAMTS13-deficient mice, C57BL/6J mice, VWF-deficient mice, rats, rabbits, cynomolgus monkeys	na	Y rats, von Willebrand factor deficient pigs, cynomolgus monkeys	14d	18d	Y	rabbits and Cynomolgus monkeys	Y, **IV**
Kovaltry	Y rats, NZW rabbits	Y	Y rats, NZW rabbits	5d	4w	Y[3]	rats, NZW rabbits	Y, **IV bolus**
Rixubis	Y Mice IV bolus	Y	Y rats, cynomolgus monkeys	28 d	2w	Y[3]	rats, NZW rabbits	Y, **IV bolus**
NovoEight	Y male cynomolgus monkeys	Y	Y SD rats, cynomolgus monkeys[1]	2w	na for rats, 6d for monkeys	Y[3]	SD rats, male Cynomolgus monkeys	Y, **IV bolus**
Esperoct	Y wistar rats, IV	Y	Y Wistar rats; Rowlett nude rats; cynomolgus monkeys	52w; 2w	2w; na	Y[3]	wistar rats, Cynomolgus monkeys	Y, **IV**

Vihuma	Reference to Module 4, Nuwiq							
PTH analogs								
Movymia	Y Rats	Y	Y rats	4w	na	na	na	Y, **SC**
Natpar	N	Y	Y rats; dogs; cynomolgus monkeys	26w	na	na	na	Y, **SC**
Terrosa	Y Rats	Y	Y rats	4w	na	na	na	Y, **SC**
Fusion proteins								
Benepali	N*	na	Y cynomolgus monkeys	4w	na	Y[3]	cynomnolgus monkey	Y, **SC**
Erelzi	N	na	Y cynomolgus monkey	4w		Y[3]	cynomnolgus monkey	Y, **SC**
Eylea	Y Cynomolgus	N, MLD, MTD	Y mice (SCID and CD-1); rats (Sprague Dawley and nude); cynomolgus monkey	8m	na	Y	mice (SCID and CD-1); rats (Sprague Dawley and nude); cynomolgus monkey	Y, **IVT**
Nulojix	Y monkeys.	na	Y monkeys[1] 3x; mice; rats	4w,6m, 1y;6m; 3m	6w,3m,13 w,4m,3m	Y[3]	Human, monkey, mice, rats	Y, **IV**
Strensiq	Y juvenile cynomolgus monkeys, IV	na	Y juvenile rats and juvenile cynomolgus monkeys	6m	4w	Y	rat, rabbit, and monkey	Y, **SC,** IV
Zaltrap	Y SD rats, IV	Y	Y rats 2x, SC; cynomolgus monkeys 4x	3m; 26w	6w; 22w	Y	cynomolgus monkey	Y, **IV**
Interferons								
Besremi	N[ß]	Y	Y rats and cynomolgus monkeys	4w	4w	Y[3]	rats, cynomolgus monkeys	Y, **SC**
Plegridy	N, ICHS6	na	Y Rhesus monkeys	5w	4w	Y[3]	Rhesus monkeys	Y, **SC**
FSH analogs								
Bemfola	Y rats	na	Y rats, comparative to Gonal-F	4w	na	Y[3]	rats	Y, **SC**
Ovaleap	Y rats	na	Y rats 2x, Comparative to Gonal-F; dogs 1x	4w	na	Y[3]	rats, dogs	Y, **SC**
Rekovelle	Y mice and rats, IV, SC	Y	Y rats and cynomolgus monkeys[1]	4w; 4w	na	Y	cynomolgus monkeys	Y, **SC**

Unclassified recombinant proteins								
Brineura	Y cynomolgus monkeys, ICV, IT-L infusion[&&&]		Y juvenile TPP1-null mutant, WT longhaired dachshund dogs[1]	3m, 18m	na	na	na	Y, **ICV**
Jetrea	Y Dutch belted rabbits, Cynomolgus monkeys, Gottingen Mini-Pigs, SD rats, Beagle dog	N[$$]	Y cynomolgus monkey			Y	rats, dogs	Y, **IVT** (intravitreal)
Kanuma	Y Cynomolgus monkey IV infusion	Y	Y rats; juvenile cynomolgus monkeys IV infusion	4w;6m	na	Y[3]	rat, monkey, and rabbits	Y, **IV infusion**
Lamzede	N[###] juvenile rat, rabbits and cynomolgus monkeys	Y	Y Tg+/α-mKO mice; cynomolgus monkeys, 3x	26w; 13w	na	Y[3]	mice, cynomolgus monkeys	Y, **IV**
Mepsevii	Y SD rats	na	Y MPS VII Mouse Model; young cynomolgus monkeys	26w	4w	Y[3]	SD rats[§§], NZW rabbits[§§]	Y; **IV**
Myalepta	N	Y	Y mice 4x; rats 1x, dogs 5x, rhesus monkeys, 1x	6m; 6m	28d;4m	Y	mice, beagle dogs	Y, **SC**
Oxervate	Y rats, rabbits; ocular, IV, SC	Na	Y rats; rabbits	26w; 3m	na	Y	rats, rabbits	Y, **ocular** (topical RoA)
Palynziq	Y Rats, Monkeys, IV, SC	Y	Y rats; cynomolgus monkeys	26w; 39w	2-3w	Y[3]	rats, cynomolgus monkeys	Y, **SC**
Ruconest	Y rats, IV	Y	Y rats 2x; cynomolgus monkeys 1x, marmosets 1x; dogs 1x	14d; 2w	2w; na	Y[3]	rats, dogs, cynomolgus monkeys	Y, **IV**
Oncaspar	Y rats, dogs and mice	Y	Y Swiss webster mice 1x; wistar rats 2x; SD rats 1x; rats 1x; beagle dogs 2x; dogs 1x	17 w	na	Y[3]	rats; dogs	Y **IM i.p**
Spectrila	Y CD rats, IV	N, MTD	Y CD rats 2x; Beagle dogs 1x	4w; 4w		Y	Beagle dogs	Y, **IV bolus**
Vimizim	Y SD rats, IV bolus	Y	Y	26w; 52w	4w; na	Y[3]	SD rats	Y, **IV**

			rats 1x, cynomolgus monkeys[1] 2x					
Vpriv	Y SD rats	Y	Y SD rats 2x; rhesus monkeys 1x	6m; 6m	4w;4w	Y[3]	SD rats; rhesus monkeys	Y, **IV bolus**

Y: study conducted; N: study not conducted; na: no information in EPAR. **bold font**: clinically relevant RoA

IV: intravenously; SC: subcutaneously; IM: intramuscularly; IT: intratumorally; IP: intraperitoneally; ICV: Intracerebroventricular; IVT: intravitreal; MTD: Maximum tolerated dose; d: days; m: months; w: weeks; Gd: gestational day; wt: wild type. STDS: single dose toxicity studies; RTDS: repeat dose toxicity studies; TK: toxicokinetics; RoA; route of administration.

EMA (CHMP/SWP/302413/08 and EMA/CHMP/SWP/81714/2010)

[2] This is not in line with CPMP/SWP/1094/04 "Guideline on the evaluation of control samples in non-clinical safety studies" but the study was conducted before the release of the guideline

[3] as a part of single/repeat dose toxicity studies or other studies

& use of single species acceptable

ß as a part of dose finding study

other studies carried out with individual components and not the combination.

§ restricted to 2 w after SA obtained by the applicant from CHMP and other regulatory authorities,

**** no data available on pivotal studies and dosages on some toxicity studies in the tabulated reference in EPAR

###': data extracted from acute toxicity studies

$: other pivotal repeat dose studies also cited with individual components of Ryzodeg

&&&: results of the studies considered inconclusive due to high variability in results and have limited value for repeat dose regimen planned for clinical use.

$$: NOAEL could not be established due to the presence of multiple findings following one or two administrations of ocriplasmin, and at both 75 and 125 µg doses

Annex XLII: Data analysis for toxicology studies for 70 recombinant proteins

Insulin analogs		SDTS		RDTS		TK	
	N	n	%	n	%	n	%
All products	9	3	33	7	78	4	44
New entities	5	2	40	3	60	0	0
Biosimilars	4	1	25	4	100	4	100
GLP agonists		SDTS		RDTS		TK	
	N	n	%	n	%	n	%
All products	5	3	60	5	100	4	80
New entities	5	3	60	5	100	4	80
Filgrastim (peg/lipe)		SDTS		RDTS		TK	
	N	n	%	n	%	n	%
All products	12	3	25	9	75	9	75
New entity	2	1	50	1	50	1	50
Biosimilars	10	2	20	8	80	8	80
Blood factors		SDTS		RDTS		TK	
	N	n	%	n	%	n	%
All products	17	14	82	16	94	13	76
New entity	17	14	82	16	94	13	76
PTH analogs		SDTS		RDTS		TK	
	N	n	%	n	%	n	%
All products	3	2	67	3	100	0	0
New entity	1	0	0	1	100	0	0
Biosimilars	2	2	100	2	100	0	0
Fusion/cimeric proteins		SDTS		RDTS		TK	
	N	n	%	n	%	n	%
All products	6	4	67	6	100	6	100
New entity	4	4	100	4	100	4	100
Biosimilars	2	0	0	2	100	2	100
Interferons		SDTS		RDTS		TK	
	N	n	%	n	%	n	%
All products	2	0	0	2	100	2	100
New entity	2	0	0	2	100	2	100
FSH analogs		SDTS		RDTS		TK	
	N	n	%	n	%	n	%
All products	3	3	100	3	100	3	100
New entity	1	1	100	1	100	1	100
Biosimilars	2	2	100	2	100	2	100
Unclassified recombinant proteins		SDTS		RDTS		TK	
	N	n	%	n	%	n	%
All products	13	11	85	13	100	12	92
New entity	13	11	85	13	100	12	92

SDTS: single dose toxicity studies; RDTS: repeat dose toxicity studies; Sp: species; RoA: route of administration

Annex XLIII: Details on genotoxicity and carcinogenicity studies for 80 analysed medicinal products.

Medicine Name	Biosimilar	Genotoxicity	Kind of studies/ Justification for lack of studies	Carcinogenicity	Kind of studies/ Justification for lack of studies
ATMPs					
Alofisel	No	N	Not applicable considering the cell-based nature of the product, which precludes interaction directly with DNA or other chromosomal material.	N	No conventional rodent studies, only tumorigenic potential
Provenge	No	N	Autologous product, genotoxicity not expected	N	No such effects are expected
Glybera (Withdrawn)	No	N	insertional mutagenesis and Oncogenicity investigated for the vector	N	Studies to assess integration sufficient to address risk of carcinogenicity
Imlygic	No	N	ICH S6(R1) guideline; The genotoxic potential of Imlygic not evaluated in long-term animal studies. As wild-type HSV-1 does not integrate into the host genome, the risk of insertional mutagenesis with Imlygic is negligible (SmPC section 5.3)	N	Review of published literature; as per ICH S9 guideline [26]
Kymriah	No	N, only with respect to germline integration. No mouse studies are conducted as rodents are not appropriate to assess the risk of insertional mutagenesis for genetically modified cell therapy products. No alternative adequate animal models are available (SmPC, section 5.3)	Integration pattern consistent with LV infection, high degree of polyclonality and no evidence for preferential integration near genes of concern or preferential outgrowth of cells harbouring the integration sites. The risk of inadvertent germline transmission of the CD19 CAR construct **not been addressed**. As per Guideline EMEA/273974/2005 [13] indicates that the risk of germline transmission associated with GM human cells is considered low …., non-clinical germline transmission studies of human GM cells are not recommended.	N	rodents are not appropriate to assess the risk cell therapy products. No alternative adequate
Luxturna	No	N	Not relevant	N	Not appropriate to assess the risk of insertional mutagenesis for GM cell therapy products
Strimvelis	No	N	Conventional genotoxicity assays are inappropriate to detect insertional events and would not provide	N	Performed with conditions on acceptance as appropriate conditions could not be attained[##]

Medicine Name	Biosimilar	Genotoxicity	Kind of studies/ Justification for lack of studies	Carcinogenicity	Kind of studies/ Justification for lack of studies
			additional information to inform a quantitative risk assessment in humans		
Yescarta	No	N	Literature evidence on resistance of mature mouse T cells to transformation induced by genomic integration of γ-retroviral vectors, clinical evidence	N	Addressed in *SA* procedure
Zalmoxis	No	N	Other studies related to vector specific integration conducted.	N	In vitro studies on clonality and risk of insertional mutagenesis
Zynteglo	No	Y	Genotoxic potential in vitro and in vivo studies.	N	The final product intended for single IV administration, conventional carcinogenicity studies in rodents do not apply to ATMPs.
Insulin analogs					
Abasaglar (Absaria)	Yes	N	Guidelines on the development of similar biological medicinal products	N	Guidelines on similar biological medicinal products, PtC document on the non-clinical assessment of the carcinogenic potential of insulin analogues [CPMP/SWP/372/01], Discussion on carcinogenic potential of the product and CHMP agreed that carcinogenic risk should be similar to that of reference Lantus (therefore acceptable).
Fiasp (new formulation)	No	N	reference to NovoRapid/NovoLog; Insulin aspart showed no potential for mutagenicity or clastogenicity in the standard battery of genotoxicity tests in the presence and absence of rat liver S9 fraction.	N	ICH S6, reference to two 52-week studies in rats for NovoRapid/NovoLog
Insulin lispro Sanofi	Yes	N	Guidelines on the development of similar biological medicinal products	N	Guidelines on the development of similar biological medicinal products
Lusduna (withdrawn MA)	Yes	na		na	
Ryzodeg (insulin aspart + insulin degludec)	No	N	ICH S6 guideline, Biotech derived product	Y	In vitro and iv vivo studies in rats-52w duration as 2 yr carcinogenicity studies are not required for biotech-derived proteins.
Semglee	Yes	N	As per applicable guidelines	N	As per applicable guidelines
Suliqua (combination product Insulin glargine +Lixisenatide)	No	Y	Negative in a standard battery of genotoxicity tests (Ames test, human lymphocyte chromosome aberration test, mouse bone marrow micronucleus test).	Y	Two-year carcinogenicity studies in mice and rats
Tresiba	No	N	ICH S6 guideline, Biotech derived product	N	Standard 2-year carcinogenicity bioassay is inappropriate for biotechnology-derived pharmaceuticals such as insulin degludec (ICH S6).
Xultophy (fixed combination of insulin degludec	No	N	na	Y	Cell proliferation observed in a mouse pancreatic beta cell line. Pancreatic cancer

Medicine Name	Biosimilar	Genotoxicity	Kind of studies/ Justification for lack of studies	Carcinogenicity	Kind of studies/ Justification for lack of studies
(Tresiba) and Victoza (liraglutide)					has been included as a potential risk within the RMP
GLP agonists					
Eperzan (withdrawn)	No	na	na	N	As clearing anti-albiglutide antibodies emerged by 14 days in rodents, meaningful 2-year studies in rats or mice are not feasible, characterization of GLP-1R distribution in rodent, monkey and humans thyroid ongoing.
Ozempic	No	Y	Non-genotoxic	Y	thyroid C-cell adenomas and carcinomas observed at all dose levels. This is an expected, also seen with other GLP-1 agonists.
Revestive	No	Y	Negative standard in vitro & in vivo genotoxicity studies. ICH S6	Y	Adoption of a recommendation by the CHMP to include the final study report for the 2-year mouse carcinogenicity study to be provided within the framework of the RMP.
Saxenda	No	Y	Ames test, the in vitro chromosome aberrations assay and the in vivo micronucleus tests indicate no genotoxic potential	Y	Relevant risks added to Section 5.3 of the SmPC
Trulicity	No	na	na	Y	findings have been included in Section 5.3 of the SmPC
Filfrastim (PEG/Lipe)					
Accofil	Yes	N		N	True copy of Grastofil MAA, not required
Cegfila) Pegfilgrastim Mundipharma	Yes	N	Guidelines CHMP/437/04 Rev. 1, EMEA/CHMP/BWP/247713/2012, EMEA/CHMP/BMWP/42832/2005 Rev.1 and annex to Guideline on similar biological medicinal products EMEA/CHMP/BMWP/31329/2005 [41]	N	Guidelines CHMP/437/04 Rev. 1, EMEA/CHMP/BWP/247713/2012, EMEA/CHMP/BMWP/42832/2005 Rev. 1 and the annex to Guideline on similar biological medicinal products: EMEA/CHMP/BMWP/31329/2005 [41]
Fulphila	Yes	N	EMEA/CHMP/BMWP/31329/2005 [41]	N	EMEA/CHMP/BMWP/31329/2005 [41]
Grastofil	Yes	N	(EMEA/CHMP/BMWP/31329/2005)	N	(EMEA/CHMP/BMWP/31329/2005)
Grasustek	Yes	N	EMEA/CHMP/BMWP/42832/2005 Rev. 1 and EMEA/CHMP/BMWP/31329/2005 [41].	N	EMEA/CHMP/BMWP/42832/2005 Rev. 1 and EMEA/CHMP/BMWP/31329/2005. [41]
Lonquex	No	N	ICH S6, Not expected to interact directly with DNA or other chromosomal material.	N	ICH S6,
Nivestim	Yes	N	(EMEA/CHMP/BMWP/31329/2005)	N	(EMEA/CHMP/BMWP/31329/2005)
Pelgraz	Yes	N	Not required for biosimilar products	N	Not required for biosimilar products
Pelmeg	Yes	N	Guidelines CHMP/437/04 Rev. 1, EMEA/CHMP/BWP/247713/2012,	N	Guidelines CHMP/437/04 Rev. 1, EMEA/CHMP/BWP/247713/2012,

Medicine Name	Biosimilar	Genotoxicity	Kind of studies/ Justification for lack of studies	Carcinogenicity	Kind of studies/ Justification for lack of studies
			EMEA/CHMP/BMWP/42832/2005 Rev. 1 and EMEA/CHMP/BMWP/31329/2005		EMEA/CHMP/BMWP/42832/2005 Rev. 1 and EMEA/CHMP/BMWP/31329/2005
Ristempa (Withdrawn)	No	N	biotechnological origin of the product and the reassuring clinical data on filgrastim	N	biotechnological origin of the product and the reassuring clinical data on filgrastim
Udenyca	Yes	N	No justifications given	N	
Ziextenzo	Yes	N	EMEA/CHMP/BMWP/42832/2005 Rev.1 EMEA/CHMP/BMWP/31329/2005	N	EMEA/CHMP/BMWP/42832/2005 Rev.1 EMEA/CHMP/BMWP/31329/2005
Blood factors					
Adynovi	No	N	ICH S6 R1	N	ICH S6(R1) Guideline "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals"
Afstyla	No	N	Active components of rVIII-SingleChain are recombinant counterparts of naturally occurring human plasma proteins. mutagenic effects of FVIII not expected, since there is no direct interaction with DNA or DNA binding proteins is anticipated.	N	ICH S6 R1
Alprolix	No	N	ICH S6(R1)	N	Carcinogenicity studies have not been conducted with rFIXFc as based on a weight-of-evidence approach and consistent with the ICH S6 Guideline The lack of such data is addressed in the SmPC section 5.3.
Elocta	No	N	ICH S6	N	ICH S6
Idelvion	No	Y	Bacterial Reverse Mutation Test (Ames test); In vitro mammalian chromosome aberration Test in Human Lymphocytes	N	Naturally occurring protein in human body
Jivi	No	N	ICH S6, Biotech compound	N	ICH S6, Biotech compound
NovoThirteen	No	N	ICH S6	N	ICH S6
Nuwiq	No	N	ICH S6	N	ICH S6
Obizur	No	N	ICH S6	N	ICH S6
Ondexxya	No	N	ICH S6,	N	ICH S1A and S6, Literature information, MoA
Refixia	No	N		N	
Veyvondi	No	Y	S. typhimurium reverse mutation test (Ames Test) with and without metabolic activation and chromosome aberration test; In vitro mammalian chromosomal aberration test in human lymphocytes; MN test with mice	N	ICH S6(R1), No cause for concern
Vihuma	No	Reference to Module 4 for Nuwiq		N, reference to Module 4, Nuwiq	Reference to Module 4 for Nuwiq

Medicine Name	Biosimilar	Genotoxicity	Kind of studies/ Justification for lack of studies	Carcinogenicity	Kind of studies/ Justification for lack of studies
Kovaltry	No	Y	in vitro mammalian gene mutation assay	N	FVIII is endogenous human protein. ADA development in animals results in loss of exposure over time, preventing the conduct of meaningful long-term studies.
Rixubis	No	N	ICH S6 guideline	N	There is no in vitro or in vivo evidence that rFIX has biologic properties other than those similar to endogenous FIX, ICH S6
NovoEight	No	N	ICH S6 guideline	N	ICH S6 guideline
Esperoct	No	N	ICH S6(R1)	N	ICH S6(R1)
PTH analogs					
Movymia	Yes	N	ICH guidance	N	ICH guidance (EMEA/CHMP/BMWP/42832)
Natpar	No	Y	rhPTH (1-84) negative for in vitro mutagenic potential in two assays (bacteria and mammalian cells).	Y	A 2-year rat carcinogenicity study showed increase in bone tumors. Although a growing body of evidence indicate that increased risk for osteosarcoma is of little clinical relevance
Terrosa	Yes	N	ICH guidance	N	ICH guidance, EMEA/CHMP/BMWP/42832)
Fusion/Chimeric proteins					
Benepali	Yes	N	EMEA/CHMP/BMWP/42832/2005	N	MEA/CHMP/BMWP/42832/2005
Erelzi	Yes	N	EMEA/CHMP/BMWP/42832/2005 Rev. 1	N	EMEA/CHMP/BMWP/42832/2005 Rev. 1
Eylea	No	N	biotechnology-derived product	N	SA (EMEA/CHMP/SAWP/310870/2007) and the requirements of ICH S6 (R1)
Nulojix	No	N	In vitro tests on abatacept, not required for proteins	N	only lifetime study in mice on abatacept (more potent version)
Strensiq	No	N	Considered acceptable	N	Considered acceptable
Zaltrap	No	N	ICH S6 and ICH S9 guidelines (EMA/CHMP/ICH/731268/1998 and EMEA/CHMP/ICH/646107/2008) [26]	N	ICH S6 (R1) and ICH S9 guidelines (EMA/CHMP/ICH/731268/1998 and EMEA/CHMP/ICH/646107/2008), EMEA/CHMP/SAWP/310870/2007)
Interferons					
Besremi	No	Y	In vitro genotoxicity studies (Ames and chromosomal aberrations) due to structural alert, both negative	N	ICH S6
Plegridy	No	Y	In silico results from analysis of the 20 kDa mPEG-O-2-methylpropionaldehyde moiety revealed structural alert for chromosomal damage, genotoxicity, and mutagenicity. Ames test and chromosomal aberration assay negative, ICH S6 guidance	N	ICHS6 Addendum
FSH analogs					
Bemfola	Yes	N	ICH S6, EMEA/CHMP/BMWP/42832/2005	N	ICH S6, EMEA/CHMP/BMWP/42832/2005

Medicine Name	Biosimilar	Genotoxicity	Kind of studies/ Justification for lack of studies	Carcinogenicity	Kind of studies/ Justification for lack of studies
Rekovelle	No	N	primary structure identical with the endogenous protein hormone	N	Primary structure identical with the endogenous protein hormone
Ovaleap	Yes	N	ICH S6, NfG on preclinical safety evaluation of biotechnology-derived pharmaceuticals; CPMP/ICH/302/95) and the CHMP guideline on biosimilar products. (EMEA/CHMP/BMWP/42832/2005).	N	ICH S6 guideline, NfG on preclinical safety evaluation of biotechnology-derived pharmaceuticals) CPMP/ICH/302/95) and the CHMP guideline on biosimilar products (EMEA/CHMP/BMWP/42832/2005).
Unclassified Recombinant Proteins					
Brineura	No	N	Not anticipated to be genotoxic due to its molecular structure and its MoA	N	Carcinogenicity is not anticipated, single administration only.
Jetrea	No	N	Biotech product, Ocriplasmin is also unlikely to interact with DNA or chromosomal material	N	ICH S6R1
Kanuma	No	N	As per guidelines	N	As per Guidelines
Lamzede	No	N	ICHS6 (R1) guidance on Preclinical Safety Evaluation of Biotechnology- Derived Pharmaceuticals (EMA/CHMP/ICH/731268/1998)	N	ICHS6 (R1), (EMA/CHMP/ICH/731268/1998). Previous clinical experience with similar products, rhuman GUS is endogenously expressed and not expected to be carcinogenic
Mepsevii	No	N	Being a large protein, not expected to enter the nucleus, interact with DNA (deoxyribonucleic acid) or chromosomes	N	ICH S1A, product is meant for enzyme replacement therapy to maintain physiological levels.
Myalepta	No	Y	Gene mutations in bacteria, salmonella and E. coli strains; Gene mutations in mammalian (CHO) cells; Chromosomal aberrations in mammalian cells: mice MN, human peripheral blood lymphocytes; Chromosomal aberrations in vivo	N	Pharmacological profile of metreleptin is identical to endogenous leptin; metreleptin is devoid of genotoxic potential and pre-neoplastic, proliferative lesions were not reported in chronic subcutaneous toxicity studies in mice and dogs.
Oxervate	No	N	NGF is a protein, not expected to interact directly with DNA	N	ICH S6(R1). However, the guideline does make reference to studies required depending on the biological activity of the product with specific reference made to growth factors. NGF is a growth factor with proliferative effects and may play a role in tumorigenesis. The applicant referred to RDTS in animals, in which rhNGF was administered systemically for up to 6 months. Considered advisable not to use rhNGF eye drops in patients with eye tumours, and a related warning is included in SmPC section 4.4
Oncaspar	No	N	Considered acceptable	N	ICH S6

Medicine Name	Biosimilar	Genotoxicity	Kind of studies/ Justification for lack of studies	Carcinogenicity	Kind of studies/ Justification for lack of studies
Palynziq	No	Y	Ames test, karyotype assessments	N	ICH S6.
Ruconest	No	N	ICH S6 (R1)	N	ICH S6 (R1)
Spectrila	No	N	Literature data submitted	N	ICH S6, $ post-marketing experience with native asparaginase and its protein nature, lack of carcinogenicity study is acceptable. Overall, evidence from published data with asparaginase renders the mutagenic, clastogenic and carcinogenic potential of asparaginase negligible, reference to SmPC section 5.3.
Vimizim	No	N	due to the nature of the product, genotoxic effects are not expected	N	Carcinogenicity is not anticipated
Vpriv	No	N	ICH S6	N	ICH S6

Y: study conducted; N: study not conducted; na: no information in EPAR. MNA: micronucleus assay; CHO; Chinese hamster ovary
SA: Scientific advice; NGF: nerve growth factor
##Agreed in previous SA that tumorgenicity risk assessment could be based on clinical data and literature on similar vectors if tumorgenicity/general toxicity studies are unsuccessful in the determination of optimal conditions for a full tumorgenicity study. Reflected in SmPC due to non-availability of a suitable animal model for Strimvelis due to the inability to achieve long-term engraftment of transduced cells in mice. Conventional genotoxicity assays inappropriate to detect insertional events. The RMP updated and the risk of malignancy will be assessed via ADRs received during the ongoing long-term follow-up study and proposed patient registry.
Agreed in Scientific advice
§:3 approaches- Karyotype analysis, Growth factor dependence, Soft agar assay

Annex XLIV: Analysis of genotoxicity and carcinogenicity studies for 80 medicinal products (summary of available studies.

		Genotoxicity		Carcinogenicity	
ATMPs	N	n	%	n	%
All products	10	1	10	0	0
New entities	10	1	10	0	0
		Genotoxicity		Carcinogenicity	
Insulin analogs	N	n	%	n	%
All products	9	1	11	3	33
New entities	5	1	20	3	60
Biosimilars	4	0	0	0	0
		Genotoxicity		Carcinogenicity	
GLP agonists	N	n	%	n	%
All products	5	3	60	4	80
New entities	5	3	60	4	80
		Genotoxicity		Carcinogenicity	
Active substance Filgrastim	N	n	%	n	%
All products	12	0	0	0	0
New entities	2	0	0	0	0
Biosimilars	10	0	0	0	0
		Genotoxicity		Carcinogenicity	
Blood factors	N	n	%	n	%
All products	17	3	18	0	0
New entities	17	3	18	0	0
		Genotoxicity		Carcinogenicity	
Parathyroid Hormone/analog	N	n	%	n	%
All products	3	1	33	1	33
New entities	1	1	100	1	100
Biosimilars	2	0	0	0	0
		Genotoxicity		Carcinogenicity	
Fusion proteins / chimeric proteins	N	n	%	n	%
All products	6	0	0	0	0
New entities	4	0	0	0	0
Biosimilars	2	0	0	0	0
		Genotoxicity		Carcinogenicity	
Interferons	N	n	%	n	%
All products	2	2	100	0	0
New entities	2	2	100	0	0
		Genotoxicity		Carcinogenicity	
FSH analogues	N	n	%	n	%
All products	3	0	0	0	0
New entities	1	0	0	0	0
Biosimilars	2	0	0	0	0
		Genotoxicity		Carcinogenicity	
Unclassified Recombinant Proteins	N	n	%	n	%
All products	13	2	15	0	0
New entities	13	2	15	0	0

Annex XLV: Fertility and developmental toxicity studies for 70 recombinant proteins.

Medicine Name	FEED	Species	EFD	Species	PPND	Species	JAS	Species	Justifications/comments in EPAR
Insulin analogs									
Abasaglar (Absaria)	N	-	N	-	N	-	N	-	similar biological medicinal products
Fiasp (new formulation)	N	-	N	-	N	-	N	-	
Insulin lispro Sanofi	N	-	N	-	N	-	N	-	similar biological medicinal products
Lusduna (withdrawn MA)	na		na	na		na			
Ryzodeg (insulin aspart + insulin degludec)	Y###	rats	Y	rats, rabbits	Y	rats	na	na	FEED and PPND in insulin degludec only, EFD in the combination product.
Semglee	N	-	N	-	N	-	N	-	similar biological medicinal products
Suliqua (combination product Insulin glargine +Lixisenatide)	N	rats, rabbits	N	rats and rabbits	N	rats	na	na	Studies on individual components of combination, addressed in previous submissions
Tresiba	Y	rats	Y	rats and rabbits	Y	rats	na	na	
Xultophy (insulin degludec (Tresiba) + Victoza (liraglutide)	N	-	N	-	N	-	N	-	Studies on individual components of combination, addressed in previous submissions SmPC currently states that Xultophy should not be used in pregnant women.
GLP agonists									
Eperzan (withdrawn)	Y	mice	Y	mice	Y	mice	na	na	
Ozempic	Y	rats, rabbits, Cynomolgus monkeys	Y	rats, rabbits, Cynomolgus monkeys	Y	cynomolgus monkeys	Y	rats	
Revestive	Y	SD rats	Y	SD rats, NZW rabbits	Y	SD rats	Y	mini-pigs and rabbits	
Saxenda	Y	rats	Y	rabbits	Y	rats	N		
Trulicity	na	-	Y	rats, rabbits	na	na	na	na	excretion in breast milk not determined during and further clarification was sought; contraindicated in breast feeding and included in section 4.6 of SmPC
Active substance Filgrastim									
Accofil	N	-	N	-	N	-	N	-	Not required

Medicine Name	FEED	Species	EFD	Species	PPND	Species	JAS	Species	Justifications/comments in EPAR
Cegfila (Pegfilgrastim Mundipharma)	N	-	N	-	N	-	N	-	Guidelines CHMP/437/04 Rev. 1, EMEA/CHMP/BWP/247713/2012 EMEA/CHMP/BMWP/42832/2005 Rev. 1 EMEA/CHMP/BMWP/31329/2005
Fulphila	N	-	N	-	N	-	N	-	EMEA/CHMP/BMWP/31329/2005, concept paper published on 27/07/2015
Grastofil	N	-	N	-	N	-	N	-	(EMEA/CHMP/BMWP/31329/2005)
Grasustek	N	-	na	-	N	-	N	-	Biosimilar, Not required as per EMEA/CHMP/BMWP/42832/2005 Rev. 1 and EMEA/CHMP/BMWP/31329/2005
Lonquex	N	-	Y	NZW rabbits	N	-	N	-	based on the available information on G-CSF products and the clinical indication which requires myelotoxic chemotherapy,
Nivestim	N	-	N	-	N	-	N	-	Biosimilar
Pelgraz	N	-	N	-	N	-	N	-	not required for biosimilar products
Pelmeg	N	-	N	-	N	-	N	-	Biosimilar, not required as per EMEA/CHMP/BMWP/42832/2005 Rev. 1 and EMEA/CHMP/BMWP/31329/2005
Ristempa (Withdrawn)	N		na		N		N		No Independent studies, reference to Neulasta
Udenyca	N	-	N	-	N	-	N	-	na
Ziextenzo	N	-	Y	Rabbits (Himalayan white)	N	-	N	-	EMEA/CHMP/BMWP/42832/2005 Rev. 1 and EMEA/CHMP/BMWP/31329/2005
Blood factors									
Adynovi	N	-	N	-	N	-	N	-	Not representative for human situation due to of incompatibility based on an antigen/antibody reaction
Afstyla	N	-	N	-	N	-	N	-	Macro and histopathological investigations of M/F reproductive organs included in the SDTS and RDTS (rats and monkeys)
Alprolix	N	-	na	-	N	-	N	-	The lack of information on fertility, EFD, pregnancy and breastfeeding reflected in the SmPC section 4.6
Elocta	N	-	na	-	N	-	N	-	ICH S6, Elocta has been shown to cross the placenta in small amounts in mice. This information has been included in the SmPC section 5.3.
Esperoct	N	-	N	-	N	-	N	-	no indication of adverse effects on reproductive organs in histopathological evaluation in sexually mature M/F, ICH S6(R1)

Medicine Name	FEED	Species	EFD	Species	PPND	Species	JAS	Species	Justifications/comments in EPAR
Idelvion	N	-	na	-	N	-	N	-	Not necessary to conduct EFD as the patient population is male, lack of information on fertility included in section 4.6)
Jivi	N	-	na	-	N	-	Y	neonatal male rats, 2x	males comprise the vast majority of patients with Hem A, and only male patients recruited in clinical trials.
Kovaltry	N	-	na	-	N	-	N	-	As males comprise >99% of the patient population for Hem A, and clinical trials of octocog alfa enrolled only male subjects. JAS not conducted as no adverse effects observed on organ systems in PPND studies, safety data in patients 12 years and older are available, and target organ sensitivity not expected to differ.
NovoEight	Y**	rats	N	-	N	-	N	-	No effects observed on reproductive organs in repeat dose toxicity studies, ICH S6
NovoThirteen	N	-	na	-	N	-	N	-	No effects on reproductive organs in RDTS
Nuwiq	N	-	na	-	N	-	N	-	ICH S6, not considered relevant as Human-cl rhFVIII is a replacement protein for use in the treatment of deficiencies
Obizur	N	-	na	-	N	-	N	-	information is included in section 4.6 and 5.2 of the SmPC
Ondexxya	N	-	na	-	N	-	N	-	Intended for single-dose administration, and has limited potential for reprotoxicity. It does not cross placental barrier, is a modification of an endogenous protein and has a very short half-life (1-2 hour). No findings on reproductive organs observed in the 2-week toxicity studies.
Refixia	N	-	na	-	N	-	N	-	ICH S6, not required for factor IX, JAS not conducted as pediatric population not included in indication due to lack of suitable animal model.
Rixubis	N	-	na	-	N	-	N	-	ICH S6 (R1), (EMA/CHMP/ICH/731268/1998) 'The need for reproductive/developmental toxicity studies is dependent upon the product, clinical indication and intended patient population'. Hem A is a hereditary sex-linked disease, mostly affecting men
Veyvondi	N	-	na	-	N	-	N	-	Generally, not required for rVWF
Vihuma	N	-	na	-	N	-	N	-	
PTH analogs									
Movymia	N	-	na	-	N	-	N	-	(EMEA/CHMP/BMWP/42832)

Medicine Name	FEED	Species	EFD	Species	PPND	Species	JAS	Species	Justifications/comments in EPAR
Natpar	Y	rats	Y	rats, rabbits	Y	rats	N		In the agreed paediatric investigation plan, a juvenile study is planned to study the impact of PTH in children
Terrosa	N	-	N	-	N	-	N	-	EMEA/CHMP/BMWP/42832
Fusion/Chimeric proteins									
Benepali	N	-	N	-	N	-	N	-	Guideline on similar biological medicinal products containing monoclonal antibodies (EMA/CHMP/BMWP/ 403543/2010), and the CHMP guidance on biosimilar medicinal products (EMEA/CHMP/BMWP/42832/2005).
Erelzi	N	-	N	-	N	-	na	-	(EMEA/CHMP/BMWP/42832/2005 Rev. 1)
Eylea	Y	Cynomolgus monkeys	Y	mated female NZW rabbits	N, indication of AMD and target population, PPND studies not required.	-	Y	young, skeletally immature Cynomolgus monkeys	A pregnancy warning is included in Section 4.6 of the SmPC
Nulojix	Y	rats	Y	time-mated rats, rabbits	Y	time-mated rats	Y$$	rats	Data on toxicity to juveniles described in section 5.3 of the SmPC
Strensiq	Y	rats	Y	rats, pregnant NZW female rabbits	Y	female rats	Y	juvenile rats, juvenile monkeys	
Zaltrap	Y §	Cynomolgus monkeys	Y	NZW rabbits	na	-	Y	cynomolgus monkeys	
Interferons									
Besremi	N	-	na	-	N	-	N	-	based on the MoA of IFN, DART studies with ropeginterferon alfa-2b are not considered to add value to understanding of its safety and toxicity profile.
Plegridy	N	-	na	-	N	-	N	-	not conducted due to known abortifacient activity of interferon beta-1a in rhesus monkeys (Avonex EPAR)
FSH analogs									
Bemfola	N	-	N	-	N	-	N	-	well-known MoA of FSH and in accordance with the CHMP guideline on biosimilar products EMEA/CHMP/BMWP/42832/2005

Medicine Name	FEED	Species	EFD	Species	PPND	Species	JAS	Species	Justifications/comments in EPAR
Rekovelle	Y	female rats	N	-	N	-	N	-	Concerning EED, information added in the SmPC for contraindication in pregnant and lactating women, other studies not conducted based on the indication.
Ovaleap	N	-	N	-	N	-	N	-	Biosimilar, (EMEA/CHMP/BMWP/42832/2005)
Unclassified recombinant proteins									
Brineura	Y[$]	dachshund dog	N	-	N	-	Y	Dachshund dogs -	CHMP recommended to perform a fertility assessment during scientific advice. Limited histopathological data from male and female reproductive organs in TPP1-null dachshund dogs do not indicate adverse effects. The lack of data on fertility studies is adequately labelled in the Product information.
Jetrea	N	-	N	-	N	-	N	-	justified by ocriplasmin characteristics and intended use
Kanuma	Y	rats	Y	pregnant female rats during gestation, rabbit	Y	pregnant female rats postcoitum and postpartum	Y	Juvenile monkeys	no adverse effects on reproductive function, fertility, or EED in either male or female rats. In the offspring, there were no adverse effects on survival, physical or sensory/behavioural development, or reproductive competence
Lamzede	Y	rat	Y	rats, rabbits	Y	rat	Y	rats	Full panel of studies conducted
Mepsevii	Y	SD rats	Y	rats, rabbits	N[&]	-	Y	Juvenile cynomolgus monkeys -	A Developmental and PPND study in rats is recommended to further characterise the potential reproductive toxicity effect of vestronidase alfa in line with the ICH S6 (R1) guidance. ICH S6 (R1) guidance states that the conduct of such a study in rats is recommended, to be able to detect any potential effects of Mepsevii in offspring development
Myalepta	Y	CD-1 mice	Y	CD-1 mice, NZW rabbits	Y	CD-1 mice	na	-	No significant adverse findings reported, PIP expected to be completed by 2024
Oncaspar	N	-	N	-	N	-	N Clinical data are available to establish the safety profile in the paediatric population	-	Information included in SmPC sections 4.6 and 5.3 and RMP

Medicine Name	FEED	Species	EFD	Species	PPND	Species	JAS	Species	Justifications/comments in EPAR
Oxervate	Y	rats, rabbits	Y	pregnant rats, rabbits	N	-	N	-	Although there are deviations from the reprotoxicity studies design recommended in guideline ICH S5(R2), such as the lack of an EFD study in another species in addition to rabbits (rat) or that males in the fertility study started treatment 2 weeks before mating (4 weeks is recommended), considering the good safety profile of rhNGF and negligible patient exposure, no additional reproductive toxicity studies were considered necessary
Palynziq	Y	rats	Y	rats, rabbits	Y	rats	na	-	A PPND study in rats and EFD study in rabbits is expected to provide information on maternal phenylalanine depletion in the incidence of EFD effects. Foetal toxicity is included in the RMP as an important potential risk and will be further characterised by a PPND study in rats and an EFD in rabbits.
Ruconest	N	-	Y	rats	N	-	N	-	It could not be excluded that rhC1INH will cross the placenta; foetal exposure and transfer in milk in lactating patients could not be excluded as there were no data to support this view. However, rhC1INH was rapidly eliminated by receptor-mediated endocytosis.
Spectrila	N		N		N		N		Literature data only, indicated in lymphoma
Vimizim	Y	SD rats	Y	NZW rabbits	Y	SD rats	Y	Juvenile monkeys -	Pre-treatment with diphenhydramine (DPH) made interpretation difficult. CHMP requested to include an appropriate statement in the SmPC informing the prescriber that animal studies are of limited relevance
Vpriv	Y	rats	Y	rats, rabbits	Y	rats	na	-	

Y: study conducted; N: study not conducted; na: no information in EPAR.
FEED: fertility and early embryonic studies; EFD: embryo-foetal developmental studies; PPND: Pre- and post-natal development studies; JAS: juvenile animal studies

other studies carried out with individual components and not the combination.
**only fertility studies conducted
$: as a part of multiple dose PK studies
$$: conducted in rats with a more potent version abatacept. Belatacept is a further development of abatacept (centrally approved in 2007 as Orencia, and is indicated in rheumatoid arthritis and polyarticular juvenile idiopathic arthritis)
§: as a part of repeat dose toxicity

Annex XLVI: Data Analysis for Reproduction toxicity and Juvenile animal studies for 70 Medicinal Products. Summary of available studies (excluding studies not conducted and not available from EPARs.

Developmental and reproductive toxicity studies conducted (excluding not conducted, no information)									
		FEED		**EFD**		**PPND**		**JAS**	
Insulin analogs	**N**	n	%	n	%	n	%	n	%
All products	9	2	22	2	22	2	22	0	0
New entities	4	2	50	2	50	2	50	0	0
Biosimilars	5	0	0	0	0	0	0	0	0
		FEED		**EFD**		**PPND**		**JAS**	
GLP agonists	**N**	n	%	n	%	n	%	n	%
All products	5	4	80	5	100	4	80	2	40
New entities	5	4	80	5	100	4	80	2	40
		FEED		**EFD**		**PPND**		**JAS**	
Active substance Filgrastim	**N**	**n**	**%**	**n**	**%**	n	%	n	%
All products	12	0	0	2	17	0	0	0	0
New entities	2	0	0	1	50	0	0	0	0
Biosimilars	10	0	0	1	10	0	0	0	0
		FEED		**EFD**		**PPND**		**JAS**	
Blood factors	**N**	n	%	n	%	n	%	n	%
All products	17	1	6	0	0	0	0	1	6
New entities	17	1	6	0	0	0	0	1	6
PTH analogs	**N**	n	%	n	%	n	%	n	%
All products	3	1	33	1	33	1	33	0	0
New entities	1	1	100	1	100	1	100	0	0
Biosimilars	2	0	0	0	0	0	0	0	0
		FEED		**EFD**		**PPND**		**JAS**	
Fusion /chimeric proteins	**N**	n	%	n	**%**	n	%	n	%
All products	6	4	67	4	67	2	33	4	67
New entities	4	4	100	4	100	2	50	4	100
Biosimilars	2	0	0	0	0	0	0	0	0
Interferons	**N**	n	%	n	%	n	%	n	%
All products	2	0	0	0	0	0	0	0	0
New entities	2	0	0	0	0	0	0	0	0
		FEED		**EFD**		**PPND**		**JAS**	
FSH analogs	**N**	n	%	n	**%**	n	%	n	%
All products	3	1	33	0	0	0	0	0	0
New entities	1	1	100	0	0	0	0	0	0
Biosimilars	2	0	0	0	0	0	0	0	0
		FEED		**EFD**		**PPND**		**JAS**	
Unclassified recombinant proteins	**N**	n	%	n	%	n	%	n	%
All products	13	9	69	9	69	6	46	5	38
New entities	13	9	69	9	69	6	46	5	38

FEED: fertility and early embryonic studies; EFD: embryo-foetal developmental studies; PPND: Pre- and post-natal development studies; JAS: juvenile animal studies

Annex XLVII: Details on the studies for immunogenicity, local tolerance and ERA for 70 recombinant proteins.

Medicine Name	Biosimilar	Immunogenicity Y/N	Human TCR	Local tolerance/RoA	Species used	Local tolerance as a part of other studies	ERA	Justification for no ERA
Insulin analogs								
Abasaglar (Absaria)	Y	Y	na	Y	rats	1	N	(EMEA/CHMP/SWP/4447/00)
Fiasp (new formulation)	N	Y	na	Y	rats, rabbits and minipigs		N	protein consisting of amino acids derived from a biological system, expected to be readily biodegradable
Insulin lispro Sanofi	Y	Y	na	Y/ SC, IV, PV, IM	rabbits		N	(EMEA/CHMP/SWP/4447/00 corr 2)
Lusduna (withdrawn MA)	Y	Y	na	N			N	Insulin glargine is a recombinant human basal insulin analog. EMEA/CHMP/SWP/4447/00 [35]
Ryzodeg (insulin aspart + insulin degludec)	N	Y	na	Y/ SC, IM, IV, IA	pig/minipig model, rabbits	1	N	Protein, no ERA required
Semglee	Y	Y	na	Y/ SC	Wistar rats, NZW rabbits	1	N	EMEA/CHMP/SWP/4447/00 corr 1
Suliqua (combination product Insulin glargine +Lixisenatide)	N	Y	na	Y/ SC, IV, IM, PV	rabbits		N	protein and peptide structure, not expected to pose a risk to the environment.
Tresiba	N	Y	na	Y/ IM, IA, IV	pig/minipig model, rabbits	1	N	protein, and a fatty acid chain coupled via an amino acid spacer
Xultophy (fixed combination insulin degludec (Tresiba)+ Victoza (liraglutide)	N	na	na	Y	pigs, rabbits		N	two drug substances, insulin degludec and liraglutide are peptides and therefore exempted from ERA.
GLP agonists								
Eperzan (withdrawn)	N	Y	na	na			N	active substance is a peptide.
Ozempic	N	Y	na	Y/ IA, IV, IM SC	pigs		N	recombinant protein.
Revestive	N	Y	na	Y/ IV, PV, IA	Göttingen minipig, NZW rabbits		N	EMEA/CHMP/SWP/4447/00
Saxenda	N	N	na	Y/ SC, IA, IV, IA	pigs		N	Liraglutide is a peptide, consisting of natural amino acids and a natural fatty acid.

Medicine Name	Biosimilar	Immunogenicity Y/N	Human TCR	Local tolerance/RoA	Species used	Local tolerance as a part of other studies	ERA	Justification for no ERA
								Therefore, liraglutide is not expected to pose a risk to the environment
Trulicity	N	na	na	na	-		N	Dulaglutide is a recombinant protein. No risk to the environment from the use of dulaglutide is expected
Active substance Filgrastim								
Accofil	Y	na	na	Y/ PV, IM	Rabbits		N	Apo-Filgrastim is intended to substitute other identical products on the market
Cegfila) Pegfilgrastim Mundipharma	Y	na	na	N PI aligned with reference Neulasta	--		N	(EMEA/CHMP/SWP/4447/00 corr. 2) [35], PEG is unlikely to result in a significant risk to the environment because of metabolic breakdown before excretion in patients
Fulphila	Y	N	na	Y/ comparative to Neulasta	rats	1	N	(EMEA/CHMP/SWP/4447/00 Corr 2)
Grastofil	Y	na	na	Y/ comparative to Neupogen, PV, IM	NZW rabbits	N	N	intended to substitute other identical products on the market, so this product is not expected to cause any additional environmental risk
Grasustek	Y	Y	na	Y/ IM, IV, IA, SC, PV	NZW rabbits		N	(CHMP/SWP/4447/00 corr. 2
Lonquex	N	Y	na	Y/IA, IV, PV	rabbits		N	(EMEA/CHMP/SWP/4447/00)[35]
Nivestim	Y	Y	na	Y/ IV, SC,	NZW rabbits		N	(EMEA/CHMP/SWP/4447/00) [35], biosimilar
Pelgraz	Y	Y	na	Y/ SC, IV, IA, PV, IM	NZW rabbits		N	(EMEA/CHMP/SWP/4447/00 corr. 2)[35]
Pelmeg	Y	N	na	N	-		N	(EMEA/CHMP/SWP/4447/00 corr. 2)[35]
Ristempa (Withdrawn)	N	No independent studies, reference to Neulasta	na		-		N	(EMEA/CHMP/SWP/4447/00)
Udenyca	Y	Y	na	Y	Cynomolgus monkeys	3 -	N	(EMEA/CHMP/SWP/4447/00 corr. 2)
Ziextenzo	Y	Y	na	Y/ SC	rats	1	N	(EMEA/CHMP/SWP/4447/00 corr.2)
Blood factors								
Adynovi	N	Y	Y	Y/ IV (IA, PV (misapplication routes)	rats, cynomolgus monkeys, rabbits	1, and independent study in rabbits	Y[$]	Although exempted, as per CHMP/SWP/4447/00 screening for persistence and bioaccumulation provided for PEG moiety
Afstyla	N	Y	na	Y/ IV, IA, PV	rabbits		N	EMEA/CHMP/SWP/4447/00 (1),
Alprolix	N	Y	na	Y/ IV, PV	SD rats, cynomolgus	1, and independent study in rabbits	N	Active substance is a protein, not expected to pose a risk to the environment.

Medicine Name	Biosimilar	Immunogenicity Y/N	Human TCR	Local tolerance/RoA	Species used	Local tolerance as a part of other studies	ERA	Justification for no ERA
					monkeys, NZW rabbits			
Elocta	N	Y	na	Y/ IV	rats and monkeys	1	N	EMEA/CHMP/SWP/4447/00
Esperoct	N	Y	na	Y/ IA, IV, PV	rabbits		N	EMEA/CHMP/SWP/4447/00
Idelvion	N	Y	na	Y, IV, IA, PV	rabbits		N	EMEA/CHMP/SWP/4447/00
Jivi	N	Y	Y	Y/ IV	rats and rabbits	1	N	EMEA/CHMP/SWP/4447/00 (1),
Kovaltry	N	Y	na	Y/ IV	rabbits	2	N	EMEA/CHMP/SWP/4447/00 (1)
NovoEight	N	Y	na	Y/ IA, IV, PV	rabbits		N	EMEA/CHMP/SWP/4447/00
NovoThirteen	N	Y	na	Y/ IV, IA, PV	rats, cynomolgus monkeys	1	N	Natural protein
Nuwiq	N	Y	na	Y/ PV[ßß]	rabbits		N	EMEA/CHMP/SWP/4447/00
Obizur	N	Y	na	Y/ IV	cynomolgus monkeys	1	N	ICH S6
Ondexxya	N	Y	na	Y, IV	rats, cynomolgus monkeys	2	N	Naturally occurring protein, exempted from ERA
Refixia	N	Y	na	Y/ IV, PV, IA	Rowett nude rats, cynomolgus monkeys, rabbits	1, and independent study in rabbits	N	rFIX, and the chemical linker are due to their composition expected readily biodegradable
Rixubis	N	Y	na	Y/ IA, IV, PV	rabbits		N	EMEA/CHMP/SWP/4447/00
Veyvondi	N	Y	na	Y/ IA, IV, PV	rabbits		N	protein as active pharmaceutical ingredient, which due to its nature is unlikely to result in a significant risk to the environment
Vihuma	N, Same as Nuwiq, informed consent						N	EMEA/CHMP/SWP/4447/00
Parathyroid Hormone/analog								
Movymia	Y	Y	na	Y/ SC	rats	1	N	EMEA/CHMP/SWP/4447/00)
Natpar	N	Y	na	Y/ SC, IA, IV, i.p.	rats, dogs, cynomolgus monkeys	1 and single dose administration	N	(EMEA/CHMP/SWP/4447/00)
Terrosa	Y	Y	na	Y/ SC	rats	1	N	(EMEA/CHMP/SWP/4447/00)
Fusion proteins								
Benepali	Y	Y	na	Y	Cynomolgus monkeys	1	N	(EMEA/CHMP/SWP/4447/00 corr 2)
Erelzi	Y	Y	na	Y/ SC	Rabbits, Monkeys	2	N	EMEA/CHMP/SWP/4447/00 corr 2

Medicine Name	Biosimilar	Immunogenicity Y/N	Human TCR	Local tolerance/RoA	Species used	Local tolerance as a part of other studies	ERA	Justification for no ERA
Eylea	N	Y	na	Y/ IM,IV, IM, SC	monkeys, NZW rabbits	1, and independent study in rabbits	N	exempted from an environmental risk assessment
Nulojix	N	Y	na	Y/ IV, IA, SC	na (several species)		N	The active substance is a protein
Strensiq	N	Y	na	Y/ SC	male juvenile rats		N	recombinant protein
Zaltrap	N	Y	Y, 33 human tissues	Y/ IM, IV, SC	NZW rabbits, Cynomolgus monkeys	1, 4-w, 13-w and 6-m toxicity studies in cynomolgus monkeys	N	(EMEA/CHMP/SWP/4447/00)
Interferons								
Besremi	N	Y	na	Y	Cynomolgus Monkeys	1	N	(EMEA/CHMP/SWP/4447/00 corr 2)
Plegridy	N	Y	na	Y	rhesus monkey	1	Y	Phase I ERA: Persistence, bio accumulative and toxic potential less than 4.5. PEC in surface water lea than 0.01ug/L, no further studies carried out as per (EMEA/CHMP/SWP/4447/00 corr 1)
FSH Analogs								
Bemfola	Y	Y	na	Y/SC	male and female rats	1	N	Bemfola has the same structure and activity as endogenous FSH. The performance of specific studies for ERA was not requested.
Ovaleap	Y	Y	na	Y/ PV,SC, IM	NZW rabbits		N	not expected to pose a risk to the environment
Rekovelle	N	Y	na	Y,na	female rabbits		N	active substance is a protein and unlikely to result in a significant risk to the environment
Unclassified recombinant proteins								
Brineura	N	na	na	N ICH S6(R1)	-	catheter-mediated delivery evaluated after single dose in Cynomolgus monkeys and repeated dose in TPP1-null and WT dachshund dogs.	N	EMEA/CHMP/SWP/4447/00 corr. 1
Jetrea	N	Y	na	Y/ PV	NZW rabbits		N	EMEA/CHMP/SWP/4447/00
Kanuma	N	Y	na	Y/ IV	rats, juvenile cynomolgus monkey	1	N	EMEA/CHMP/SWP/4447/0

Medicine Name	Biosimilar	Immunogenicity Y/N	Human TCR	Local tolerance/RoA	Species used	Local tolerance as a part of other studies	ERA	Justification for no ERA
Lamzede	N	Y	na	Y/infusion site effects and repeated catheterisation effects	Juvenile rats, TG+KO mouse, monkey	1, 5	N	EMEA/CHMP/SWP/4447/00
Mepsevii	N	na	na	N	-	2***	N	EMEA/CHMP/SWP/4447/00
Myalepta	N	Y	na	Y/ SC	rats, NZW rabbits	-	N	unlikely to pose a significant risk to the environment
Oncaspar	N	Y	na	Y/ IM	Mice	1	Y	Only phase I ERA: calculation of the PEC in surface water (PECsw) for PEG, less than 0.01 µg/L. (EMEA/CHMP/SWP/4447/00 corr 2*)
Oxervate	N	Y	na	Y/ ocular administration	rats and rabbits	4	N	EMEA/CHMP/SWP/4447/00 corr 2
Palynziq	N	Y	na	Y/ SC	ENU2 mice	3	N	not expected to pose a risk to the environment
Ruconest	N	Y	na	Y/ IV	NZW rabbits	-	N	EMEA/CHMP/SWP/447/00
Spectrila	N	Y	na	Y/ IM, IA, IV, PV	rats	-	N	EMEA/CHMP/SWP/4447/00
Vimizim	N	na	na	N as RoA is IV	-	-	N	not expected to pose a risk to the environment
Vpriv	N	Y**	na	Y/ bolus IV	SD rats & Rhesus monkeys	1	N	Glycoprotein, does not pose a risk for the environment

Y: study conducted; N: study not conducted; na: no information in EPAR; PEC: Predicted Environmental concentration; PI: product information

** İmmunological response relating to the supplement to the cell culture medium was considered necessary by the CHMP and a specific Follow-up measure was agreed

***No dedicated studies, only observations from RDTS in juvenile cynomolgus monkeys and single dose studies in rats.

$: for the absence of need for further screening on the persistence and bioaccumulation potential of the drug substance PEG-rFVIII conjugate.

ßß: No other local tolerance studies deemed necessary for intended IV route as other toxicology studies provided sufficient information concerning possible local effects

1: as a part of repeat dose toxicity;
2: as a part of single and repeat dose toxicity
3: as a part of PD evaluation
4: as a part of local administration
5: as a part of developmental studies

Annex XLVIII: Data analysis- Immunogenicity, Local tolerance and ERA for 80 Medicinal Products.

		Immunogenicity		**Local tolerance**		**ERA**	
ATMPs	**N**	**n**	**%**	**n**	**%**	n	%
All products	10	4	40	5	50	8	80
New entities	10	4	40	5	50	8	80
		Immunogenicity		**Local tolerance**		**ERA**	
Insulin analogs	**N**	n	%	n	%	n	%
All products	9	8	89	8	89	0	0
New entities	5	4	80	5	100	0	0
Biosimilars	4	4	100	3	75	0	0
		Immunogenicity		**Local tolerance**		**ERA**	
GLP agonists	**N**	n	%	n	%	n	%
All products	5	3	60	3	60	0	0
New entities	5	3	60	3	60	0	0
		Immunogenicity		**Local tolerance**		**ERA**	
Filgrastim (Peg)/ (lipe)	**N**	n	%	n	%	n	%
All products	12	6	50	9	75	0	0
New entities	2	1	50	1	50	0	0
Biosimilars	10	5	50	8	80	0	0
		Immunogenicity		**Local tolerance**		**ERA**	
Blood factors	N	n	%	n	%	n	%
All products	17	16	94	16	94	1	6
New entities	17	16	94	16	94	1	6
		Immunogenicity		**Local tolerance**		**ERA**	
PTH analogues	**N**	n	%	n	%	n	%
All products	3	3	100	3	100	0	0
New entities	1	1	100	1	100	0	0
Biosimilar	2	2	100	2	100	0	0
		Immunogenicity		**Local tolerance**		**ERA**	
Fusion proteins	**N**	n	%	n	%	n	%
All products	6	6	100	6	100	0	0
New entities	4	4	100	4	100	0	0
Biosimilar	2	2	100	2	100	0	0
		Immunogenicity		**Local tolerance**		**ERA**	
Interferons	**N**	n	%	n	%	n	%
All products	2	2	100	2	100	1	50
New entities	2	2	100	2	100	1	50
		Immunogenicity		**Local tolerance**		**ERA**	
FSH	**N**	n	%	n	%	n	%
All products	3	3	100	3	100	0	0
New entities	1	1	100	1	100	0	0
Biosimilar	2	2	100	2	100	0	0
		Immunogenicity		**Local tolerance**		**ERA**	
Unclassified recombinant proteins	**N**	n	%	n	%	n	%
All products	13	10	77	10	77	1	8
New entities	13	10	77	10	77	1	8

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Hiermit erkläre ich an Eides statt, die Arbeit selbständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.